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Field screening of biofumigant species for the reduction of potato cyst nematodes (*Globodera* sp.)

By W D J WATTS[1], I G GROVE[1], G R TOMALIN[2] and M A BACK[1]

[1]*Harper Adams University, Newport, Shropshire TF10 8NB, UK*
[2]*Vegetable Consultancy Services, Brooke, Norwich NR15 1AT, UK*
Corresponding Author Email: 00708080@harper-adams.ac.uk

Summary

Potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are the most problematic potato pests in the UK. Management of PCN is often reliant on use of nematicides; however, pesticide registration and approval is becoming increasingly stringent and the likelihood of nematicides being retracted from commercial use more probable. Biofumigation represents a potential alternative. The process involves growing a green manure for maceration and incorporation into soil whereby intracellular glucosinolate (GSL) molecules are hydrolyzed to pesticidal volatiles such as isothiocyanate gases. The experiment detailed in this paper represents one of the first studies to investigate biofumigant species and blends for efficacy against PCN in-field, and for varietal suitability to UK conditions. Statistical differences (P<0.001) in PCN egg viability were found between the untreated control and biofumigant treatments, but no difference between biofumigant treatments was recorded. Overall, biofumigation was shown to reduce encysted egg viability by approximately 42%. Additionally, fresh (P=0.008) and dry weight (P=0.044) biomass was found to be variable between biofumigant species and blends. All biofumigants were found to be suitable for UK conditions when grown between September and November.

Key words: Potato cyst nematodes, biofumigation, glucosinolate, isothiocyanate

Introduction

Potato cyst nematodes (PCN) *Globodera pallida* (Stone) and *G. rostochiensis* (Wollenweber) are the most problematic pests of commercially produced potatoes (*Solanum tuberosum L.*) in the United Kingdom (UK). They are estimated to cause between £26–50 million in economic losses to the national crop per annum which equates to 3–6% of the national crop value (Twining *et al.*, 2009; Wale *et al.*, 2011; FAO, 2014).

Potato cyst nematode associated yield loss is initially attributed to invasion and parasitism of roots by infective juveniles (Turner & Subbotin, 2013). This decreases nutrient uptake efficiency in infested plants and limits haulm development (Trudgill *et al.*, 1975). A resultant decrease in total light interception by plants causes a reduction in photosynthetic activity and limits carbohydrate synthesis; a direct inhibitor of tuber yield (Hay & Porter, 2006). Root damage and parasitism by second stage juveniles and succeeding moults also detrimentally affects the ability of potato plants to access and transport water. This causes premature senescence and greatly reduces tuber yield in many instances (Evans *et al.*, 1975).

To manage PCN and protect yield, most growers rely on synthetic granular and fumigant nematicides (Kerry *et al.*, 2002; Lainsbury, 2013). Unfortunately for growers, new European Union (EU) legislation threatens the withdrawal of all registered nematicides from industry and no new and acceptable synthetic alternatives have yet been released (Hillocks, 2012). With 64% of UK fields infested with PCN and total losses being possible in unmanaged systems, UK growers urgently require alternatives to synthetic chemicals to ensure productivity remains high (Minnis *et al.*, 2002; Turner & Subbotin, 2013).

Biofumigation is a novel pest management technique which represents a potential alternative (Lord *et al.*, 2011). It involves growing glucosinolate rich cover crops such as Indian Mustard (*Brassica juncea*) or Oil Radish (*Raphanus sativus*) to mid-flowering, followed by macerating and incorporating biomass into soil (Kirkegaard *et al.*, 1993). This process liberates glucosinolate (GSL) molecules and myrosinase enzymes from plant cells, which in conjunction with water facilitates a hydrolysis process and the production of volatiles such as isothiocyanate (ITC) gases (Agerbirk & Olsen, 2012).

Research indicates a wide spectrum of commercially available and physiologically diverse biofumigant species (Kirkegaard & Sarwar, 1998; Lord *et al.*, 2011), although only a few studies have been conducted which investigate this method for management of PCN. Of the studies conducted, volatiles from 2-propenyl GSL and 2-phenylethyl GSL are suggested to be most promising for PCN management, although reductions in PCN viability are highly variable between experiments and only one study has tested biofumigation under field conditions in the UK (Buskov *et al.*, 2002; Lord *et al.*, 2011; Brolsma *et al.*, 2014; Ngala *et al.*, 2014). In the study conducted by Ngala *et al.* (2014), incidence of *G. pallida* was reduced by approximately 50% in summer sown crops, however, biofumigation effectiveness was markedly reduced in overwintered crops on account of poor varietal suitability to environmental conditions (Ngala *et al.*, 2014; Sarwar & Kirkegaard, 1998)

This paper reports the results of a UK field experiment conducted in Norfolk over the autumn of 2011 as an addition to the currently limited literature base. Several previously undocumented biofumigant species and blends were investigated for efficacy against PCN and suitability to late summer and autumn conditions. This work represents an expansion of the varietal and efficacy testing conducted by Ngala *et al.* (2014) and Lord *et al.* (2011).

Methods

Study site selection and initial preparation

A site with mixed PCN species was selected near Thetford (Norfolk) in the summer of 2011 for autumn biofumigant planting. Initial sampling showed that PCN infestation ranged between 10 and 400 eggs g^{-1} soil. A *Solanum sisymbriifolium* trap crop was planted on the site in July 2011 by the grower. Nearly 1 ha^{-1} *S. sisymbriifolium* was desiccated at cotyledon growth stage using glyphosate herbicide in August for planting the experiment in September.

Experimental design

The experiment was based on a randomised block design with 28 experimental units over four blocks. Each unit consisted a 5 m^2 plot with neighbouring plots within blocks adjoining. Inter block walkways were 2 m wide for easy access to plots and to allow for biofumigant material movement at incorporation. Treatments included an untreated control and six biofumigant treatments which were randomly assigned within blocks using the random numbers function in Microsoft 2010. Treatments consisted of several diverse species and species blends, selection of which was based upon disparities of GSL profile, concentration and biomass as suggested by Kirkegaard & Sarwar (1998). Only species exhibiting aromatic and aliphatic GSL's were sourced on account of ITC liberating qualities. Table 1 illustrates the species and blends of species used in this experiment, biofumigant product names, supplier details and seed rates used.

Table 1. *Treatment details for the field experiment (Thetford, Norfolk) investigating biofumigant species and blends*

Product name	Abbreviation	Species	Seed-rate (kg/ha)	Supplier
Variety 2	Var. 2.	*B. juncea*	15	Joordens Zaden
Trio	Tri.	*E. sativa*	8	
Biofum Summer	Bi.F.	*R. sativus* + *B. carinata* + *S. alba*	15	
Architect	Arc.	*S. alba*	10	
Fumigrow	Fum.	*B. juncea* + *S. alba* + *E. sativa*	8	Tozers Seeds
Curegrow	Cur.	*B. juncea* + *S. alba*	10	

Planting and maintenance

The experiment was initiated on 8 September 2011 between pre-existing 36 m tramlines. A 50 m tape measure and flexi canes were used to mark the experimental area, and seed sown to plots by hand at the rates detailed in Table 1. Plots were raked for improved germination.

Inter-block walkways were sprayed with glyphosate 4 wk post sowing to reduce weeds, whilst plots required no herbicide treatment. Although irrigation was an option in this experiment, no application was required. Canes connected with string were erected within blocks and bird-scares attached at 5 m intervals. Fertiliser equivalent to 60 kg ha^{-1} nitrogen and 100 kg ha^{-1} sulphate was applied to each plot 2 wk post sowing to increase biomass, and GSL production (Falk *et al.*, 2007).

Incorporation technique

Maceration was achieved on 18 November 2011 using a John Deere (JD) 6930 with a 3 m wide front mounted flail topper and 3 m wide rear mounted terradisc. This achieved approximately 7 cm foliage sections and damaged roots. A JD 8330 followed with mouldboard plough for inversion of material to approximately 20–30 cm depth. Sealing was achieved using a JD 6930 with 3 m wide heavy flat roll. All operations moved perpendicular to blocks and used inter block walkways to reduce biofumigant contaminations between plots as a result of drag. The incorporation process achieved maceration and inversion of standing crop into soil within one minute of initiation.

Egg density assessment

A 1 kg soil sample was collected from the centre of each experimental unit immediately prior to sowing and 4 wk post biofumigant incorporation for air drying and cyst extraction using a Fenwick Can (Fenwick, 1940). Egg density and viability was assessed using Meldola's blue staining as outlined by Shepherd (1986).

Biomass assessment

Root and foliage fresh and dry weight biomass assessments were conducted on each biofumigant inclusive plot on the day of incorporation. The foliage assessment involved randomly placing a bespoke 20 cm^3 quadrat onto each plot and harvesting the foliage within the cubic quadrat. This material was weighed and combined with foliage height and ground cover percentage data to estimate the fresh weight foliar biomass t ha^{-1} for each treatment. These samples were then oven

dried at 105°C and reweighed to reveal the foliage dry weight biomass which was used to produce projections of dry-weight biomass in t ha^{-1}. Root fresh weight biomass was assessed by digging, washing and weighing all roots within a 20 cm^2 area using a standard 20 cm^2 quadrat. Roots were then dried at 105°C and reweighed to reveal the root dry weight biomass. Similarly to foliage biomass, ground cover percentage was used in conjunction with root weight per 20 cm^2 to estimate projected root biomass t ha^{-1}. Root and foliage data was then combined to indicate total projected biomass (t ha^{-1}) for each biofumigant treatment.

Statistical analyses

Data was analysed by analysis of variance using GenStat (16th Edn). Treatments were compared using the Tukey's multiple range test at 5% probability.

Results

Biofumigation effect on egg viability of PCN

All biofumigant treatments were shown to negatively affect the viability of PCN when compared to the untreated control, however, no significant differences were found between biofumigant treatments (Fig. 1). Potato cyst nematode viability was shown to have been reduced by approximately 42% through the application of biofumigation.

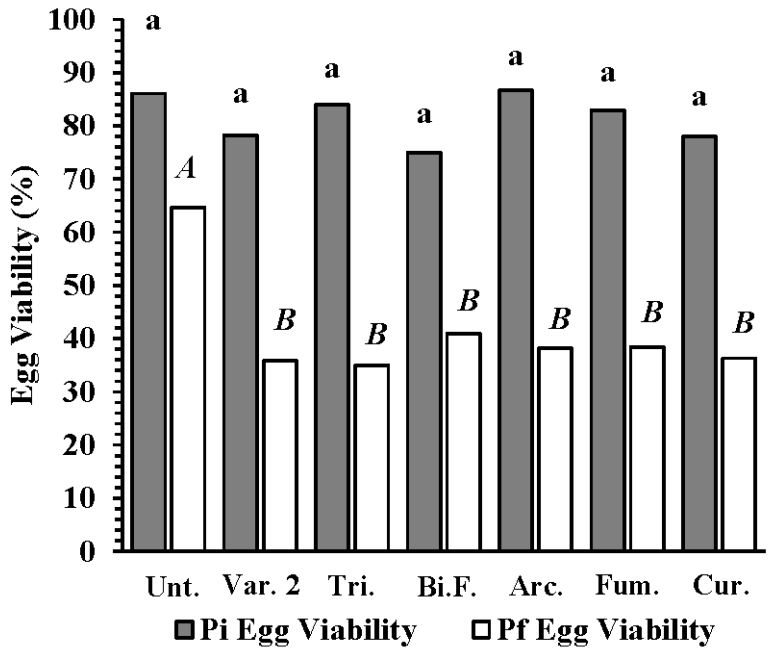

Fig. 1. Potato cyst nematode egg viability prior to sowing (P>0.05) and post incorporation of biofumigants (P<0.001) with selected species and blends. Lower case letters represent Tukey's analysis of P_i results. Italicised capital letters represent a separate Tukey's analysis of P_f results.

PCN population shift after biofumigation

Potato cyst nematode population shift was determined through calculation of P_f/P_i ratios between initial and final sampling (Fig. 2). Statistical separations were evident between biofumigant treatments and the untreated control, but not between biofumigant treatments. A negative shift of 25% was recorded in the untreated treatment, and between 45–58% under biofumigant treatments.

Biomass disparities between biofumigant species and blends

Fresh and dry weight biomass was shown to be variable between species and blends (Fig. 3). Trio illustrated lower fresh weight biomass to Architect and Fumigrow (P=0.008), and also lower dry-weight biomass to Architect (P=0.044). No other treatments were different from another.

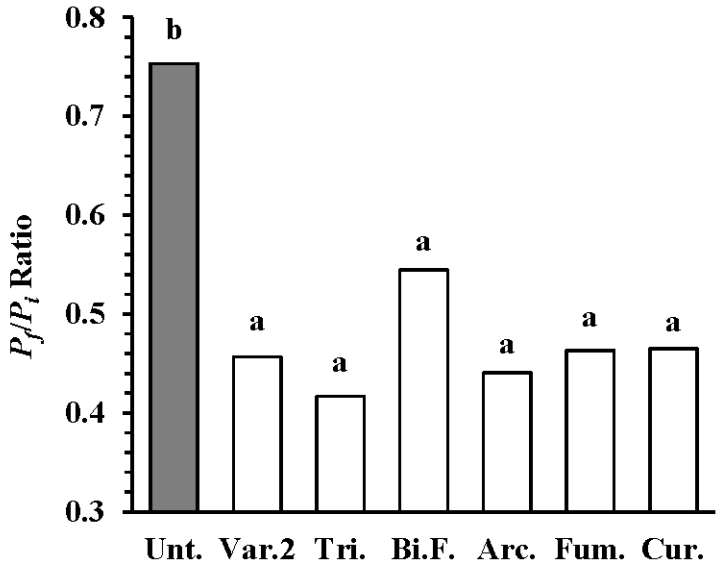

Fig. 2. Potato cyst nematode population shift as affected by biofumigation (P<0.001) with selected species and blends. Lower case letters represent Tukey's analysis.

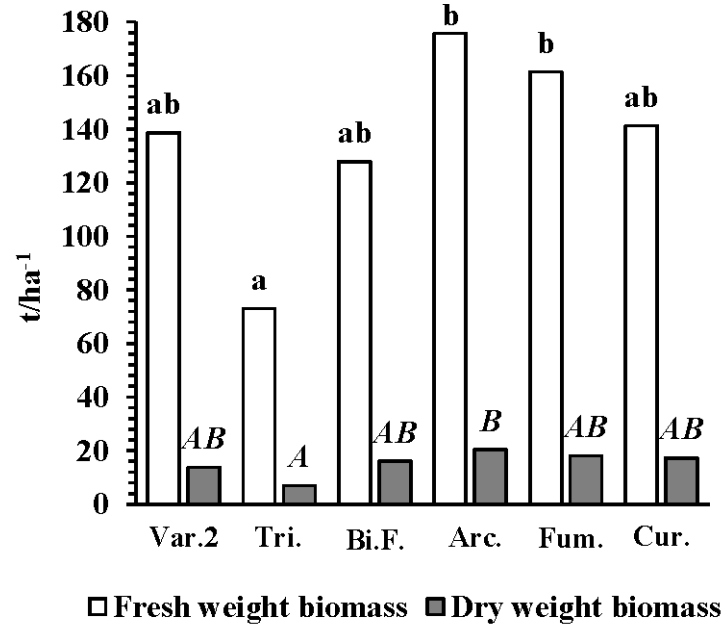

Fig. 3. Fresh and dry weight biomass of biofumigant treatments. Lower case letters represent Tukey's analysis of fresh biomass, whilst italicised letters represent analysis of dry biomass.

Discussion

This paper supports the findings of Ngala *et al.* (2014) and illustrates the potential of biofumigation for reducing the viability of PCN in the field. Results indicate field biofumigation of PCN to induce approximately 2 years of natural viability decline over a 72 day period from planting to incorporation (Wale *et al.*, 2011). This represents a useful reduction in PCN for UK growers. Moreover, the similar efficacy recorded in this paper to that recorded by Tobin *et al.* (2008) when investigating fosthiazate nematicide for reduction of PCN population densities, suggests in-field biofumigation to be a strong candidate for replacement of granular nematicides in the event of their retraction from industry.

Separation of biofumigant treatment effects on PCN egg viability was not achieved in this experiment in either the individual analysis of P_f data (Fig. 1) or the P_f/P_i analysis (Fig. 2), however, plants were late sown and had not achieved mid-flowering when incorporated which suggests that they had not achieved full biofumigation potential (Kirkegaard & Sarwar, 1998). Future field experiments in the UK should be sown in July or August to ensure mid-flowering is achieved before incorporation. This might aid separation of biofumigant treatment effects on PCN egg viability and population shift. Earlier sowing might also be expected to increase the level of PCN reduction across all treatments on account of increased biomass and improved biofumigation potential (Sarwar & Kirkegaard, 1998; Kirkegaard & Sarwar, 1998).

A negative 25% shift in PCN population under the control treatment was found (Fig. 2). This decrease was not anticipated, however, several factors and/or interactions could be responsible; the subjectivity of the Meldola's blue stain technique, mechanical damage to cysts and eggs during biofumigant incorporation and natural viability decline (Shepherd, 1986; Wale *et al.*, 2011).

Biofumigant species were shown to vary significantly in fresh and dry biomass (Fig. 3). This could suggest the GSL profile and/or concentration of tested varieties to also be variable as biofumigants induced inseparable effects on PCN viability and population dynamics. Trio is a particularly promising variety as it achieves comparable efficacy with all other varieties with half the biomass of Architect. Trio should be screened using HPLC for GSL profile and concentration as it represents a promising biofumigant for PCN management.

Overall the results of this study suggest that all biofumigants are effective in reducing PCN viability in-field under UK conditions and that approximately 42% viability decline in PCN can be achieved after incorporation of an early autumn sown biofumigant crop. All varieties were found suitable for UK implementation, although, this work should be replicated again and species and blends assessed for GSL content using HPLC as in the work of Lord *et al.* (2011) and Ngala *et al.* (2014). Analysis of ITC's from soil and air would also be desirable and could be analysed using GCMS equipment.

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