Appendix to R476 - Effectiveness of biofumigant crops for the management of PCN in GB

Field experiments to evaluate nitrogen and sulphur inputs on Indian mustard (*Brassica juncea*) cv. Caliente 99 used for suppressing potato cyst nematodes (2016-2017) – Glucosinolate assessments for field experiment sites in Shropshire and Lincolnshire

Table A1 shows the sinigrin concentrations found in composite stem and leaf tissue samples collected just prior to the maceration and incorporation of *Brassica juncea* cv. Caliente 99. Increasing nitrogen inputs from 0 to 150 kg/ha were found to cause a significant reduction (P=0.020) in both GSL and sinigrin concentration. In contrast, an increase of sulphur from 0 to 25 kg/ha resulted in a significant increase (P=0.008) in sinigrin concentration.

Tables A2 and A3 show the sinigrin concentrations found in stem and leaf tissue respectively for samples of *Brassica juncea* cv. Caliente 99 collected at the Lincolnshire site, just prior to the maceration and incorporation of the biofumigant. For leaf tissue (Table A2), nitrogen and sulphur did not affect sinigrin content independently. However, a significant interaction (P=0.001) was found between the two factors (N and S). Tukey's post hoc test revealed that at a dose of 50 kg/ha of nitrogen there was a significant difference between 0 and 25 kg/ha of sulphur, with the latter dose resulting in a higher concentration of sinigrin. In stem tissue (Table A3) there was also a significant interaction between nitrogen and sulphur for sinigrin concentration (P=0.001).

Table A1: *Sinigrin (2-propenyl glucosinolate) concentration (µmol g^{-1} DW) in the aboveground biomass of* Brassica juncea *cv. Caliente 99 sampled pre-incorporation (Shropshire site). Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Separate Tukey's multiple range tests (95%) conducted for nitrogen (lower case letters) and sulphur (upper case letters). Values with the same letter are not significantly different*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	20.71	23.37	30.34	24.8a
50	15.49	20.07	18.57	18.0ab
100	10.47	22.11	20.24	17.6ab
150	6.20	18.84	17.43	14.2b
Mean	13.2A	21.1B	21.6B	
Grand Mean	18.7			
Significance (P-value): N	0.020		SED: N	3.26
Significance (P-value): S	0.008		SED: S	2.82
Significance (P-value): N*S	0.733		SED: N*S	5.64
CV%	42.8			

Table A2: *Sinigrin (2-propenyl glucosinolate) concentration (μmol g^{-1} DW) in the leaf tissue of* Brassica juncea *cv. Caliente 99 sampled pre-incorporation (Lincolnshire site). Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Means with the same value are not significantly different according to Tukey's multiple range test (95%)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	57.3ab	49.2ab	49.8ab	54.6
50	42.4a	63.1b	57.6ab	54.4
100	51.7ab	60.6ab	46.7ab	53.0
150	57.2ab	46.6ab	60.1ab	52.1
Mean	52.2	54.9	53.5	
Grand Mean	53.5			
Significance (P-value): N	0.853		SED: N	3.29
Significance (P-value): S	0.642		SED: S	2.85
Significance (P-value): N*S	0.001		SED: N*S	5.70
CV%	15.1			

Table A3: *Sinigrin (2-propenyl glucosinolate) concentration (μmol g^{-1} DW) in the stem tissue of* Brassica juncea *cv. Caliente 99 sampled pre-incorporation (Lincolnshire site). Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Means with the same value are not significantly different according to Tukey's multiple range test (95%)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	13.1abc	15.6abc	20.3c	16.3
50	17.4bc	16.7bc	7.8ab	14.0
100	15.9abc	16.1abc	5.1a	12.3
150	14.6abc	16.5bc	18.1bc	16.4
Mean	15.3	16.2	12.8	
Grand Mean	14.8			
Significance (P-value): N	0.094		SED: N	1.83
Significance (P-value): S	0.103		SED: S	1.59
Significance (P-value): N*S	0.001		SED: N*S	3.18
CV%	30.4			

Conclusions

Previous findings from the experiments conducted in Lincolnshire and Shropshire highlight that an addition of 50 and 100 kg/ha of N respectively was sufficient to produce a significant increase in both leaf and stem fresh or dry weight (Tables 125-128, 146-149). For optimal biofumigation, brassica cover crops should produce high biomasses of up to 10 t DW above ground biomass per hectare (Lazzeri *et al*., 2004) and also high GSL concentration. Moreover, biofumigants should be selected on the type of GSLs produced, with reference to the biocidal activity of their respective isothiocyanates. The dominant glucosinolate in Indian mustard (*B. juncea*) is sinigrin (2-propenyl GSL) and this is hydrolysed by the enzyme (EC 3.2.1.147) thioglucoside glucohydrolase (myrosinase) to produce allyl (2 propenyl) isothiocyanate (ITC). Allyl ITC is known to have toxic effects on the eggs and juveniles of *Globodera* spp. (Buskov *et al*., 2002; Wood *et al*., 2017).

At the site in Shropshire, sinigrin concentration was increased through the addition of 25 kg of sulphur per hectare but decreased by nitrogen inputs of 150 kg/ha. Similar effects associated with these nutrients are described and discussed in the review by Bjorkman *et al*. (2011). The site at Lincolnshire did not present such clear trends but similar, yet non-significant, effects can be seen, with the highest sinigrin concentration found in the leaf tissue of *B. juncea* receiving 50 kg/ha of nitrogen and 25 kg/ha of sulphur.

Taking into account the results on sinigrin concentration and biomass production, it is reasonable to suggest that an input of 100 kg/ha nitrogen and 25 kg/ha sulphur would be optimal for biofumigation. This recommendation is further supported by the reduction in PCN egg viability (%) found in the treatment where *B. juncea* was provided with this ratio of nutrients at the site in Shropshire (see Table 123). Whilst these findings are interesting, further repetition of the studies over time are needed to fully substantiate the results.