Biofumigation: a significant opportunity for OSR and other brassica species with high glucosinolate contents?

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Glucosinolates are sulphur and nitrogen containing compounds that occur naturally in brassicas and closely related families. In rapeseed (*Brassica napus*), there are aliphatic glucosinolates which include 3-butenyl (Gluconapin), *R*-2-hydroxy-3-bunenyl (Progoitrin/Glucorapiferin) and 4-pentenyl (Glucobrassicanapin) which mainly occur within the foliage and aromatic glucosinolates such as 2-phenylethyl (Gluconasturtiin) which mainly occur in the the roots (Figure 1).

Aliphatic glucosinolates

3-butenyl (Gluconapin)

CH_2=CH-CH_2-CH_2

[$CH_2CHCH_2CH_2$]

R-2-hydroxy-3-bunenyl (Progoitrin)

CH_2=CH-CH-CH_2-

[CH_2CH(OH)$CHCH_2$]

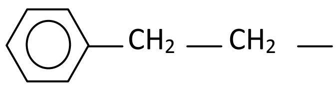
4- pentenyl (Glucobrassicanapin)

CH_2=CH-CH_2-CH_2-CH_2

[$CH_2CH(CH_2)_3$]

Aromatic glucosinolates

2-phenylethyl (Gluconasturtiin)



[$C_6H_5(CH_2)_2$]

Figure 1 Structural (top) and empirical (bottom) formulae of the R-groups in the main glucosinolates occurring in *Brassica napus*

Older varieties of rapeseed, for example those used in commercial production during the early 1970’s, contained reasonably high levels of glucosinolates from both types. Although, following research in Canada indicating potential problems in the use of oil-extracted rapeseed meal in the diet of some livestock, efforts were made to reduce glucosinolate content through breeding, including the utilisation of the ‘Bronowski’ gene, and subsequently standards to maintain reduced levels were introduced in EU. That standard is now 18 micromoles glucosinolates per gm defatted meal.

The occurrence of specific glucosinolates is not uniform throughout the brassica family and some species have different types and concentrations of molecules. Species such as Indian/brown Mustard (*Brassica juncea*), black mustard (*Brassica nigra*), white mustard (*Sinapis alba*) and oil radish (*Raphanus sativus*) are known to have a higher glucosinolate content.

Glucosinolates and the enzyme myrosinase are naturally partitioned from one another in living cells. However, tissue disruption such as that caused during insect feeding, mechanical damage or infection facilitates the mixing of myrosinase and glucosinolates. Myrosinase degrades glucosinolates through hydrolysis to produce a variety of volatile products including isothiocyanates (ITC’s), organic cyanides, ionic thiocynates and oxazolidinethiones, which appear to have some potentially exploitable biological activity. Indeed there is an increasing body of work on the use of the ‘glucosinolate-myrosinase system’ in crop protection. The vast majority of this work has focussed on the production of ITC’s from aliphatic and aromatic glucosinolates. This process is widely referred to as biofumigation. Interestingly, brassica’s have been used for many years by developing nations to control soil-borne pests and diseases. To date research has indicated that biofumigation can suppress weeds, nematodes, insects, bacteria and soil borne fungi such as *Rhizoctonia*, *Fusarium* and *Gaeumannomyces graminis* var. *tritici* (causal pathogen of take-all in wheat).

Developments in the EU, mainly driven by ‘Green’ parties in the European Parliament have debated the utilisation of pesticides (i.e. all types of agrochemical used for plant protection) and the debate seems to have moved the basis for assessing impact on potential to produce a hazard rather than a traditional, balanced risk analysis. The upshot of this outcome has been that many pesticides will cease to be available and that in turn will lead to potential reductions in plant production, at a time when food supplies are predicted to be under increasing strain due to increasing populations. Hence alternatives to conventional pesticides may be required quite quickly and the potential of

ITC's to fulfil some of the roles of the pesticides that are withdrawn comes to the fore. It appears then that the inclusion of green cropped brassicas may be the partial resolution to this challenge. Certainly a rotational 'pesticide' would obviate many of the problems and costs associated with toxicity testing of individual new molecules intended for use as pesticides and would of course be under individual grower/farmer control.

However, much yet needs to be undertaken in terms of developing a full understanding of this opportunity. For example, would '00' rapeseed residues alone be sufficient to have some significant impact on pathogens or pests? How significant are the concentrations of various ITC's in terms of biological activity against specific pest or pathogens and do current all crop pests and pathogens react in the same way to all of these molecules? What is the most effective method of maceration and incorporation into the soil of brassicaceous leaf tissues, stems and other aerial and root parts in terms of delivering optimal biofumigation? Additionally, when is the most appropriate time to grow and incorporate brassica crops in current crop rotations? Further, will that change as global warming progresses?

Optimistic indicators for exploitable activity of glucosinolates/ITC's have been shown in Australia, USA, South Africa and elsewhere on a range of different crops and much was summed up by Professor Venturi and Dr Palmieri at a biofumigation conference held in Florence, Italy some years ago. Similarly anecdotal evidence has been accrued in UK, indicating some decline in potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in potato crops, where closely cropped brassicas (e.g. cabbage; cauliflower) have been in the same rotation.

So, what are the next steps that should be taken? Clearly there is a need for quantification of the data on efficacy of ITC's derived from brassicas on a range of crop pests and pathogens. Also, the practical implication for incorporation of green material needs elucidation. Further, the ideal brassica for the production of glucosinolates/ITC requires identification too, or perhaps some new thought needs to be directed towards rapeseed varieties and their glucosinolate content, such that normal rotations may be limiting to pests and diseases for the crops in that rotation?

Work at Harper Adams University College (Shropshire, UK) is currently investigating the use of Indian mustard for the biofumigation of potato cyst nematodes (PCN). PCN are major economic pests of the potato crop in the UK and elsewhere, causing annual losses in excess of £50 million in the UK alone. Soil- borne populations of PCN can rapidly increase during repeated exposure to potato crops, but are slow to decline in the absence of the crop. Management of these organisms poses a significant challenge to potato growers due to the lack of resistant varieties (particularly for *G. pallida*) and the threat to the limited number of chemical control measures by proposed changes in EU legislation. Preliminary studies conducted by B.Sc. and M.Sc. project students, investigating the use of biofumigation for PCN management produced promising results. In one study, the incorporation of the commercially named 'Caliente' mustard residues immediately prior to potato planting were found to reduce PCN invasion of potato roots by up to 48%. In other work, a clear trend was found between the method used to macerate mustard plants and the degree of nematode invasion. Mustard foliage that was chopped, frozen and pressed produced the biggest reduction in nematode invasion, whereas chopping alone produced less of an effect on the nematodes. These results probably reflect the greater degree of tissue destruction and ultimately increased mixing of myrosinase with glucosinolates to generate ITC's.

Although ITC's appear to affect the hatched nematode juveniles, it is unclear whether they are able to diffuse through the cuticle of dormant nematode cysts which contain up to 500 eggs. This question needs to be addressed in order to establish the most appropriate time for mustard to be grown within a rotation. Whilst considering this question, it is important to note that other researchers, namely Sarwar & Kirkegaard (1997), have shown that the glucosinolate content of some brassica species is up to 10 times higher when sown in the spring in contrast to plants sown in the autumn. Brassica plants grown in the autumn inevitably experience less radiation, cooler temperatures and shorter day lengths. As a result of these conditions the plants accumulate less glucosinolates and therefore produce lower concentrations of isothiocynates when incorporated in the soil. In addition to the accumulation of glucosinolates it is also critical to consider the biomass potential of brassica's. Existing literature indicates that brassica species differ in their biomass production depending on the time of year that they are grown.

A new Ph.D. project sponsored by Harper Adams U.C., Agrovista and Plant Solutions Ltd will be investigating specific parameters affecting the efficacy of biofumigation in the management of PCN with the aim of developing practical guidelines for growers. In particular this work will be examining incorporation methods, application timing, biomass potential and soil sealing techniques. Furthermore, studies will need to be replicated on field sites with contrasting soil characteristics. This is important because the fate of ITC's in soil is known to be affected by volatilisation, organic matter, leaching and microbial degradation (Brown & Morra, 1997).

Since initiating work in this area, there has been an increased level of interest in biofumigation from local potato growers. Indeed a number of individuals, with support from their agronomists, have begun conducting their own strip trials with Indian/brown mustard (*Brassica juncea*) on land known to be heavily infested with PCN.

Given that the use of chemical control methods appears to be on the decline e.g. the failure of 1-3 Dichloropropene (Telone II) to reach Annex 1, the need for alternative crop protection strategies is more crucial than ever before. Methods such as biofumigation offer an effective yet sustainable replacement for chemical control in the longer term. However, research is clearly needed to formulate appropriate usage. In the wider context, biofumigation offers a number of advantages to crop production such as increased nutrition (green manure), improved soil structure and multi-faceted control of pests, weeds and diseases. Clearly too, if oilseed rape, as both a crop and, simultaneously in effect, a 'pesticide' producing plant could be incorporated into rotations the significant financial and environmental benefits could accrue.

NOTE: The authors of this report are presently in the process of developing a network for those with research interests in biofumigation. It is anticipated that the network will allow greater interaction between researchers in the EU. Please contact Matthew Back if you are interested in joining the network (mback@harper-adams.ac.uk).