Biofumigation in Agriculture and Horticulture

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Abstract

Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds, particularly isothiocyanates (ITCs), released from brassicaceous rotation and green manure crops when the glucosinolates (GSLs) in their tissues are hydrolysed in soil. Our research has investigated the potential to exploit biofumigation by *Brassica* rotation and green manure crops for pest and disease suppression in both broad-acre and horticultural crops. Some hydrolysis products, particularly the ITCs are known to have broad biocidal activity including insecticidal, nematicidal, fungicidal, antibiotic and phytotoxic effects.

We have shown that the roots of canola (*B. napus*) oilseed crops contain an aromatic GSL, 2-phenylethyl GSL (2PE-GSL) which is hydrolysed in soil to release 2-phenylethyl ITC, a compound highly toxic to cereal pathogens such as take-all (*Gaeumannomyces graminis*) and *Pratylenchus* nematodes. Studies in pots and in the field have shown that canola varieties with higher levels of 2PE-GSL in the roots reduce populations of these disease pathogens resulting in less disease development in subsequent wheat crops. Breeding studies have also demonstrated the high heritability of 2PE-GSL in the roots of *B. napus* which was unlinked to seed GSL levels, presenting the opportunity to develop varieties with enhanced biofumigation potential in agricultural systems. Current research is concerned with determination of the amounts and timing of ITC production in the soil, and investigating the wider impacts on soil organisms and how this might affect plant growth either in the presence or absence of pathogens. Initial results

indicate that changes in rhizosphere microbial communities, related to GSL levels of varieties, persist into following crops

In high value horticultural crops, interest in the potential use of 'biofumigant' brassicas for disease control has grown as a result of the phase-out of methyl bromide as a soil fumigant, and the banning of several other synthetic pesticides. One of the main alternative chemicals in use is metham sodium, a precursor of methyl ITC, a compound similar to those produced naturally by brassicas. Significant empirical evidence suggests potential for suppression of a range of soil pests by *Brassica* green manures. Previous research has often failed to take account of the variation in GSL types in various species and/or plant tissues, differential sensitivity of different organisms to ITCs, and opportunities to increase the efficiency of ITC release in soil during incorporation. We have focussed on these issues in collaboration with groups world-wide including investigations of pest control in potatoes, vineyards, orchards, cotton, sugarcane, tobacco, carrots, tree seedling nurseries, strawberries and others. Several seed companies have released 'biofumigant' brassicas onto the market in Australia and elsewhere, but significant potential exists to improve their efficacy based on current research.

Introduction

The term biofumigation refers to the allelopathic effects of biocidal glucosinolate GSL hydrolysis products, principally isothiocyanates (ITC), on soil organisms (Angus *et al.* 1994). This review briefly describes the chemistry underlying the concept of "biofumigation", summarises previous field applications, and considers approaches necessary to enhance the effectiveness of biofumigation as a part of IPM in broad-acre and horticultural crop production systems. A major focus is in current research in the Australian agriculture and horticulture industries. The subject has been previously reviewed by Brown and Morra (1997) and Rosa *et al.* (1997)

GSLs are hydrolysed by the myrosinase enzyme to release a range of hydrolysis products including nitriles, sulfur, oxazolidinethione, epithionitriles, thiocyanates, thiones and various forms of ITCs, which may be formed under specific conditions (Brown & Morra 1997; Gardiner *et al.* 1999; Rosa *et al*. 1997). These GSL hydrolysis products are responsible for the flavours of *Brassica* vegetable and condiments (eg mustard), the anti-nutritional properties of some seed meals (eg rapeseed), and the interactions of brassicas with certain pest organisms (eg cabbage white butterflies) (Brown and Morra 1997; Chew 1988; Fenwick *et al.* 1983; Rosa *et al*. 1997). In addition, the non-GSL derived compounds carbon-disulfide, dimethyl-disulfide, dimethyl-sulfide and methanedithiol are formed during decomposition of brassica tissues (Bending & Lincoln 1999). These are less toxic that ITCs but are formed in larger quantities with measurable microbial impact (Bending & Lincoln 1999; Bending & Lincoln 2000).

The hydrolysis of GSLs is primarily constrained by the separate localisation of GSLs in aleurone-like cells and myrosinase in myrosin cells (Bones and Rossiter 1996; Kelly *et al.* 1998). Although recent studies have shown the coexistence of both GSL and myrosinase in protein vacuoles of aleurone-like cells (Kelly *et al.* 1998). Thus although hydrolysis certainly occurs with tissue damage such as the ingress of pathogens, herbivory by insects, plant senescence, or during mechanical damage such as incorporation into the soil (Bones and Rossiter 1996) it is possible that hydrolysis occurs in the absence of damage. This would

explain why ITCs may be produced by growing roots in the apparent absence of damage (Bending and Lincoln 2000).

Biofumigation by oilseed crops in Australia

Field experiments in Australia during the early 1990's demonstrated that wheat crops grew more vigorously following *Brassica* break crops such as canola and Indian mustard than other non-legume break crops and improved vigour could not be attributed to N nutrition or residual water (Angus *et al.* 1991, Kirkegaard *et al.* 1994, 1997). The beneficial effects of brassicas were not obvious in all situations (Heenan 1995) and the magnitude of the effect varied significantly with site and seasonal conditions (Kirkegaard *et al.* 1994). These results were consistent with those reported in Europe (Schonhammer and Fischbeck *et al* 1987a,b)

One hypothesis regarding the beneficial effects of brassicas was that in addition to not hosting cereal diseases, biocidal ITCs released during breakdown of GSLs in their residues reduced disease infection in following crops. The term "biofumigation" was coined to describe this suppression of soil pathogens by compounds released from *Brassica* tissues, and implies a greater reduction in disease inoculum than that resulting from the simple absence of a host. In Australian broad-acre cropping, the *Brassica* crops are grown to maturity and the dry shoot residues remaining after harvest are low in GSL and are not usually incorporated by cultivation. Thus the decaying root system seemed the likely source of the suppressive compounds. Field studies identified 2-phenylethyl GSL (2PE-GSL) as the major GSL present in the roots of canola, comprising around 80% of the total GSL profile (Kirkegaard and Sarwar 1999).

<u>In vitro *studies*</u>

Confirmation of the toxicity of both hydrolysed *Brassica* tissues and specific GSL hydrolysis products has been extensively demonstrated (see Brown and Morra 1997). Toxicity of ITCs has been demonstrated for microbial bacteria (Brabban and Edwards 1995), post harvest fruit pathogens (Mari *et al*. 1993), soil fungi including pathogens (Drobnica *et al*. 1967; Kirkegaard *et al*. 1996; Manici *et al*. 1997, 1999; Mithen & Lewis 1986; Pryor *et al*. 1940; Walker *et al*. 1937) and saprophytic fungi (Drobnica *et al*. 1967).

In vitro toxicity tests using fresh or re-hydrated *Brassica* root tissues demonstrated suppression of a range of soil-borne cereal pathogens (Angus *et al.* 1994, Kirkegaard *et al.* 1996). Further studies using different ITCs dissolved in agar showed that 2-phenylethyl ITC (2-PE ITC) was the most toxic of several ITCs (including the commercial soil fumigant methyl ITC). This was consistent with previous studies indicating the generally higher toxicity of the aromatic ITCs (Sarwar *et al.* 1998). Although these studies provided evidence for the toxicity of ITCs under laboratory conditions, their effectiveness in soil remained uncertain, as significant losses of ITCs due to sorption and other processes may occur (Brown and Morra 1997). The efficiency of conversion of GSLs in *Brassica* tissues into ITCs can be as low as 15% in soil (Borek *et al.* 1997).

Effectiveness in soil

In pot studies, Smith *et al.* (1999) showed that incorporation of ground, freeze-dried canola root residues at realistic field rates (0.1 – 0.5% w/w) caused a 50% reduction in the infection of wheat seedlings by several root fungal pathogens, a result consistent with that found for *Pratylenchus* nematodes (Potter *et al.* 1998). In another study, Kirkegaard and Sarwar (1998) grew several different crops in pots of soil with added inoculum of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*), and incorporated the root residues after harvesting the tops at flowering. Wheat seedlings were sown into the pots eight weeks later and the level of take-all infection was assessed. The results showed lower levels of infection on wheat after the brassicas compared with linseed and higher root GSL levels increased the level of suppression (Figure 1). These results demonstrate that significant suppression of fungal inoculum can be achieved in soil by incorporated canola roots or root residues, and that higher levels of GSL increase suppression.

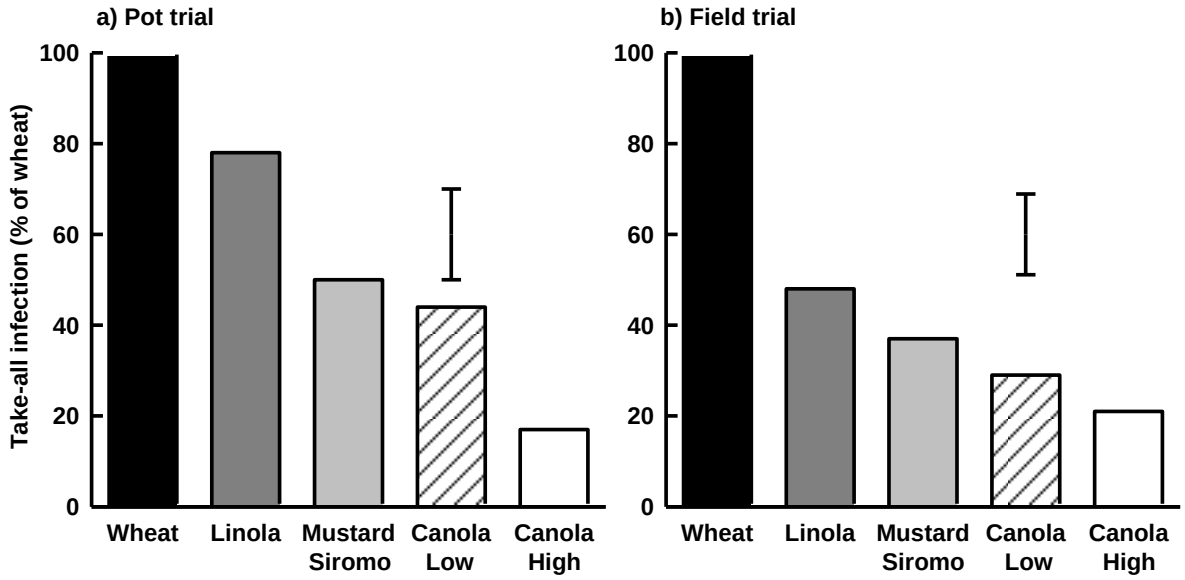

Figure 1. Infection of wheat seedlings by take-all (*Gaeumannomyces graminis*) following a range of different crops in (a) pot and (b) field experiments (15 January). The infection is expressed as a % of that found following wheat. Vertical bars show lsd (P=0.05).

Another broad-acre application of biofumigation is against the nematode *Pratlyenchus neglectus* which causes root rot in wheat. In pot studies nematode numbers are suppressed in soil by a wide variety of *Brassica* spp., and suppression was attributed primarily to 2-PE ITC (Potter *et al.* 1998). However, while the hydrolysed canola tissues are toxic to the nematode, canola is nevertheless a host for the nematode. However Potter *et al.* (1999) have showed that increased levels of 2-PE ITC had a strong effect on the susceptibility of canola to attack by *Pratylenchus neglectus*. Thus it appears that the suppression may occur in response to biofumigation effects on the nematode in the soil, and additionally by reducing hosting on the canola in previous years.

Field studies

In the field, canola roots are generally left undisturbed after harvest and there is a 4-5 month period from canola harvest (December) until the following wheat crops are sown (May). In field studies we investigated the impact of a range of crops including brassicas with high and low root GSLs on the levels of take-all

inoculum in soil during the growth of the crops, at maturity, and during the summer fallow (Kirkegaard *et al*. 2000). The experiments were conducted at sites where high initial levels of take-all inoculum had been established in the previous year. The levels of inoculum were measured using a wheat seedling bioassay on soil sampled from plots. The results showed that at harvest, the *Brassica* crops were more suppressive to the take-all fungus than linseed and that the level of suppression was greater for the high root GSL variety (Fig 1). These differences were not apparent during crop growth suggesting that the suppression occurred as a result of root decay around maturity rather than exudation from live roots. This is consistent with the fact that most 2-PE GSL is present in the large tap and lateral roots and is likely to be released during the decay of these larger roots as the crop matures (Kirkegaard and Sarwar 1999). The impact of this fungal suppression on the disease development in following wheat crops was dependent on the seasonal conditions in the subsequent summer fallow (Table 1). In the wet summer of 1996/97 the differences in take-all inoculum between different crops had disappeared by March due to the wet summer reducing inoculum to low levels after all break crops. In contrast, the dry summer in 1997/98 preserved inoculum and significant differences were apparent until mid April, when the following crops were sown. These results indicate that the benefits of biofumigation will be highly dependent on seasonal conditions.

Effects on beneficial organisms

While there are reports from overseas of brassicas being colonised by VAM fungi, a survey of *Brassica* crops across temperate south east Australia found no instances of colonisation (Ryan *et al*., 1999). Examination of many crop rotation experiments in southern Australia over a number of seasons found VAM colonisation of wheat following a *Brassica* crop to be greatly reduced compared with wheat after a mycorrhizal colonized crop. However, the reduced colonisation had no effect on wheat nutrition, growth or yield. The situation is likely to differ in the subtropical northern Australian wheatbelt, where VAM fungi play a greater role in crop nutrition and growth (Thompson 1987).

The reduction in VAM colonisation following *Brassica* crops is probably simply due to a decline in inoculum in the absence of a host. Colonisation of crops in the field is similar following brassicas with differing levels and types of glucosinolates. Moreover, addition of canola roots to soil, up to rates far

greater than would occur in the field, did not affect colonisation of clover in a glasshouse experiment (Ryan 2001). However, application of larger volumes of material, such as in the use of green manure crops in horticulture, may greatly affect VAM inoculum levels and would need to be investigated in each instance (Ryan 2001).

Opportunities to enhance the biofumigation effect

While the conversion efficiency of plant GSL to soil ITC is low the two are correlated. Thus breeding to increase plant GSL will be an effective strategy in increasing biofumigation potential. A recent study by Kirkegaard *et al.* (2001) showed high heritability for 2-phenlyethyl GSL, the predominant GSL in the roots of canola. Large additive gene action, small G×E (Kirkegaard *et al.* 2001) and the fact that there is apparently no correlation with seed GSL in *B. napus* (Kirkegaard and Sarwar 1998, 1999) indicates that early selection for high 2-PE GSL in a breeding program is possible.

Horticulture: glucosinolates a source of natural soil fumigants

Empirical evidence

In horticulture potential use of 'biofumigant' brassicas for disease control has grown as a result of the phase-out of methyl bromide as a soil fumigant, and the banning of several other synthetic pesticides. One of the main alternative chemicals in use is metham sodium, a precursor of methyl ITC, a compound similar to those produced naturally by brassicas. Significant empirical evidence suggests potential for suppression of a range of soil pests by *Brassica* green manures (Table 1). *Brassica* green manures, rotation crops or seed meal amendments have been reported to suppress pest and disease organisms when grown or incorporated in the soil. Table 1 summarises results from a range of published papers in which various pest organisms have been suppressed by Brassicaceous amendments in the field. Importantly, Table 1 also includes examples in which no effective suppression was observed, and one in which disease levels increased when *Brassica* amendments were incorporated. This variation in the response of different organisms is explicable in terms of the variation in the type, concentration and distribution of the

biofumigant pre-cursors among different *Brassica* species/varieties, the differential sensitivity of various pest organisms to the ITCs, and the often inefficient release of ITCs from incorporated tissues.

Biofumigation may be implemented by growing a green manure crop grown *in situ* and incorporating, companion planting (where root tissue is active) or utilising residues from nearby crops after harvest (eg cabbage roots, stem bases and lower leaves, radish stems and leaves). Utilising a suppressive rotation crop or a green manure may significantly reduce the time needed to lower inoculum levels.

Table 1. Examples of the impacts of various *Brassica* amendments on a range of pest organisms in field studies.

Pest	**Brassica used**	**Suppression**	**Reference**
Suppression			
Soldier fly (insect)	Kale, Raddish	76-86%	Blank *et al.* (1982)
Bacterial wilt (bacteria)	Indian mustard	40-80%	Akiew and Trevorrow (1999)
Root-knot nematode	Rapeseed	53-78%	McLeod and De Silva (1994) Mojtahedi *et al.* (1993)
Aphanomyces root rot (fungus)	White mustard	29-54%	Muehlchen *et al.* (1990) Chan and Close (1987)
Weeds	Rapeseed	50-96%	Boydston and Hang (1995)
No Suppression			
Verticillium dahliae (fungus)	Rapeseed	0%	Davis *et al.* (1996)
Rhizoctonia, *Pythium* (fungi), *Meloidogyne* (nematode)	Rapeseed	0%	Johnson *et al.* (1992)
Pathogen Stimulation			
Pythium (fungus)	Canola, mustard	(+ 8%)	Stephens and Davoren (1997)

Enhancing biofumigation potential

There are about 20 different types of GSLs commonly found in brassicas which vary in their structure depending on the type of organic side chain (aliphatic, aromatic or indolyl) on the molecule. The profile, concentration and distribution of these GSLs varies within and between *Brassica* species and in different plant tissues, and consequently the concentration and type of biocidal hydrolysis product evolved also varies (Table 2). Among the major hydrolysis products, ITCs are generally considered the most toxic, however individual ITCs also vary in their toxicity to different organisms. For example the aromatic GSLs often found in *Brassica* roots release ITCs which are up to 40 times more toxic to the eggs of black vine weevil than aliphatic types found in shoots (Borek *et al.* 1995). The range in GSL profiles, the differential toxicity of the ITCs to different pests, and the wide range in phenological and morphological diversity in brassicas provides significant scope to select or develop brassicas with enhanced biofumigation potential for particular target organisms (Kirkegaard and Sarwar 1998).

Table 2. Range of GSL types and concentrations in various field-grown Brassicaceous species and plant parts harvested at flowering in Canberra 1995. (from Kirkegaard and Sarwar 1998).

Species	Plant Part	Major ITC-liberating GSL types	Concentration range (μmole/g tissue)
Brassica napus (rapeseed, canola)	Shoot Root	3-butenyl, 4-pentenyl 2-phenylethyl	0.1 – 25.0 1.9 – 29.7
Brassica juncea (Indian mustard)	Shoot Root Seed Meal	2-propenyl 2-phenylethyl, 2-propenyl 2-propenyl	1.2 - 21.6 4.1 - 11.9 10.0 – 100
Brassica oleracea (cabbage, cauliflower)	Shoot Root	3-methylsulphinylpropyl 2-phenylethyl	1.4 –7.3 2.4 – 10.8
Raphanus sativa (Raddish)	Shoot Root	4-methylsulphinylbutenyl 4-methylsulphinylbutenyl	17.8 – 30.0 35.2 – 48.2
Sinapis alba (white mustard)	Shoot Root	ρ-hydroxybenzyl, benzyl benzyl, 2-phenylethyl	16.0 – 26.0 8.0 – 14.7

As a result of the variation shown in Table 2, it is difficult to interpret the role of ITCs in the suppressive effects reported in many previous field studies such as those summarised in Table 1. In most cases, no information is provided on the type or concentration of the precursor GSLs in the incorporated tissues. Furthermore, the contribution of root tissues in incorporated green manures has often been overlooked, despite the high concentrations of highly toxic aromatic ITCs derived from the roots (Sarwar *et al.* 1998). Although useful to indicate the potential for pest control, empirical studies provide limited scope to both assess the basis of the observed effects and to pursue opportunities to enhance them. The risks of this empiricism are two-fold; firstly without information on GSL types or concentrations biofumigation may be disregarded as an option when different species, cultivars or plant tissues may be suppressive; secondly biofumigation may be pursued when the observed suppression is unrelated to GSL hydrolysis products as in the case of nematode suppression by *Brassica* leaf tissues reported by Potter *et al.* (1998). Despite this uncertainty, the magnitude and range of the suppressive effects reported in Table 1, with no purposeful selection of biofumigant types, suggests significant potential to improve biofumigation efficacy if information on the most effective compounds for particular target pests is sought.

Biofumigation in practice

There are many reports in the literature of laboratory and glasshouse studies demonstrating the suppressive potential of pure ITCs or *Brassica* tissues (reviewed by Brown and Morra 1997), but fewer reporting its successful use under field conditions. This is in part due to the problems mentioned above, but also to the many other considerations associated with success in the field. The soil type, climate and time of year in which the crop is to be grown will influence the choice of *Brassica* species. Many brassicas are adapted to temperate climates and flower quickly in response to longer days, providing limited biomass (Sarwar and Kirkegaard 1998). The *Brassica* crops may also be susceptible to local pests and pathogens for which control is prohibitively expensive and management of the crop needs to be compatible with equipment available on farm. The brassicas may even host the pathogen of interest (e.g. *Rhizoctonia*, some nematodes) which will limit their potential to reduce the pathogen populations (McLoed and Steel 1999). Fortunately, the wide variety of phenological and morphological diversity within the Brassicacae provides

scope to select those most appropriate for different environments and applications (Kirkegaard and Sarwar 1998).

Once there is good field evidence for suppressive effects of brassicas linked to the GSL hydrolysis products there are two key strategies available to enhance the suppressive effects. Firstly by selecting brassicas that produce the greatest amount of the GSL-precursors most toxic to target organisms, and secondly by managing the incorporation process to maximise the exposure of the organisms to toxic compounds at the most vulnerable stage. Selecting appropriate brassicas requires information on the types of GSL products to which the pathogen is most sensitive. For cereal pathogens the aromatic GSLs present in canola roots have been shown to be highly toxic to cereal fungal pathogens and genetic diversity within Australian canola varieties allows selection for higher root GSL levels (Kirkegaard and Sarwar 1999). Our preliminary studies on the bacterial wilt pathogen (*Ralstonia solanacearum* indicate that tissues of mustards (*B. juncea, B. nigra* and *B. carinata*) are more suppressive than those of other brassicas tested suggesting that 2-propenyl GSL is the active compound (Akiew, unpublished). This compound is also present in kale and cabbage indicating residues from these crops may also have activity against BW. This type of information linking GSL types and concentration with impacts on the pest organism is fundamental to maximising the suppressive potential of biofumigation.

Increasing the efficiency of tissue incorporation involves matching the timing of incorporation and release of biocides to the most vulnerable stages of the pest organisms life-cycle. For example white-fringed weevil exists in the soil for a long period as a small larvae in the pasture phase preceding potato crops, a time when biofumigant crops can be grown and incorporated (Matthiessen and Kirkegaard 1998). Sufficient time must be provided for the organic material to decompose to avoid both physical and potential allelopathic interferences in the following crop, while waiting too long may allow some pathogens to recover. The efficiency of release of ITCs from incorporated tissues is also influenced by a number of factors including soil type, moisture content, GSL concentration, degree of tissue disruption, type of tissue (leaf/root/shoot) (Brown and Morra 1997). These factors can be manipulated to develop incorporation

strategies that maximise biocide release from the tissues. In developing countries, small scale farmers may have significant scope to manipulate the collection, placement, timing and method of tissue incorporation for maximum effect compared with the broader scale mechanised systems. Recent studies as yet unpublished undertaken by M. Morra (Personal communication) have identified tissue disruption and increased soil moisture as the two most critical factors in increasing conversion efficiency of GSL to ITC in the soil.

Biofumigation, like most cultural control techniques will not be a "silver bullet" and will need to be integrated with other approaches such as resistant varieties, crop rotation, solarisation, biocontrol agents or augmented chemical applications. Previous research has demonstrated that the impact of incorporated cabbage residues can be enhanced by combining it with solarisation (Keniath 1996). Recent studies at CSIRO also indicate that the widely used biocontrol fungus *Trichoderma* is considerably more resistant to the ITCs released from canola roots than cereal pathogens which may provide opportunities to combine biofumigant rotation crops with *Trichoderma* inoculants to increase control efficiency (Smith and Kirkegaard unpublished). In the USA, the use of rapeseed green manures planted in the fall and incorporated prior to spring potato crops has reduced soil erosion, reduced infection by *Rhizoctonia* and suppressed red-root pigweed sufficiently to reduce the number of herbicide sprays required (M. Morra personal communication 2000)

Potential problems

Some difficulties may arise in fitting a biofumigant green manure crop into the cropping systems, especially in intensive horticultural production systems. Biofumigation will not be as straightforward and easy as applying a single chemical fumigant at high doses typically recommended to ensure a high degree of effectiveness across a wide range of soil types and climatic zones. However the need for long periods of rotation with non-hosts in many horticultural systems and the increasing use of legume and cereal cover crops indicates scope to include biofumigants in some systems.

Brassicas are also susceptible to their own suite of pests and diseases, with GSLs having a role in both attracting and repelling insects and other organisms (Giamoustairs and Mithen 1995). Thus the possibility

of brassicas attracting their own pests or acting as refugia for pests and diseases of other crops must be carefully monitored as costly control measures within a biofumigant crop will be a major disincentive. Brassicas may influence beneficial organisms such as mycorrhizal fungi, although their non-host status may be more to do with the absence of exuded growth stimuli rather than to ITC release as originally thought (Glenn *et al.* 1988).

Current Research

The major aim of the current CSIRO Biofumigation research is to improve the efficacy observed in previous empirical studies by identifying the GSLs most toxic to specific target organisms, selecting brassicas with high concentrations of those compounds and developing incorporation strategies to enhance biocide release in soil. Similar studies are being undertaken in laboratories in Italy, Idaho USA and UK. An example of the novel results that can arise from this approach is shown by the recent work on suppression of white-fringed weevil, an important pest of potatoes in Western Australia (Matthiessen and Kirkegaard 1998). Studies on the toxicity of various *Brassica* tissues revealed that the roots of some fodder rape varieties were more toxic than high GSL mustard seed meal that is generally considered one of the most potent sources of ITCs. The aromatic compound 2-phenylethyl GSL present in the roots appears to be highly toxic to the organisms and quite persistent in the soil following incorporation of fodder rape plants in the field (Gardiner *et al.* 1999). Various fodder rape varieties are now being tested for field effectiveness and breeding efforts to enhance levels are underway (Wrightson HRDC HG98036). The CSIRO group have now accumulated a wide range of brassicas with known GSL profiles and groups of individual species which vary from high to low in single GSL types. This material provides an excellent resource for testing GSL efficacy and to test release efficiency in the soil. The work is providing a growing body of data on the pattern of release, persistence and fate of *Brassica*-derived ITCs in soil (Matthiessen *et al.* 2001).

Several seed companies have released ‘biofumigant’ brassicas onto the market in Australia and elsewhere (Table 3), but we believe significant potential exists to improve their efficacy based on current research. We have focussed on these issues in collaboration with groups worldwide including investigations of pest

control in potatoes, vineyards, orchards, cotton, sugarcane, tobacco, carrots, tree seedling nurseries, strawberries and others.

Table 3. Some biofumigant brassicas currently marketed.

Variety	**Species**	**Country**
Fumus	*B. juncea*	Australia
BQ Mulch	*B. napus*/*B. campestris*	Australia
Weedcheck	*R. satwa*	Australia
Nem Con	*B. napus*	Australia
Humus	*B. napus*	Idaho USA
Musclean	*B. juncea*	Australia
ISC120	*B juncea*	Italy

Conclusion

Biofumigation is not a replacement for fumigation in horticulture, however its potential is presently unrealised. Further purposeful selection of species and varieties with high biofumigation potential coupled with improved incorporation strategies based on an understanding of ITC release patterns in soil should increase field efficacy. The applications of biofumigation to broad acre-cropping appear more limited than in horticulture, however potential benefits even small may be significant when applied over large areas.

References

Akiew S, Trevorrow P, (1999) Biofumigation of Bacterial Wilt of Tobacco. In: Magarey RC ed. *Proceedings of the First Australasian Soilborne Disease Symposium.* Brisbane, Australia: Bureau of Sugar Experiment Stations, 207-8.

Angus JF, van Herwaarden AF, Howe GN, (1991) Productivity and break-crop effect of winter growing oilseeds. *Australian Journal of Experimental Agriculture* **31**, 669-77.

Angus JF, Gardner PA, Kirkegaard JA, Desmarchelier JM, (1994) Biofumigation: isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. *Plant and Soil* **162**, 107-12.

Bending GD, Lincoln SD, (1999) Characterisation of volatile sulphur-containing compounds produced during the decomposition of *Brassica juncea* tissues. *Soil Biology and Biochemistry* **31**, 695-703.

Bending GD, Lincoln SD, (2000) Inhibition of soil nitrifying bacterial communities and their activities by glucosinolate hydrolysis products. *Soil Biology and Biochemistry* **32**, 1261-69.

Blank RH, Robertson LN, McMeikean WB, Piggot GJ, (1982) Summer crops of direct-drilled kale (B oleracea) and fodder radish (R sativus) to control Australian soldier fly (Inopus rubriceps). *New Zealand Journal of Experimental Agriculture* **10,** 83-86.

Bones AM, Rossiter JT, (1996) The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum* **97**, 194-208.

Borek V, Elberson LR, McCaffrey JP, Morra MJ, (1995) Toxicity of aliphatic and aromatic isothiocyanates to the eggs of the black vine weevil (Coleoptera, Curculionidae). *Journal of Economic Entomology* **88**, 1192-1196.

Boydston RA, Hang A, (1995) Rapeseed green manure crop suppresses weeds in potato (Solanum tuberosum). *Weed Techniology* **9,** 669-675.

Brabban AD, Edwards C, (1995) The effects of glucosinolates and their hydrolysis products on microbial growth. *Journal of Applied Bacteriology* **79**, 171-77.

Brown PD, Morra MJ, (1997) Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy* **61**, 167 - 231.

Chan MKY, Close RC, (1987) *Aphanomyces* root rot of peas. 3. Control by the use of cruciferous amendments. *New Zealand Journal of Agricultural Research* **30**, 225-33.

Chew FS, (1988) Biological effects of glucosinolates. In: Cutler HC ed. *Biologically active Natural Products: Potential Use in Agriculture*. Washington DC, USA: American Chemical Society, 155-181.

Davis JR, Huisman OC, Westermann DT, Hafez SL, Everson DO, Sorneson LH, Schneider AT, (1996) Effects of green manures on verticillium wilt of potato. *Phytopathology* **86**, 444-453.

Drobnica L, Zemanová M, Nemec P, Antos K, Kristián P, Stullerová A, Knoppová V, Nemec Jr. P, (1967) Antifungal activity of isothiocyanates and related compounds. *Applied Microbiology* **15**, 701-9.

Fenwick GR, Heaney RK, Mullin JW, (1983) Glucosinolates and their breakdown products in food and food plants. *CRC Critical Reviews in Food Science and Nutrition* **18**, 123-201.

Gardiner JB, Morra MJ, Eberlein CV, Brown PD, Borek V, (1999) Allelochemicals released in soil following incorporation of rapeseed (Brassica napus) green manures. *Journal of Agricultural Food Chemistry* **47**, 3837-3842.

Giamoustris A, Mithen R, (1995) The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. *Annals Applied Biology* **126**, 347-363.

Glenn MG, Chew FS, Williams PH, (1988) Influence of glucosinolate content of Brasica (Cruciferae) roots on growth of vesicular0arbuscular mycorrhizal fungi. *New Phytologist* **110**, 217-225.

Heenan DP, (1995) Effects of broad-leaf crops and their sowing time on subsequent wheat production. *Field Crops Research* **43**, 19-29.

Johnson AW, Golden AM, Auld DL, Sumner DR, (1992) Effects of rapeseed and vetch as green manure crops and fallow on nematodes and soil-borne pathogens. *Journal of Nematology* **24**, 117-126.

Kelly PJ, Bones A, Rossiter JT, (1998) Sub-cellular immunolocalization of the glucosinolate sinigrin in seedlings of *Brassica juncea*. *Planta* **206**, 370-77.

Keniath AP, (1996) Soil amendment with cabbage residue and crop rotation to reduce gummy stem blight and increase growth and yield of watermelon. *Plant Disease* **80**, 564-570.

Kirkegaard JA, Gardner PA, Desmarchelier JM, Angus JF, (1994) Effects of *Brassica* crops on the growth and yield of wheat. *Australian Journal of Agricultural Research* **45**, *529-45*.

Kirkegaard JA, Wong PTW, Desmarchelier JM, (1996) *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathology* **45**, 593-603.

Kirkegaard JA, Hocking PJ, Angus JF, Howe GN, Gardner PA, 1997. Comparison of canola, indian mustard and linola in two contrasting environments. II. Break-crop and nitrogen effects on subsequent wheat crops. *Field Crops Research* **52**, 179-91.

Kirkegaard JA, Sarwar M, (1999) Glucosinolate profiles of Australian canola (Brassica napus annua L.) and Indian mustard (Brassica junceaL.) cultivars: implications for biofumigation. *Australian Journal of Agricultural Research* **50**, 315-324.

Kirkegaard JA, Sarwar M, Wong PTW, Mead A, Howe, GN, Newell M, (2000) Field studies on the biofumigation of take-all by *Brassica* break crops. *Australian Journal of Agricultural Research* **51**, 445-56.

Manici LM, Lazzeri L, Palmieri S, (1997) *In vitro* fungitoxic activity of some glucosinolates and their enzyme-derived products towards plant pathogenic fungi. *Journal of Agricultural and Food Chemistry* **45**, 2768-73.

Manici LM, Leoni O, Lazzeri L, Galletti S, Palmieri S, (1999) Fungitoxic activity of four thio-functionalised glucosinolate enzyme-derived products on ten soil-borne pathogens. *Pesticide Science* **55**, 486-503.

Mari M, Iori O, Marchi A, (1993) *In vitro* activity of glucosinolate-derived isothiocyanates against postharvest fruit pathogens. *Annals of Applied Biology* **123**, 155-64.

Matthiessen JN, Kirkegaard JA, (1998) Biofumigation for managing soil-borne pests – progress, pitfalls and prospects. In: Zalucki MP, Drew RAI, White GG eds. *Pest management – future challenges, Proceedings of the 6th Australasian Applied Entomological Research Conference, Brisbane*, Brisbane, Australia: The University of Queensland, 364-372.

Matthiessen JN, Kirkegaard JA, Morra MJ, (2001) Taking Biofumigation research beyond the empirical – towards linking isothiocyanate release and presence in the soil to pesticide efficacy. In: Porter IJ, *et al.* eds. *Proceedings of the Second Australasian Soilborne Diseases Symposium.* Melbourne, Australia: Department of Natural Resources and Environment, 144-5.

McLoed R, Desilva E, (1994) Covercrops and inter-row nematode infestation in vineyards 2. Effects of green material and of roots of cereals, legumes and crucifer crops on root knot (*Meloidogyne incognita* and *Meloidogyne javanica*) and citrus (*Tylenchus semipenetrans*) nematodes. *The Australian Grapegrower and Winemaker* **366a**, 119, 121-124.

McLoed RW, Steel CC, (1999) Effects of *Brassica*-leaf green manures and crops on activity and reproduction of *Meloidogyne javanica*. *Nematology* **1**, 613-624.

Mithen RF, Lewis BG, 1986. *In vitro* activity of glucosinolates and their products against *Leptosphaeria*

maculans. *Transactions of the British Mycological Society* **87**, 433-40.

Mojtahedi H, Santo GS, Wilson JH, Hang AN, (1993) Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. *Plant Disease* **77**, 42-46.

Muehlchen AM, Rand RE, Parke JL, (1990) Evaluation of Crucifer green manures for controlling *Aphanomyces* root rot of peas. *Plant Disease* **74**, 42-46.

Potter M, Davies K, Rathjen A, (1998) Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematodes (*Pratylenchus neglectus) Journal of Chemical Ecology* **24**, 67-80.

Pryor DE, Walker JC, Stahmann MA, (1940) Toxicity of allyl isothiocyanate vapor to certain fungi. *American Journal of Botany* **26**, 30-38.

Rosa EAS, Heaney RK, Fenwick GR, Portas CAM, (1997) Glucosinolates in crop plants. *Horticultural Review* **19**, 99-215.

Ryan, MH (2001) The effect of *Brassica* crops on the level of mycorrhizal inoculum in soil. *Proceedings of the 10th Australian Agronomy Conference, Hobart. Published on CD*.

Ryan MH, Kirkegaard JA,Angus JF, (1999) Impacts of biofumigation on VAM fungi in the southern wheatbelt - preliminary results. In: *Proceedings 10th International Rapeseed Congress, Canberra Australia* (Eds PA Salisbury, TD Potter, G McDonald, AG Green) (Published on CD-ROM). 174-175.

Sarwar M, Kirkegaard JA, (1998) Biofumigation potential of brassicas. II. Effect of environment and ontogeny on glucosinolate production and implications for screening. *Plant and Soil* **201**, 91-101.

Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM, (1998) Biofumigation potential of brassicas. III. *In-vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* **201**, 103-112.

Schonhammer A, Fischbeck B, (1987a) Untersuchungen an getreidereichen fruchtfolgen und getreidemonokulturen. II. Die differenzierung der ertragsleistung und deren struktur im verlauf von es versuchsjahren. *Bayerisches Landwirtschaftliches Jahrbuch* **64**, 175-191.

Schonhammer A, Fischbeck G, (1987b). Untersuchungen an getreidereichen fruchtfolgen und getreidemonokulturen. II. Die veränderungen an sproβ und wurzel. *Bayerisches Landwirtschaftliches Jahrbuch* **64**, 293-307.

Smith BJ, Sarwar M, Wong PTW, Kirkegaard JA (1999) Suppression of cereal pathogens by canola root tissues in soil. In: *Proceedings 10th International Rapeseed Congress, Canberra Australia* (Eds PA Salisbury, TD Potter, G McDonald, AG Green) (Published on CD-ROM)

Stephens PM, Davoren CW, (1997) Influence of soil fumigants, fungicides and biofumigant crops on vine "take" and growth in two grapevine nurseries in the Riverland, South Australia. *The Australian Grapegrower and Winemaker* **397**, 18-21.

Thompson JP, (1987) Decline of vesicular-arbuscular mycorrhizas in long fallow disorder of field crops and its expression in phosphorus deficiency in sunflower. *Australian Journal of Agricultural Research* **38**, 847-867.

Potter MJ, Davies KA, Rathjen AJ, (1998) Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematodes (*Pratylenchus neglectus*). *Journal of Chemical Ecology* **24,** 67-80.

Potter MJ, Vanstone VA, Davies KA, Kirkegaard JA, Rathjen AJ, (1999) Reduced susceptibility of *Brassica napus* to *Pratylenchus neglectus* in plants with elevated root levels of 2-phenylethyl glucosinolate. *Journal of Nematology* **31**, 291-298.

Smith BJ, Sarwar M, Wong PTW, Kirkegaard JA, (1999) Suppression of cereal pathogens by canola root tissues in soil. In: *Proceedings 10th International Rapeseed Congress, Canberra Australia* (Eds PA Salisbury, TD Potter, G McDonald, AG Green) (Published on CD-ROM)

Walker JC, Morell S, Forster HH, (1937). Toxicity of mustard oils and related sulfur compounds to certain fungi. *American Journal of Botany* **24**, 536-41.