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Biofumigation - optimising biotoxic Brassica rotations for soil-borne pest and disease management

John Matthiessen
CSIRO Entomology

Project Number: VX00013

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Final Report – Project VX00013

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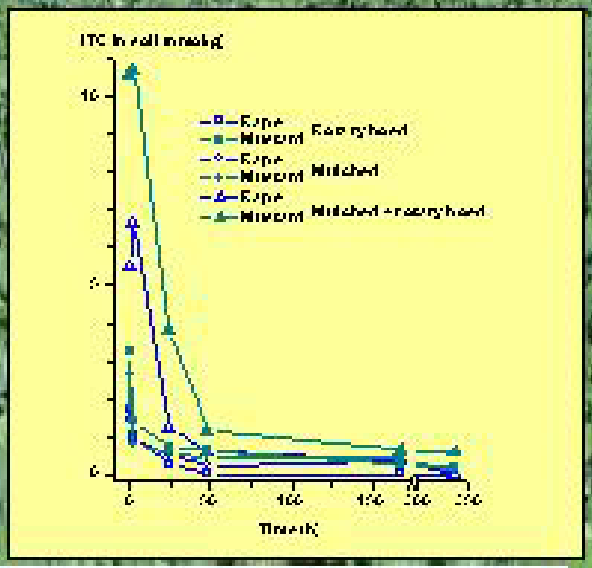

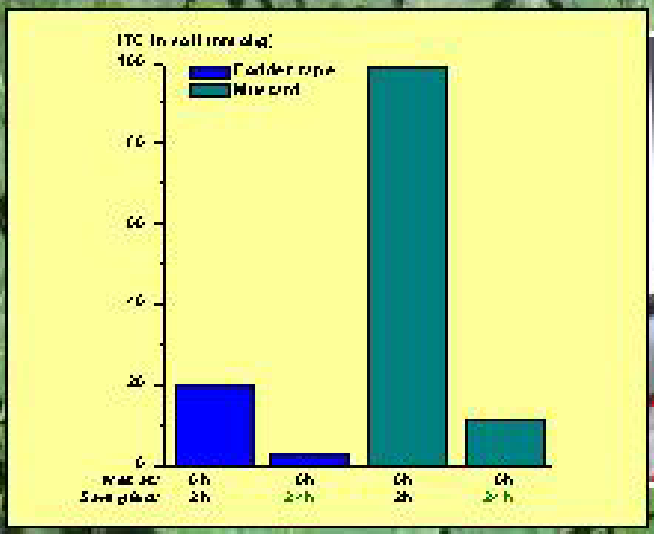

Research carried out 1 July 2000 – 30 June 2003

Horticulture Australia Limited
CSIRO Entomology

Project Final Report - HAL Project VX00013

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Research carried out by CSIRO Entomology

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John Matthiessen, Principal Research Scientist, CSIRO Entomology Perth (project leader).
Mark Shackleton, senior technician, CSIRO Entomology, employed on this project.
Ben Warton, Post-Doctoral Chemist, appointed to CSIRO Entomology for the duration of concurrent project HG98034 (Enhanced biodegradation of soil-applied pesticides), but developed involvement in this project through application of analytical chemistry knowledge and skills.
Various casual staff assisted with experiments and analyses during the project.

Keith and Paula Taylor, potato growers, Busselton WA keenly worked with us to develop practical methods that horticultural producers not equipped with small seeds machinery can use for growing brassicas, and freely allowed access to their property for sampling.

MEDIA SUMMARY

Biofumigation – optimising biotoxic *Brassica* rotations for soil-borne pest and disease suppression.

Biofumigation refers to the use of plants in the Brassica family to produce natural chemicals that are toxic and behave like some pesticides used to fumigate soil. The aim is to achieve a more generally acceptable and biologically-based method of suppressing pests and diseases in soil.

Soil-borne pests and diseases are a major problem for many of Australia's horticultural producers, decreasing yields and affecting quality. Some industries can afford to use expensive synthetic fumigants and pesticides, but many cannot. Most producers would prefer to avoid or at least limit their use of such chemicals.

Rotation crops of various kinds are often used to break the life cycle of pests and diseases, but the purpose of this research project was to achieve greater effects by using brassicas and treating them in such a way that maximised the release of the fumigant-like chemicals. It also aimed to find simple methods for producers to grow brassicas without specialist machinery.

The research found that dense stands of rapes and mustards could be produced after sowing seeds onto well-prepared land using simple equipment. The most significant findings centred on achieving maximum release of the fumigant chemicals from the plants, where amounts over ten times greater than had been previously obtained were achieved.

To achieve the maximum chemical release it is most important to thoroughly pulverise the plant material using a mulching machine fitted with club blades. Cutting blades are inadequate as they only chop the plants into fragments. It is then vital to water the mulched plant material thoroughly to assist the reactions that produce the chemicals, and carries them into the soil.

The results of this research project give the basic knowledge that will assist producers in making decisions about the various alternatives for managing soil pests and diseases and implementing them in their various production systems.

TECHNICAL SUMMARY

Biofumigation – optimising biotoxic *Brassica* rotations for soil-borne pest and disease suppression.

Soil-borne pests and diseases are a major problem for many of Australia's horticultural producers, decreasing yields and affecting quality. Some industries can afford to use expensive synthetic fumigants and pesticides, but many cannot. Most producers would prefer to avoid or at least limit their use of such chemicals.

Biofumigation refers to the use of plants in the Brassica family to produce various toxic isothiocyanates (ITCs) from the range of glucosinolates that can occur in high concentrations in different species and varieties. The aim of this project was optimise the chances of achieving high levels of ITC release from brassicas and incorporation into soil to provide horticultural producers with more options for suppressing pests and diseases in soil. It also aimed to find simple methods for producers such as potato growers, who are unlikely to have equipment for handling small seeds, to grow brassicas without specialist machinery.

Dense stands of rapes and mustards could be produced after sowing seeds through a fertiliser spreader onto a well-prepared seedbed. The most significant findings centred on methods for achieving maximum release of ITCs from the plants by ensuring that tissue disruption was at the cellular level. Amounts of ITCs measured in soil 10-100 times greater than had been previously obtained were achieved.

The high levels of ITC release were achieved in the field by thoroughly pulverise the plant material using a mulching machine fitted with club blades. Cutting blades are inadequate as they only chop the plants into fragments. But it is then vital to water the mulched plant material thoroughly to assist and promote the hydrolysis that produce the chemicals, and carries them into the soil.

Mustards, which produce high levels of above-ground biomass rich in propenyl ITC produced the greatest concentrations of ITC in the soil after mulching and substantial watering. Levels of propenyl ITC in soil reached 50% of the levels of methyl ITC produced by application of the recommended 500 L/ha of the commercial pesticide metham sodium.

The results provide the basic knowledge that optimises the production and incorporation into soil of ITCs from brassicas. This will assist horticultural producers by broadening their options and helping them in making decisions about the various alternatives for managing soil pests and diseases and implementing them in their various production systems.

INTRODUCTION

One of the most critical issues facing producers of underground vegetable crops is the management of soil-borne pests and diseases in sustainable ways. The subterranean nature of crops such as potatoes, carrots and onions exposes the final product to a wide variety of noxious organisms – insects, nematodes, fungi, bacteria. These reduce yield through plant destruction, or they damage the product directly, making it unmarketable. Such organisms can also have a major deleterious effect on above-ground crops through damage to roots, causing reduced plant growth and yield.

Predicting the threat of soil-borne pests and diseases is highly challenging because they occur hidden in soil. They are particularly difficult to diagnose or scout for, making these basic approaches for implementation of classical integrated pest management (IPM) that are adopted in above-ground crops almost impossible to implement practically and economically.

Control is also difficult because very low populations are often highly damaging, the organisms are hidden and unevenly distributed in the soil, they are often microscopic in size, they often occur in resting stages and are triggered to erupt by a particular congruence of unpredictable environmental circumstances, and damage can be cumulative from early in crop development.

Often the risk of attack or infection is so pervasive that prophylactic application of pesticides prior to planting the crop is common, simply as insurance against the unexpected. It is usually too late and impractical to effectively apply pesticides for control of soil-borne pest organisms during crop growth.

The use of residual pesticides to control such things as soil insects is no longer permitted. Recently there has been an accelerating trend to the use of the soil fumigant metham sodium to obtain broad-spectrum control of a wide range of soil-borne pests and diseases in horticulture in Australia. This appears to have been driven by two main factors: the phaseout of methyl bromide under the Montreal Protocol for reduction of ozone-depleting substances, to which Australia is a signatory, and increasing market demands for high quality blemish-free produce.

Metham sodium is the precursor for the toxin methyl isothiocyanate, which is generated when metham sodium contacts moisture in the soil. Metham sodium is a relatively expensive product, costing around $800 per hectare. Despite benefits that can derive from its use, it is uneconomical for vegetable and potato production in many parts of Australia.

In common with many commonly-used and effective pesticides, there are concerns about over-reliance on the use of metham sodium. It is a broad-spectrum biocide toxic to almost all soil-dwelling organisms and to plants until fully dissipated. It is also unpleasant to use, and it is susceptible to the phenomenon of enhanced biodegradation where repeated use leads to selection of microbial populations in the soil that degrade the pesticide so rapidly that the desired pesticidal effect becomes problematic.

Producers would undoubtedly prefer to minimise their use of highly toxic and expensive materials. Also, the frequent use of broad-spectrum biocides in soil would be widely viewed as not the most biologically desirable practice to adopt in the long term. Heavy pesticide use, in sometimes apparently indiscriminate ways associated with prophylactic use, also does not fit well with the 'clean and green' image that Australian horticultural industries wish to convey as they promote products and develop markets, particularly those that are export-focussed.

Also, it is widely and commonly known that there are no new synthetic soil fumigants on the horizon from the agrochemicals industry, and that development of soil fumigants is not an area that industry actively pursues. Unsustainable practices in the way metham sodium is used could result in its absence for use in situations of very high soil-borne pest and disease pressure, where such powerful methods arguably still have a role.

The characteristics of soil pests and diseases makes for a major challenge in developing management techniques that do not impact unnecessarily severely on diverse range of organisms that make up the soil biota, but that can offer the virtual elimination of risk that producers desire. Reliance solely on incorporation of pesticides, especially such compounds as broad-spectrum fumigants into soil for the control of soil-borne pests and diseases is a practice many would see as being not sympathetic to the ideals of managing agricultural systems for long-term sustainability.

This research project was predicated on the principle that producers have a strong desire, and need to be offered more options, for management of soil-borne pest and disease problems. This is in order to have the capacity to make choices that meet their particular pest management needs and production system ideals, and to fit with general industry strategic objectives of reducing reliance on a limited range of chemical pesticides.

These needs can only be satisfactorily addressed by seeking a biologically-based solution to the issue. The focus of this research project was to develop the use of plants that have biological activity against other organisms through their capacity to produce fumigant-like compounds as biological fumigants to act against soil-borne pests and diseases.

Biofumigation refers to the use of biologically-active plants as green manures, cover crops or rotation crops for suppression of soil-borne pests and diseases. In the context used here, biofumigation refers specifically to the use of *Brassica* species. The concept stems principally from the knowledge that many *Brassica* species have within their tissues glucosinolates that are the precursors of isothiocyanates.

These are often volatile chemicals known to be powerful toxins. They form from glucosinolates when the plant tissue is damaged, which allows the enzyme myrosinase to catalyse the chemical reaction. The particular link that makes the concept of harnessing plants that have a natural ability to produce isothiocyanates attractive is that methyl isothiocyanate is the well-known and effective active compound resulting from breakdown of the commercial soil fumigant metham sodium.

The notion that these properties could be used as a pest and disease management technique came initially from research results that wheat crops in the south-eastern Australian cereals belt often grew more productively and yielded better when grown after a *Brassica* crop such as canola. The effect appeared related, at least in part, to the presence of healthier roots on the wheat plants. The effects were often more pronounced where wheat was grown following mustard.

Unlike canola, which is a selection of oilseed rape to contain extremely low levels of glucosinolates in its seed in order to produce edible oil, mustard is valued for its pungent characteristics which are related to high isothiocyanate production. Tests of volatiles emitted from pieces of *Brassica* root material confirmed that they were toxic and suppressive to the growth of cereal pathogens such as the Take-all fungus.

Together, these results gave rise to the idea that appropriate *Brassicas* could be selected and used for soil-borne pest and disease suppression in cropping systems, through the biological 'fumigation' effect. Various accounts, either in the literature dating back some time, or anecdotally from people in industry tended to corroborate the research findings in the cereals industry, with comments about 'cleaner' crops following such plants as *Brassica* vegetable and fodder crops.

Investigations of the phenomenon in earlier GRDC and HRDC-funded projects revealed the widely varying types and levels of occurrence of glucosinolates in different species and cultivars and in different plant tissues, compared the growth of different types of brassicas in various regions, developed analytical and bioassay techniques, and assessed biological activity.

The measurement of glucosinolate profiles was used as a measure of biofumigation potential of *Brassica* tissues. That work revealed wide variation in 80 or so diverse commercial *Brassica* species and cultivars. Associated studies examined the effects of environment and plant development on glucosinolate production and the vapour and contact toxicity of various isothiocyanates to growth of fungal pathogens. A range of impact was measured, indicating varying toxicity of different isothiocyanates, and different modes of action. Less volatile isothiocyanates were intrinsically more toxic, but generally were more efficacious as toxins when allowed to directly contact organisms rather than be exposed only as vapour.

The earlier work suggested the potential for being able to harness brassicas for biofumigation, revealed the complexity in type and amount of isothiocyanates likely to be produced, through the glucosinolate profiles in the range of species, and laid the groundwork for more detailed studies to enable the biofumigation potential to be harnessed for beneficial use. It also developed bioassay and analytical techniques that provided the capacity to better evaluate the biofumigation potential of new lines of brassicas.

In particular, those techniques offered the capacity to link back to plant breeders to evaluate parent breeding lines and crosses for biofumigation potential, with the aim of developing superior types for this purpose. A great proportion of commercial brassicas have been selected for *reduced* glucosinolate content in order to enhance palatability for edible oils (eg. canola) or for livestock grazing (eg. fodder rapes). It followed, therefore, that there should exist good scope for directing breeding in

essence the opposite way, to develop lines high in the most appropriate glucosinolates for maximising biofumigation capacity.

In large part, the research effort in earlier work was directed to determining benchmark information to establish a sound foundation for future work. It also specifically set out to engage a broad group of collaborators and associates also interested in developing rotation, green manure and cover crops for management of various soil pests and diseases in various production systems. This extended particularly to broadening the skills base for this complex work by developing interactions and collaborations with plant breeders and seed companies to help the process of making the best selections and choices for research, development and implementation of biofumigation.

Because of this broad interest in extending or enhancing the use or potency of rotations with the concept of biofumigation, the team conducting the research in this project took an active role in acting as a focus for coordination of effort. A key aspect of this coordination, and to enhance technology transfer, was continued production of the regular newsletter, the 'Biofumigation Update' that was instigated very early in the previous project. The newsletter met with excellent acceptance by industry and its circulation continued to grow. It was designed to provide information on various studies being conducted in different areas and cropping systems, as well as to facilitate networking and direct contact between researchers, growers and consultants.

The core thrust of the work carried out in this project was to further develop biofumigation to enhance its efficacy through developing practical ways in which horticultural producers could grow and handle brassicas to optimise biofumigation effects, and to focus on developing methods to maximise and measure the release of isothiocyanates from the plants into the soil. In this way it was aimed at providing growers with more choice in their soil pest management needs and in meeting their personal production system objectives, while offering potential cost savings and additional rotation benefits associated with green manuring, and to fit with industry objectives of securing 'clean and green' and sustainable production systems.

SECTION 1

Practical aspects of growing brassicas and biofumigation potential

Background

Brassicas are very small-seeded and can present agronomic challenges to growers not accustomed to dealing with small seeds. In cropping situations brassicas, such as canola in broadacre, are sown with precision air seeders, or as transplants in vegetable production. This is impractical for brassicas used as green manure and biofumigant rotations.

In collaboration with potato farmer Keith Taylor of Vasse near Busselton WA, we focussed a component of this project on developing practical methods of growing biofumigant brassicas. Feedback and discussions with many vegetable and potato growers indicated that this was an important aspect to help or advise growers on.

Methods

Practical growing

Keith sowed several hectares of BQMulch® fodder rape and Fumus® mustard, on both sandy loam soil and a coarse sand. The loam had a long fertiliser history from horticulture, whereas the sand was old pasture. The loam had been under sweetcorn in the previous summer.

Residues of the sweet corn were rotary hoed to break them up in March, and the ground was irrigated. The winter rainy season began in mid autumn.

Best results were obtained in the sandy loam by mouldboard ploughing in early June to invert the soil profile. This produced a very clean seedbed, and left the surface somewhat rough ('crumb' – like). About 1-2 weeks later, with rain forecast, the seed was mixed with double superphosphate in a rotary fertiliser spreader and applied to the surface. The flow rate was cut right back, and two passes were made in order to achieve as even a distribution as possible. The seed was applied at around 10 Kg/ha and the fertiliser at 70 Kg/ha.

Biomass and isothiocyanate measurement

Plots of BQMulch, a mixture of two fodder rape lines (referred to as 'B' and 'C'), and Fumus mustard were grown in the way described in winter of each year of this project (2000-2003). In 2000 a plot of BQMulch and Fumus mixed was grown to examine the biomass of each type, as they have distinctly different ITC profiles and growers had enquired whether advantages could be gained from a mixture.

In 2000 and 2001 regular samples of plants were taken every three weeks. All plants in five 0.5 sq m plots were removed, the two components of the BQMulch mixture were separated by the distinct appearance of their foliage, roots and tops were separated and the plants dried and weighed.

Samples of shoot and root tissue were freeze-dried and a method was developed to analyse them for isothiocyanates (ITCs) directly (see separate section below). Typically, the biofumigation potential of brassicas had been determined by analysing the concentration of glucosinolates (GSLs) in the plant tissue. However, this method requires time-consuming wet chemistry preparation and HPLC analysis. As we had GC set up to analyse ITCs, as part of HAL project HG98034 (enhanced biodegradation of metham sodium), there was considerable attraction in developing a method that analysed directly for the ITCs that arise from hydrolysis of GSLs.

Results and discussion

General observations

The following series of photographs shows the establishment and growth of the brassicas sown in the way described, for plots sown in winter 2000.

The seed could be seen on the surface of the ground. Following a substantial fall of rain, the surface smoothed down. Many seeds were lightly buried in the process, although many also germinated on the surface.

Continued rain and cool weather ensured that the surface never dried, and the soil remained uncompacted for a lengthy period. The soil has a sufficient clay fraction (8-12%) that it can pug quite firmly if compressed when wet and be difficult for small-seeded plants to establish and grow. This combination of factors resulted in excellent establishment and uniform growth. The wheel tracks from the sowing were followed during the growing season for four applications of approximately 50 Kg/ha of urea each.

Growth is typically quite slow initially during mid-winter, but takes off rapidly in spring. Both the fodder rape and mustard form a dense canopy by early September. The fodder rape does not flower when planted after about June and in mild conditions, as it requires a vernalisation chill to initiate that process. Mustard begins to flower in about late September.

A dense stand of both *Brassica* types resulted, giving a total biomass of Fumus of 15.7 t dry matter/ha, and of BQMulch of 11.2 t dry matter/ha by early November. Keith's conclusion is that on this loamy soil, the most important aspect is achieving a good seed bed and that there is not need to harrow seeds in.

Incorporation (dealt with in detail below) was typically carried out in the Busselton environment in the first week of October. At that time, the mustard is about 25% flowering, the brassicas are still at a stage of lush foliage, without too much woodiness in the mustard stems. This quickly changes during the period of extremely rapid growth in October.

Biomass measurements

Figures 1 and 2 show the seasonal changes in biomass of BQMulch in two separate plots in 2000 (photographs below show plot 4). In 2000 sampling was carried on until summer to fully chart the growth through until complete maturity.

Total above-ground BQMulch biomass peaked at around 14 t dm/ha in early December and then plateaued (Fig. 1). The total plant biomass (shoots plus roots) peaked at around 15-16 t/ha.

BQMulch plot 2000

9 July 2000

13 August 2000

27 August 2000

10 September 2000

24 September 2000

2 October 2000 (time of first incorporation of some sections)

17 October 2000 – BQMulch and Fumus in the background

17 October 2000 – Fumus plot

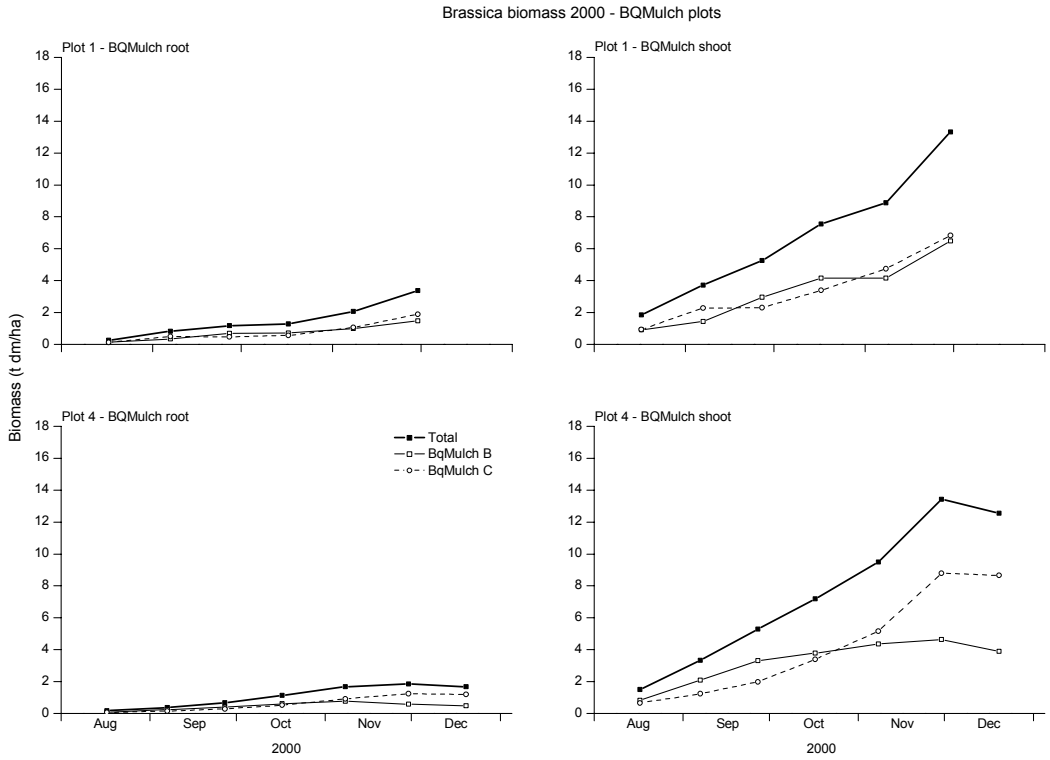


Figure 1. Seasonal change in biomass of BQMulch plant parts and mixture components, year 2000 plots, loam soil at Busselton WA.

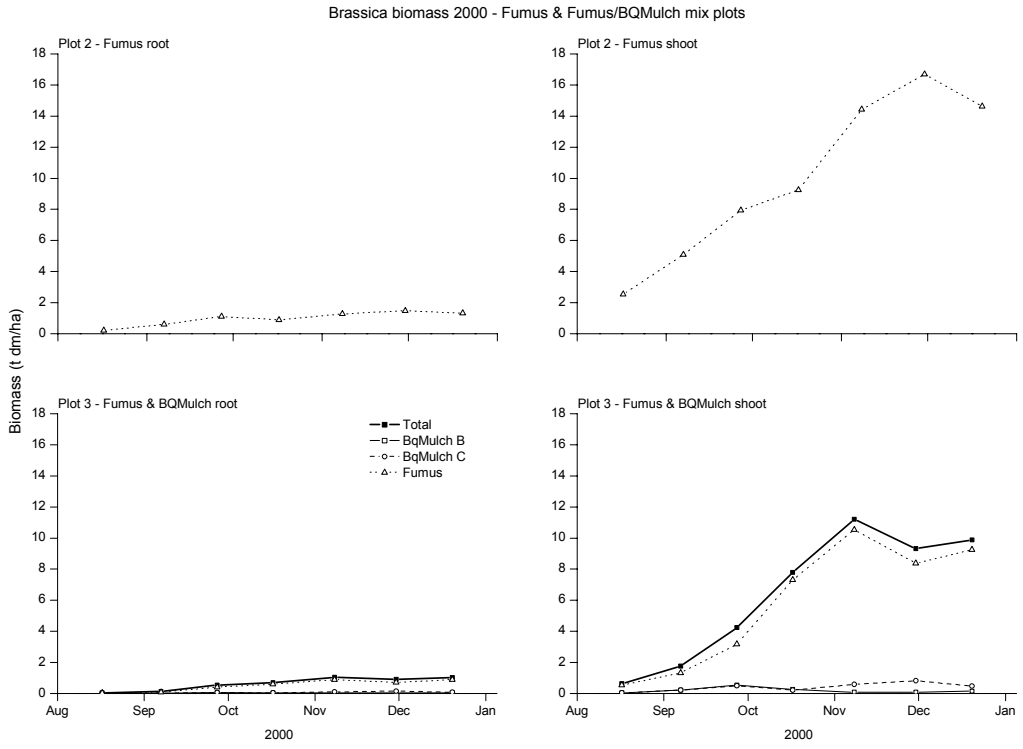


Figure 2. Seasonal change in biomass of Fumus plant parts and mixture of Fumus and BQMulch, year 2000 plots, loam soil at Busselton WA

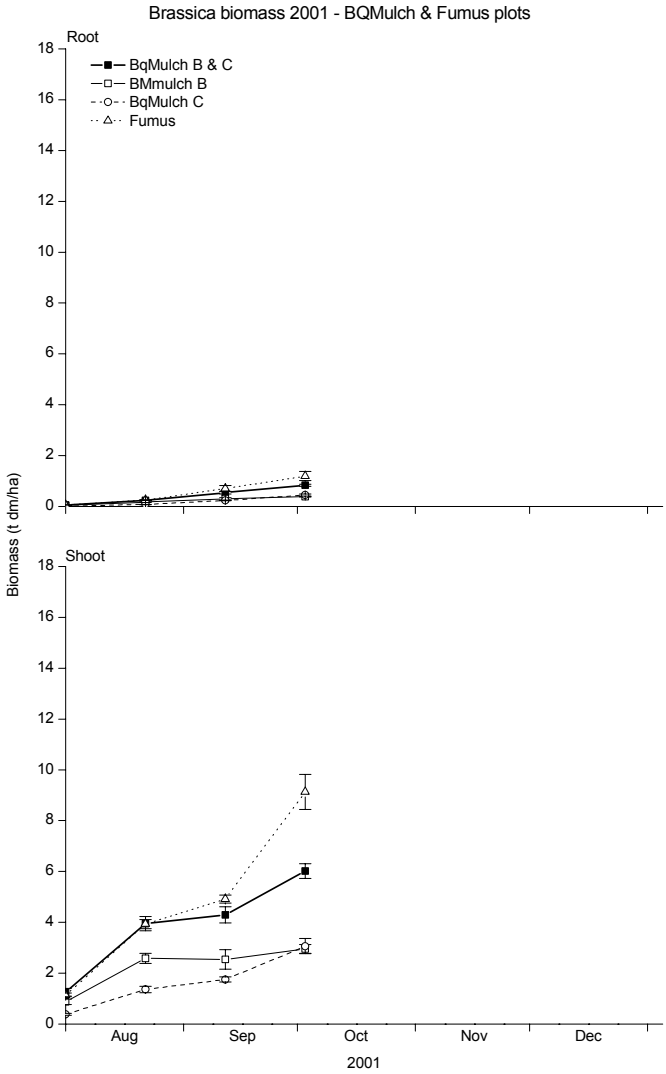


Figure 3. Seasonal change in biomass of BQMulch and Fumus plant parts and mixture components, year 2001 plots, loam soil at Busselton WA.

Fumus biomass also peaked in early December, at around 16 t/ha, with about another 1 t/ha of root biomass (Fig. 2). When BQMulch and Fumus were mixed, the taller Fumus clearly out-competed the BQMulch and dominated the total biomass (Fig. 2). It is clear that mixing these two highly *Brassica* types that are of highly contrasting growth habit is not a practical way to capture the benefits of both.

The general assessment from observation was that the most practical time to treat or incorporate the brassicas in the Busselton environment was before they became too woody or dry in very early October. (All details of incorporation strategy are dealt with below). Reference to Figs. 1 & 2 shows that at that time the plants had reached between about 35 and 50% of the maximum biomass achieved.

If the fodder rape is left too long, the lower stems become very thick and hard and are difficult to break up, while if mustard is left too late into its growth cycle the stems become very coarse, dry and straw-like, making them very sinewy and difficult to macerate. Early October coincided with approximately 25% flowering in the Fumus

mustard.

In 2001, the biomass in the BQMulch and Fumus plots was measured only until early October when the crop areas were totally mulched and incorporated (detailed below). Similar biomass levels of around 5 t dm/ha for BQMulch and around 8 t dm/ha for Fumus were achieved in 2001 as had occurred at the same time in 2000 (Figs.1-3).

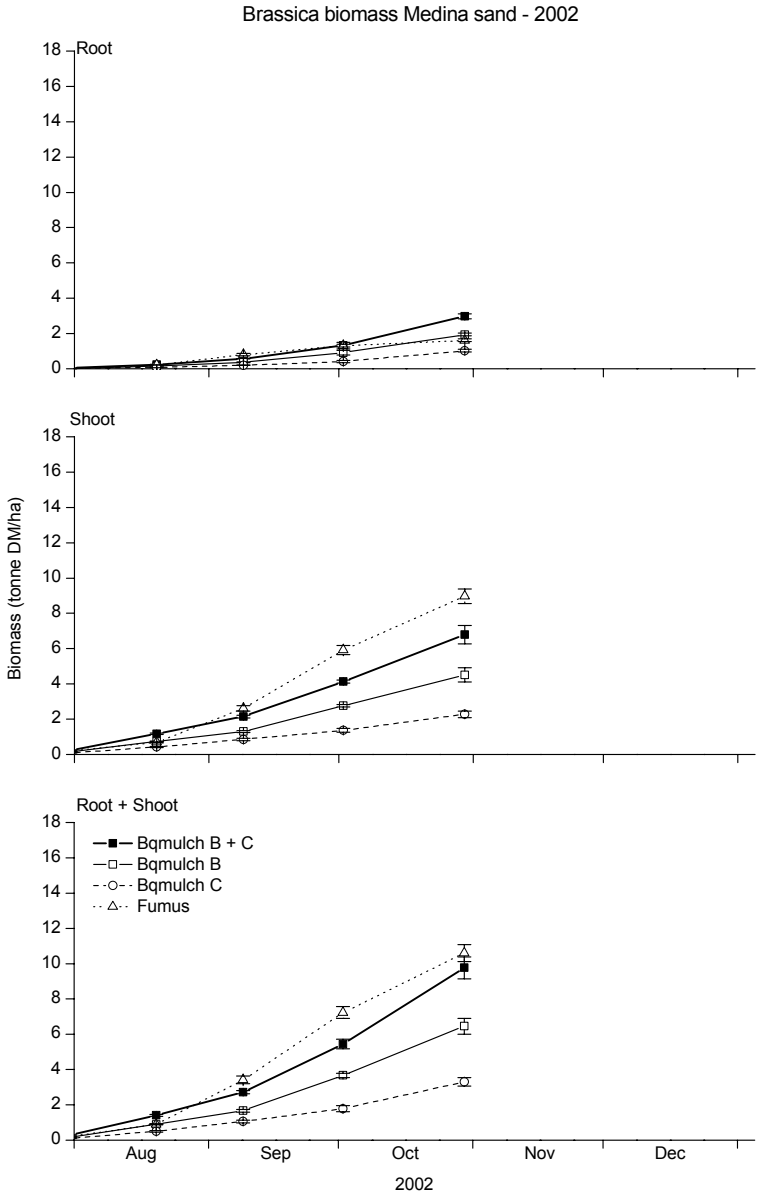


Figure 4. Seasonal change in biomass of BQMulch and Fumus plant parts and mixture components, year 2002 plots, sand soil at Medina WA.

ITC profile and concentration

Using the methodology developed and described in detail in the published paper that makes up the following section, ITC levels were measured in the shoots and roots of both BQMulch components and in Fumus.

Figure 4 shows the general ITC profile of the BQMulch components and of Fumus in mid October 2000 in the Busselton plots, derived from the mean of samples taken in all the plots. Both BQMulch components had low levels of 2-propenyl ITC in comparison to Fumus, which had highest concentrations in the shoots. Fumus was devoid of any significant quantities of other ITCs except for some 2-phenylethyl in roots.

BQMulch B differed from BQMulch C principally in containing considerable quantities of 4-pentenyl ITC. Both had similar levels of 3-butenyl ITC, while BQMulch C had more 2-phenylethyl in roots, with both BQMulch components having a greater concentration of 2-phenylethyl ITC in roots than Fumus (Fig. 4).

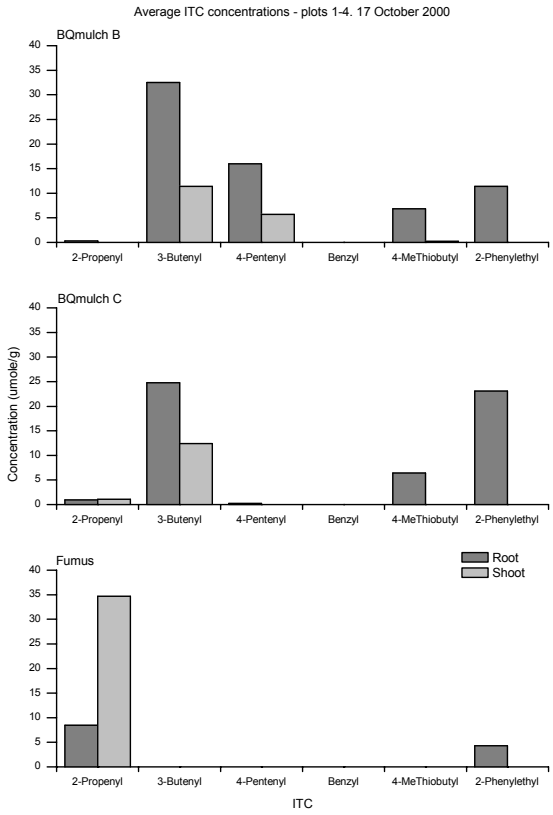


Figure 4. Mean concentration of major ITCs in shoots and roots of BQMulch components and Fumus.

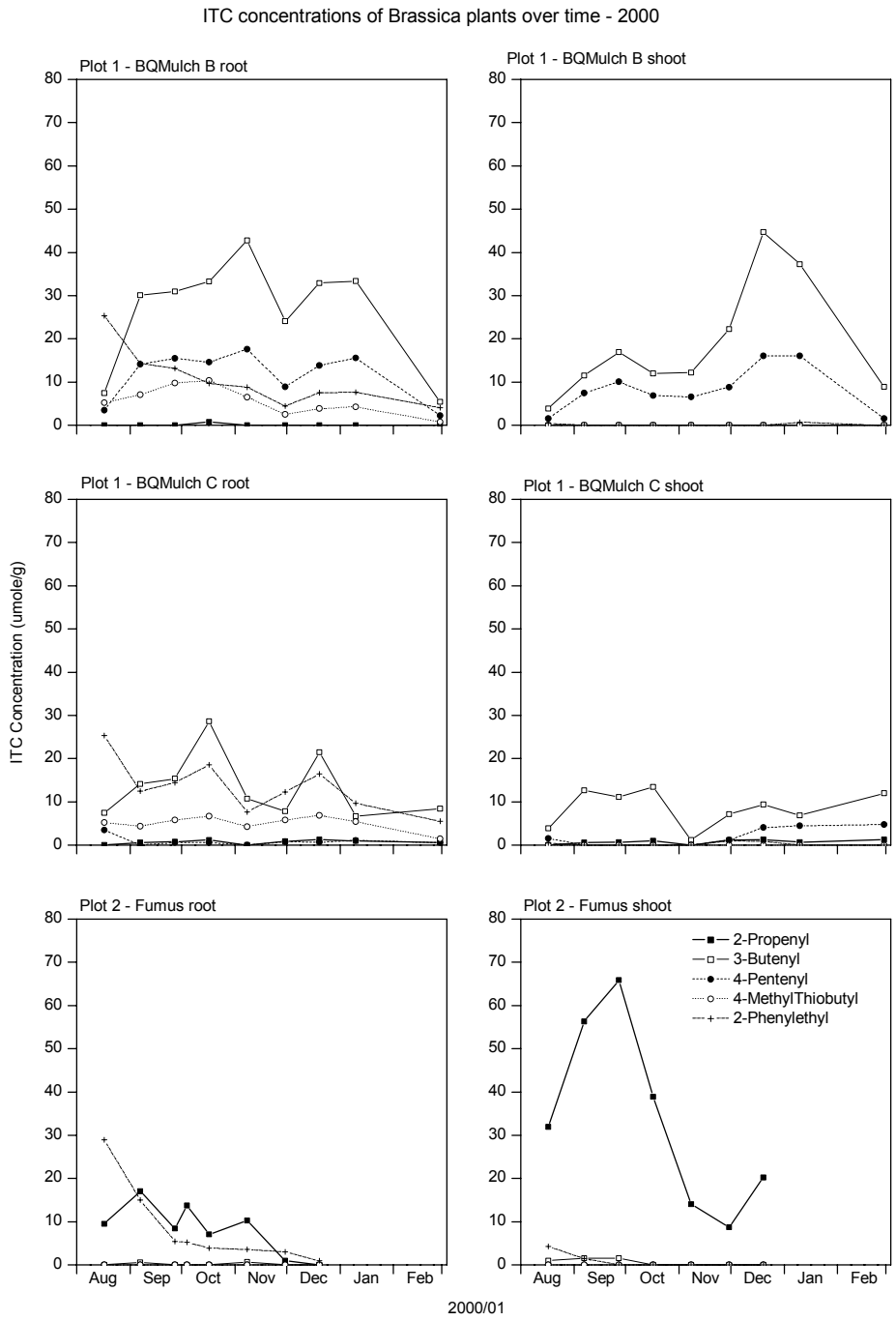
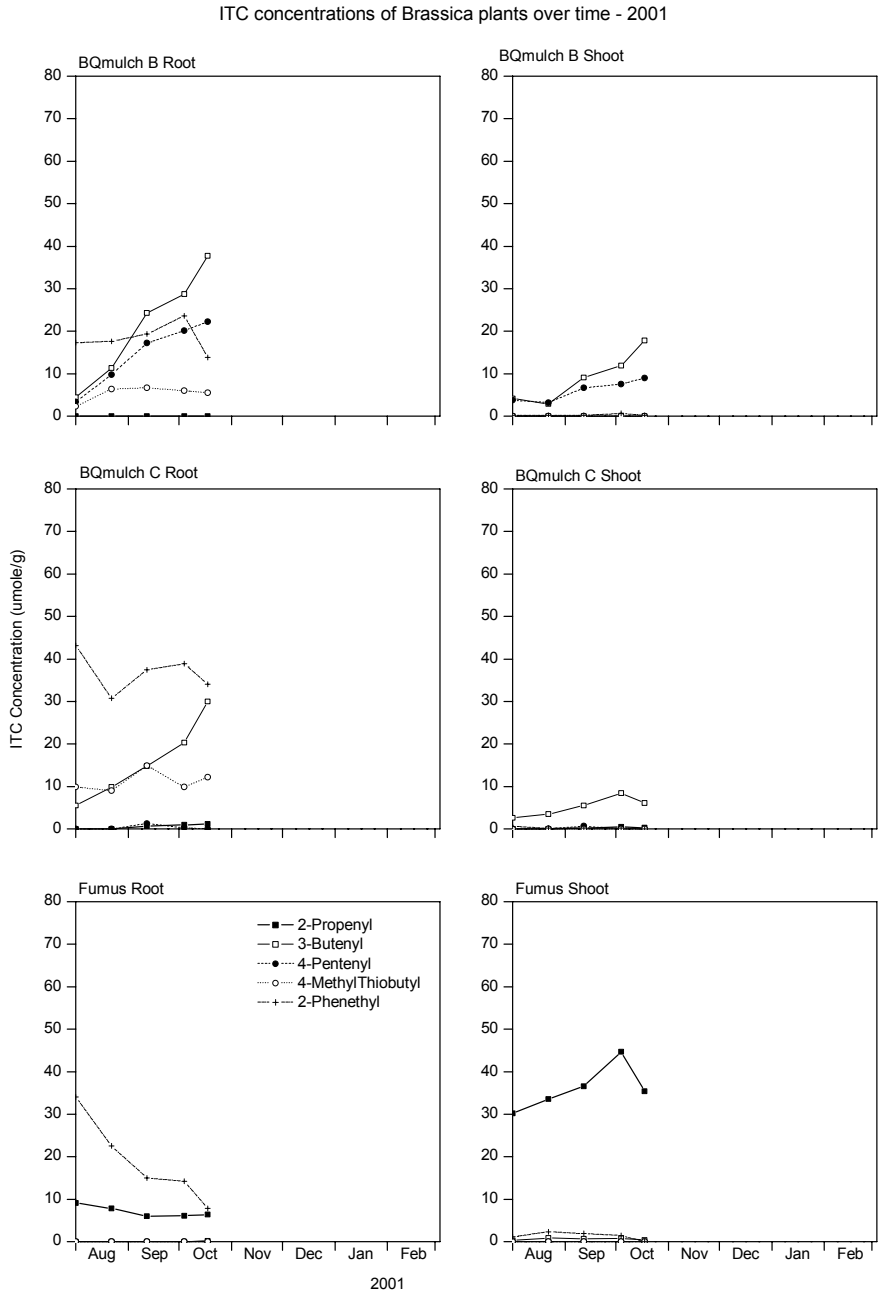


Figure 5. Seasonal changes in the concentration of the major ITCs in BQMulch components and Fumus, for brassicas grown to full maturity – 2000 Busselton plots.

The ITC profile of the shoots and roots of the BQMulch components and Fumus were measured regularly until full maturity of the plants in 2000. Fumus, the annual mustard, showed a clear decline in its ITC concentration (dominated by propenyl ITC) after early October, particularly in the shoots (Fig. 5). Fumus root 2-phenylethyl concentration was highest very early in plant growth.

BQMulch showed greater uniformity in its ITC concentrations through the season, clearly a reflection of the fact that these plants did not become senescent as did Fumus



(Fig. 5).

Figure 6. Seasonal changes in the concentration of the major ITCs in BQMulch components and Fumus, for brassicas grown only until early October – 2001 Busselton plots.

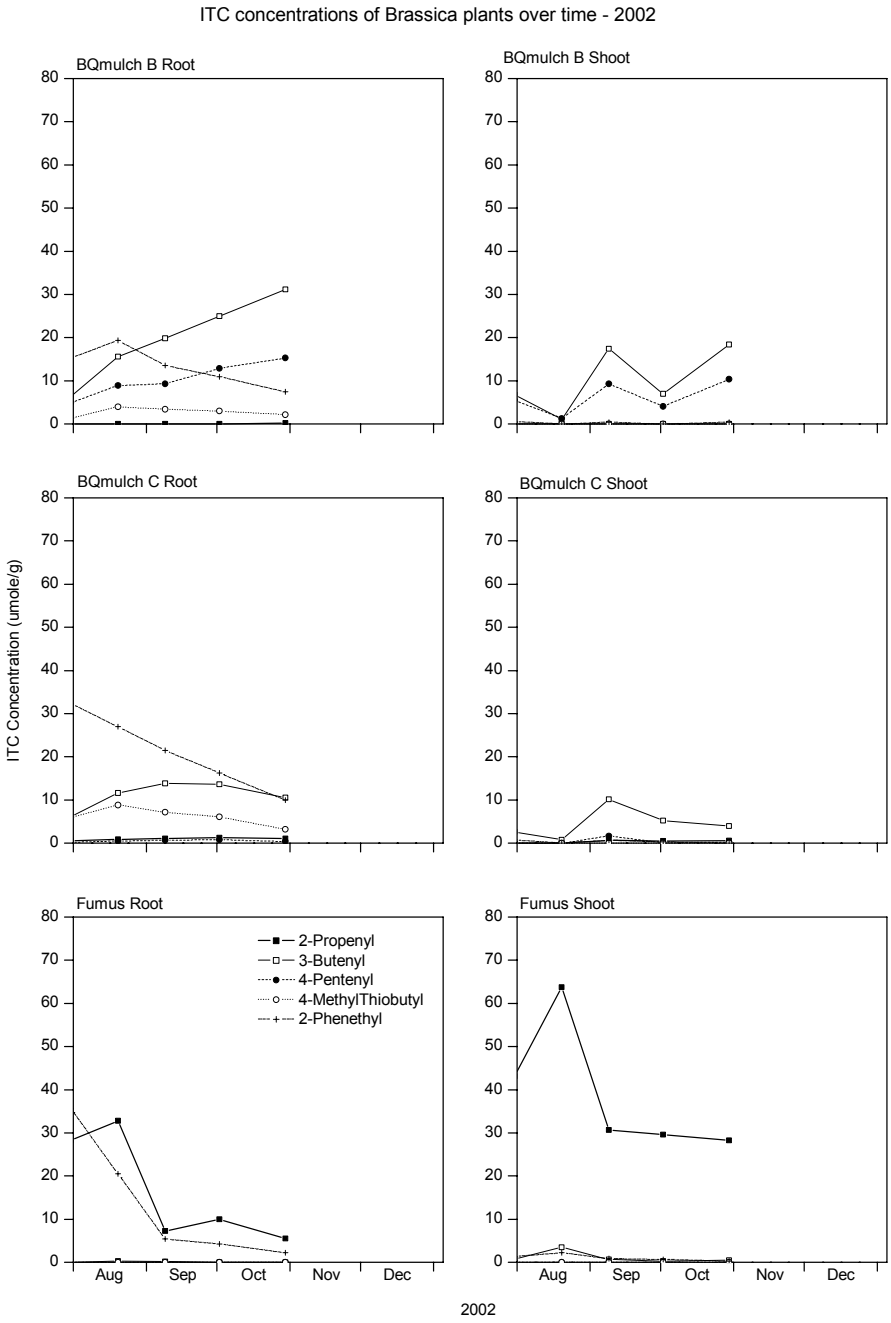


Figure 7. Seasonal changes in the concentration of the major ITCs in BQMulch components and Fumus, for brassicas grown only until early October – 2002 Medina plots.

Figures 6 and 7 show the same data as Fig. 5, but for the 2001 Busselton and 2002 Medina plots when the growth of the plants was terminated in early October as a result of mulching and incorporation (discussed in detail below). Generally, the patterns observed in the equivalent part of the 2000 season were repeated in 2001 and at the different site in 2002 (Figs. 5, 6 & 7).

Biofumigation potential

The combination of biomass and ITC concentration provides a raw measure of total

crop biofumigation potential per unit area of soil. Figures 8 and 9 show the changes in biofumigation potential in the BQMulch, Fumus and mixed BQMulch/Fumus plots over time in 2000 when the crops were grown on into the summer, while Figs. 10 and 11 show it for the BQMulch and Fumus plots from soon after sowing to early October when incorporation was carried out in 2001 at Busselton and 2002 at Medina.

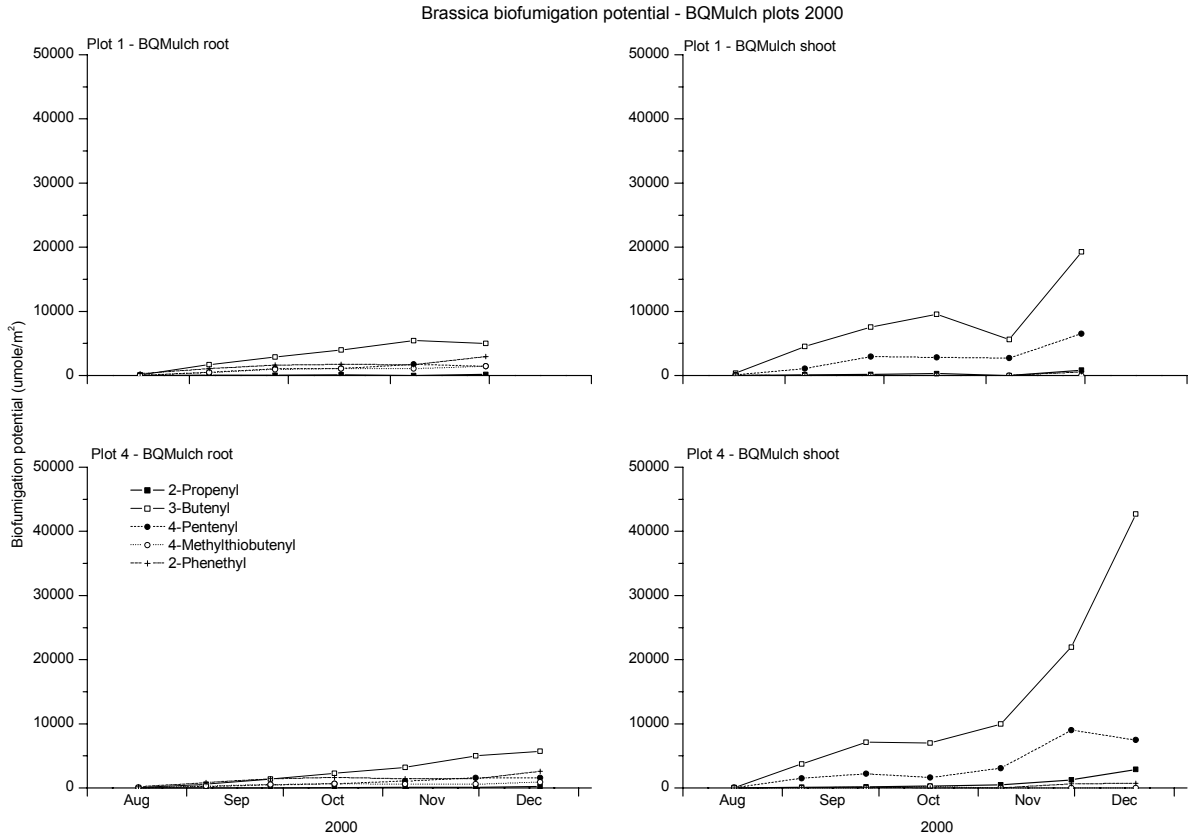


Figure 8. Raw biofumigation potential for roots and shoots of two BQMulch plots in 2000.

There was a consistent pattern of an early rise in the potential of 2-phenylethyl ITC in roots, which levelled out in early October. With the exception of 3-butenyl ITC in roots, which continued to increase for the duration of the crop, the other main ITCs detected in roots tended to show the same plateau response as 2-phenylethyl ITC (Fig. 8).

The shoots of BQMulch produced a biofumigation potential similar to that of roots until about November. The ITCs were principally of 3-butenyl and 4-pentenyl ITC, with 3-butenyl rising to very high levels late in the period. The BQMulch plants were still alive in early summer but they had been heavily attacked by insects and their stems had become very woody. It would be unrealistic to think that plants in this state were a practical proposition for incorporation treatment (Fig. 8).

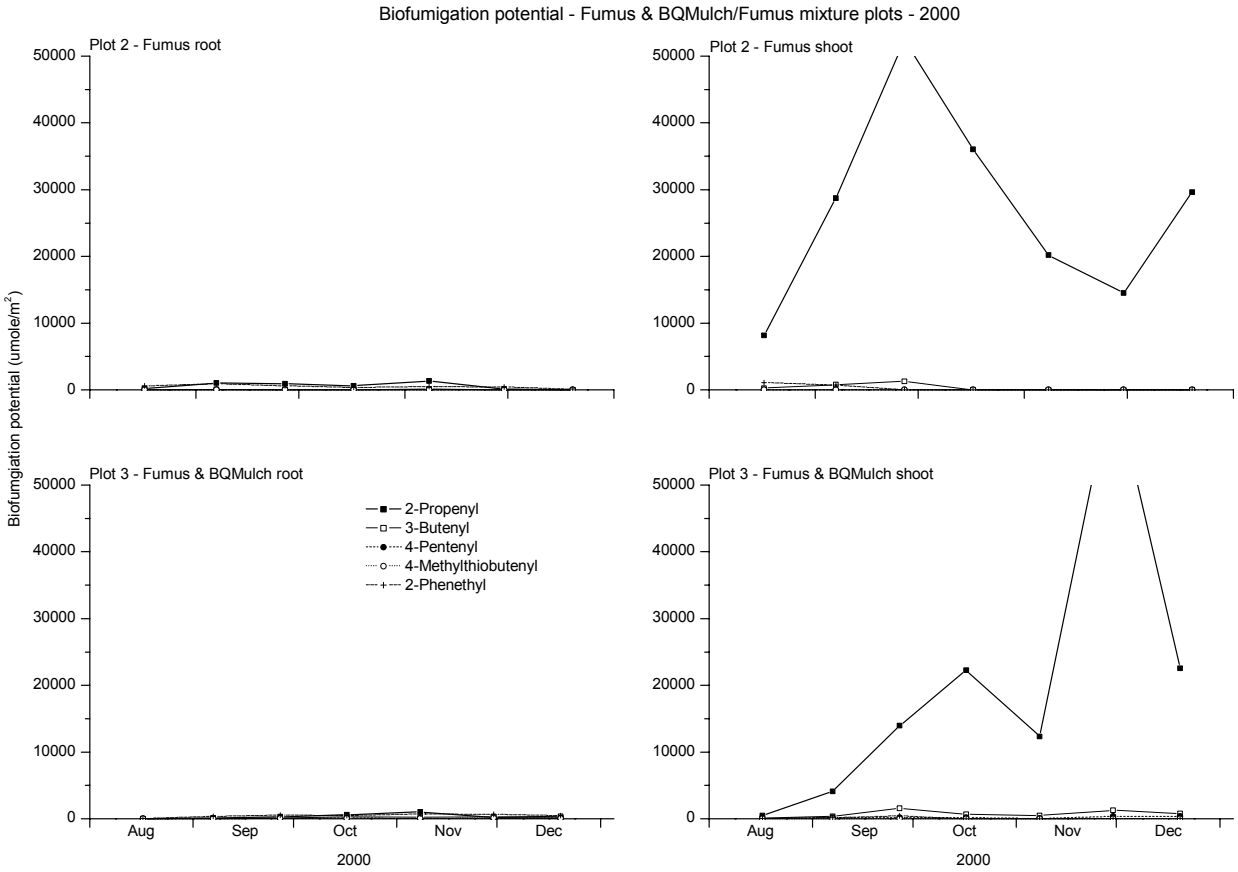


Figure 9. Raw biofumigation potential for roots and shoots of the Fumus and BQMulch/Fumus mixture plots in 2000.

Fumus root tissue produced a generally low raw biofumigation potential based on the ITC analyses, with the main ITCs being 2-propenyl and 2-phenylethyl (Fig. 9). In contrast, shoot-derived biofumigation potential in Fumus became very high and reached near peak levels by early October. Shoot biofumigation potential was very heavily dominated by 2-propenyl ITC. When BQMulch and Fumus were sown as a mixture, the ITC biofumigation potential was dominated by the Fumus component (Fig. 9).

In 2001 and 2002, the BQMulch and Fumus plots were incorporated in early October (discussed below), on the basis that this was the most practical time in terms of achieving good tissue maceration and minimising coarse plant residues. Figures 10 and 11 show the ITC-based biofumigation potential per unit area of soil from early growth until termination of the plots for Busselton in 2001 and Medina in 2002. Relative to Fumus shoots, which produced very high 2-propenyl ITC-based biofumigation potential, the BQMulch and Fumus roots had a lower potential based on raw ITC quantity.

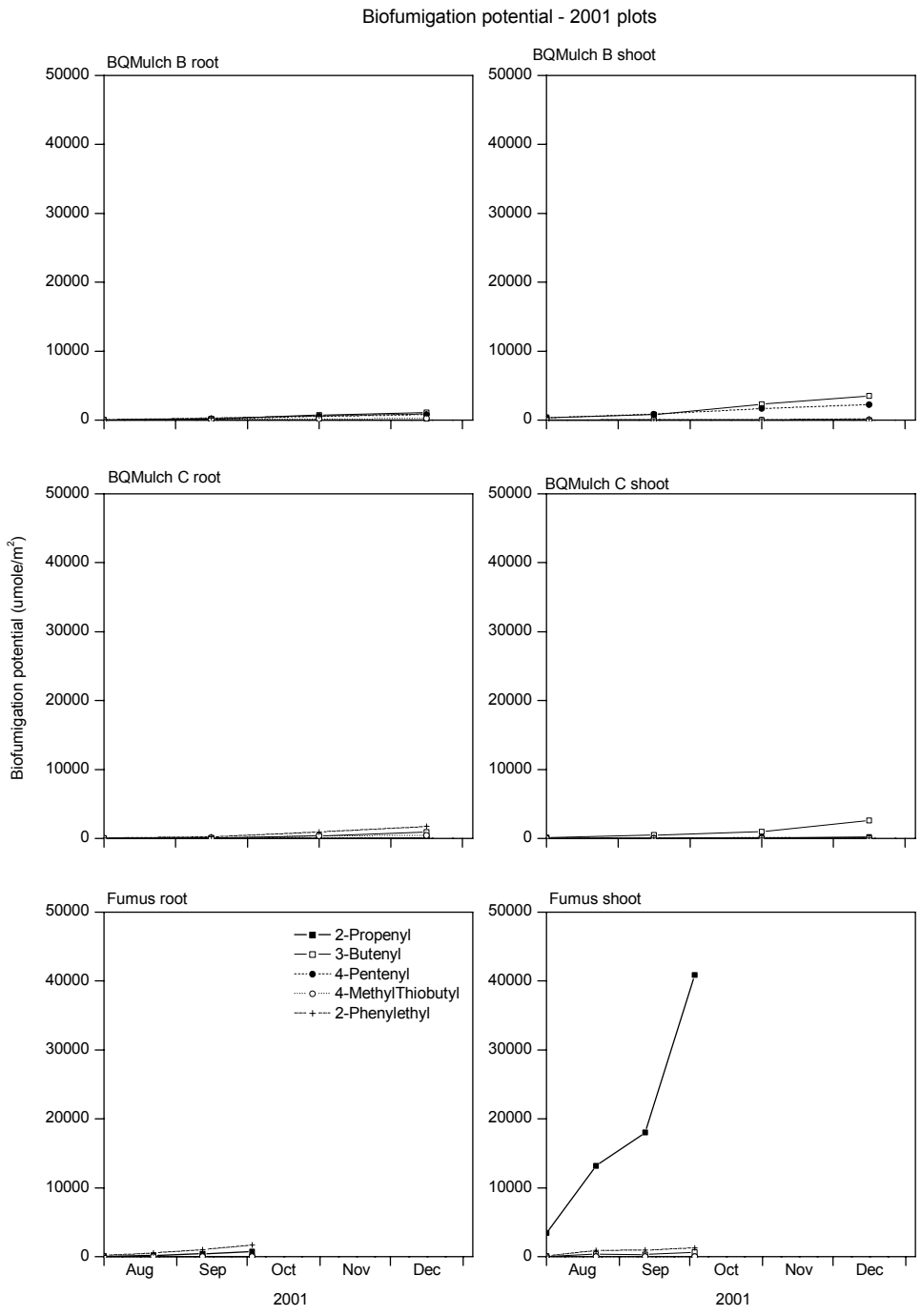


Figure 10. Biofumigation potential of BQMulch components and Fumus grown until early October 2001 when incorporation was carried out – Busselton plots.

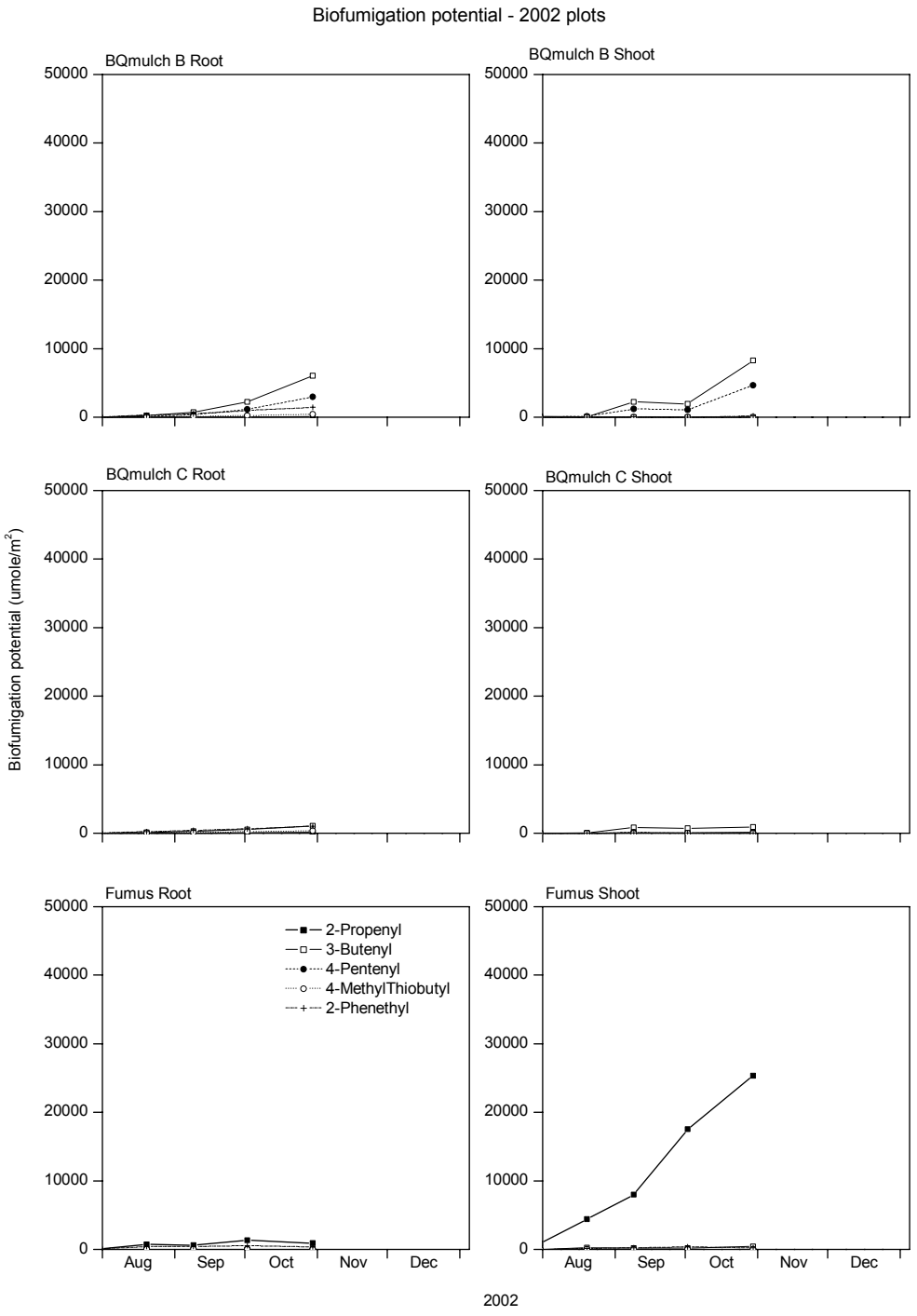


Figure 11. Biofumigation potential of BQMulch components and Fumus grown until early October 2002 when incorporation was carried out – Medina plots.

The following section describes the methodology developed to measure ITCs directly from plant tissue, instead of the more complex GSL-based procedure.

Glucosinolate content and isothiocyanate evolution – two measures of the biofumigation potential of plants

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Abstract

A total of 570 lyophilised *Brassica* root and shoot tissue samples were hydrolysed and the liberated isothiocyanates (ITCs) analysed by gas chromatography-flame photometric detection (GC-FPD). Glucosinolates (GSLs) were extracted from samples of the same tissues and analysed by high performance liquid chromatography (HPLC). The concentrations of six GSLs/ITCs, *viz:* 2-propenyl, 3-butyl, 4-pentenyl, benzyl, 4-methylthiobutyl and 2-phenylethyl, as determined by the two techniques were compared. In 79% of the samples, the concentration of the GSLs in the tissues was greater than that of the ITCs released on hydrolysis. Several possible reasons are proposed, including the effect of tissue storage time, that the hydrolysis of GSLs was less efficient than the GSL extraction procedure, or that a proportion of the ITCs formed react with plant proteins and amino acids in the sample and are therefore not detected in the extract. GSL concentration in plant tissues is used to estimate the biofumigation potential of the plant tissue, whereas the actual biofumigation effect is thought to be due to the ITCs formed by hydrolysis of the plant-based GSLs. The variation between ITC and GSL values therefore has implications for the assessment of the biofumigation potential of the plant tissue.

Introduction

Glucosinolates (GSLs) are a class of naturally occurring anionic compounds found in plants (*1*), usually as the potassium or sodium salt. They consist of a β-D-thioglucose moiety, a sulfate attached through a C=N bond and a side chain that distinguishes one GSL from another (*2*). GSLs are hydrolysed by enzymes of the family myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1) in the presence of water, to yield glucose and an unstable aglucone, which spontaneously undergoes a Lossen rearrangement to form an isothiocyanate (ITC) as the major product (*2*). Myrosinase is stored in specialised myrosin cells (*3, 4*). These cells do not contain GSLs, which are located separately within a variety of plant cells. GSLs and myrosinase come into intimate contact when the plant tissue is damaged by mechanical stress such as crushing, or during pathogen attack, with the resultant formation of ITCs.

GSLs have been the subject of much recent attention due to their involvement in the process termed 'biofumigation' (*5*). In biofumigation, plant-based GSLs are hydrolysed in field soil to form toxic products including ITCs, thiocyanates, nitriles, oxazolidinethiones and ionic thiocyanate (*2*). These GSL degradation products may exert a suppressive or control effect on a wide range of soil-borne plant pathogens including wheat take-all fungus (*5*), root-knot nematode (*6, 7*), *Rhizoctonia solani* (*8*) and *Fusarium oxysporum* (*9*). A comprehensive review of allelochemical effects of glucosinolate degradation products can be found in Brown and Morra (*2*).

Hydrolysis of GSL-containing plant tissue, or pure GSLs isolated from plant tissues, can be achieved in the laboratory using pure myrosinase isolated from *Brassicas* such as white mustard (*Sinapis alba*) (eg. (*10*)), in order that reaction conditions can be well controlled. This hydrolysis reaction has also been performed using endogenous myrosinase present in lyophilised, ground and rehydrated *Brassica* plant tissue (*11, 12*). In the former study, seed meal was wetted and incubated for 10-15 min followed by extraction using organic solvents (*11*).

In the present study, a modified GSL hydrolysis procedure is reported, in which the hydrolysis and solvent extraction steps are combined. After a simple clean-up procedure, the hydrolysis products in the organic phase were qualitatively and quantitatively determined using gas chromatography with a flame photometric detector (GC-FPD). Molar concentrations of ITCs liberated from the original plant tissues were calculated, and compared to GSL concentrations determined by high performance liquid chromatographic (HPLC) analysis of desulfoglucosinolates extracted from the same plant tissues, a method previously used to assess the biofumigation potential of *Brassica* plants (*13*). The potential of these two procedures as indicators of the biofumigation potential of plant tissues is discussed.

Experimental

Reagents and solvents

Propenyl GSL, sephadex, sulfatase and methyl ITC were purchased from Sigma-Aldrich (Castle Hill, Australia), benzyl GSL was purchased from Merck (Kilsyth, Australia). All reagents and solvents were used as purchased.

GSL and ITC comparisons

A total of 570 root and shoot tissue samples were taken from *Brassica* plants grown at different locations in Australia and New Zealand, freeze-dried and ground (Wiley mill with 1mm screen), and stored (15°C) for different periods. The majority of samples were taken from mature plants as they began to flower. The GSL content of, and ITC quantity liberated from, these samples were measured according to the procedures outlined below, and then compared. Analyses were not replicated. Theoretically, one mole of GSL has the capacity to produce one mole of ITC under optimal conditions for the conversion of GSLs to ITCs.

Extraction and analysis of glucosinolates from Brassica *tissue samples*

Glucosinolates were extracted and analysed according to the procedure of Kirkegaard and Sarwar (*13*). Briefly, freeze-dried and ground *Brassica* root and shoot material (300 mg), HPLC grade methanol (10 ml, 70 %, preheated to 70°C) and an internal standard (15 µl, 16 mM, propenyl glucosinolate or benzyl glucosinolate as appropriate) were added to 50 ml centrifuge vials. The vials were sealed without delay, hand shaken and stood in a water bath at 70°C for 20 minutes. The samples were then agitated briefly, cooled and centrifuged at 3500 rpm for six minutes.

The supernatant (3 ml) was carefully applied to a 0.5 cm plug of A-25 sephadex in 10

ml poly-prep chromatography columns and the effluent discarded. The sephadex was washed with milli-Q water (1 ml) followed by sodium acetate (1 ml, 0.02M), with the combined effluents discarded. Prepared sulfatase (75 µl) was added to each of the columns, which were then capped and allowed to stand overnight. Subsequently, milli-Q water (1 ml) was applied to each column and the effluent collected in 1 ml HPLC vials that were frozen until analysis.

Analysis for glucosinolates was performed using HPLC according to the procedure given in Kirkegaard and Sarwar (*13*) and references therein.

Hydrolysis of Brassica tissue samples and ITC analysis

Ethyl acetate (8 ml), methyl isothiocyanate (MITC, 2 ml, 100 mg l^{-1}) in ethyl acetate (normalisation standard) and deionised water (10 ml) were added to freeze-dried Brassica root and shoot tissue (200 mg) in 100 ml Erlenmeyer flasks. The flasks were sealed without delay and placed on an orbital shaking table operating at 150 rpm. Samples were shaken for 24 h (except for the timed hydrolysis experiment), removed and allowed to settle. Aliquots (1 ml) of the upper organic layer were drawn off, then dried and filtered through a plug of anhydrous magnesium sulfate (approx 4 cm) in a pasteur pipette for analysis by GC.

In order to determine the optimal time for hydrolysis of GSLs to liberate ITCs, a *Brassica* plant tissue sample was selected that contained measurable quantities of five ITC-producing GSLs, *viz*: 2-propenyl GSL, 3-butyl GSL, 4-pentenyl GSL, 4-methylthiobutyl GSL and 2-phenylethyl GSL (*Brassica napus* root tissue). Samples of this plant tissue were shaken with water for a range of time periods, and the amount of ITC produced was measured, according to the experimental procedure detailed above.

Isothiocyanates were analysed using a Hewlett Packard 6890 GC equipped with an FPD in sulfur mode (394 nm). A 30 m × 0.32 mm i.d. wall coated open tubular fused silica capillary column coated with a 0.25 µm methylsilicone stationary phase (HP-1, Hewlett Packard) was used at an oven temperature of 50°C. Helium was used as the carrier gas at a linear velocity of 19 cm s^{-1}. The GC oven was programmed from 50-220°C at a rate of 8°C min^{-1} with a 1 min initial hold time at 50°C. Samples for analysis were injected splitless using a HP 7683 auto sampler.

Results and Discussion

Effect of hydrolysis time on ITC release from Brassica *tissue*

A total of eighteen GSLs were identified, eleven of which form ITCs on hydrolysis. Figure 1 shows ITC concentration v shaking (hydrolysis) time for five homologues. Formation was rapid for the first 6 h for 3-butenyl ITC, 4-methylthiobutyl ITC and 2-phenylethyl ITC, with little or no further ITC formation after this time. For 2-propenyl ITC and 4-pentenyl ITC, formation was rapid between 5 h and 17 h, with a further small increase to 24 h. For all of the ITCs, formation was not significant after 24 h, and on this basis 24 h was chosen as a suitable time for hydrolysis of plant tissue to release ITCs.

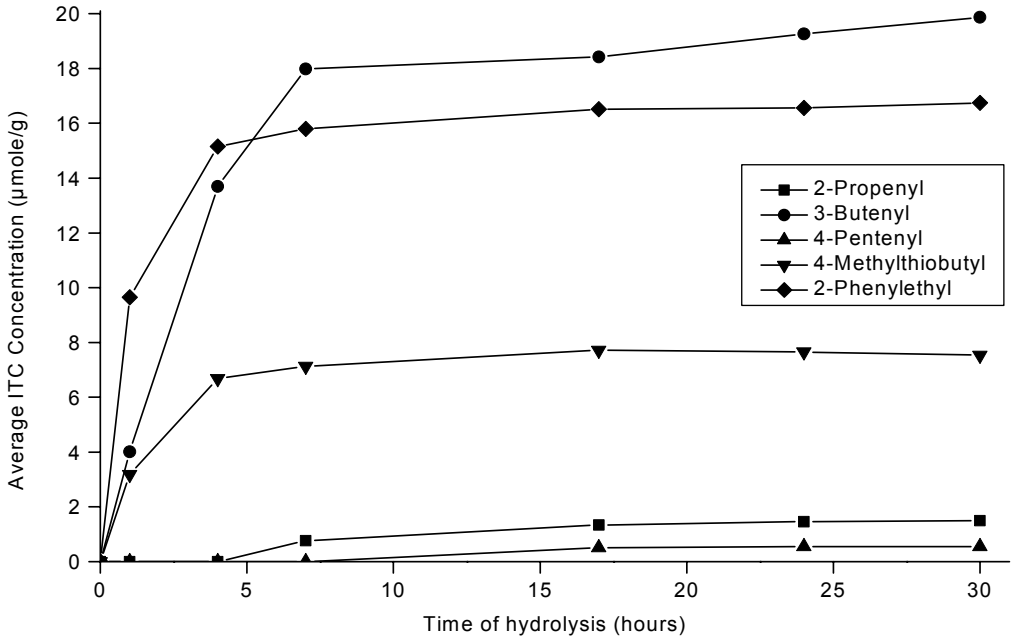

Figure 1. Concentrations versus time for five ITCs produced by the hydrolysis of a freeze-dried *Brassica* tissue sample.

Effect of tissue storage time on ITC release

GSL and ITC analyses were not performed at the same time for most of the samples – GSL analyses were performed first, and the samples freeze-dried and placed in storage prior to ITC analysis. The time interval between analyses ranged from a few days up to six years. ITC v GSL concentrations, grouped by the time interval between the analyses, were plotted to determine whether the time of storage had an effect on the GSL concentration, as has previously been reported (*14, 15*), and hence on the capacity of the tissue to liberate ITCs on hydrolysis (Figure 2).

As Figure 2 shows, the slopes of the trendlines decrease with increasing storage time. This suggests that since the time of the GSL analysis, the concentration of GSLs in the tissue has been decreasing during storage, resulting in lower concentrations of ITCs formed on hydrolysis of the plant tissue. In order to investigate other causes of variation between GSL and ITC concentrations, samples that had been stored for longer than two years (Figure 2d) were removed from the sample set. In these samples, the effect of storage time was greatest and may therefore mask other causes of variation.

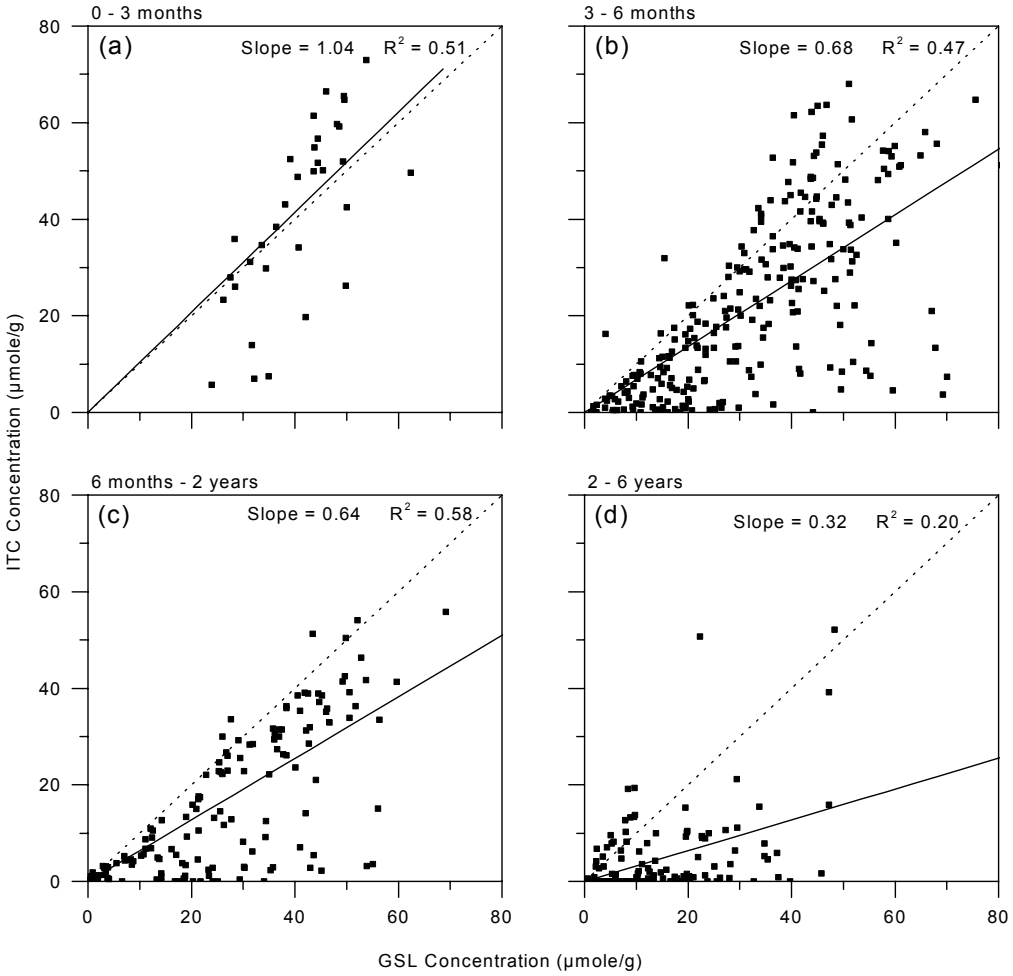

Figure 2. Sums of molar concentrations of ITCs produced by hydrolysis of a *Brassica* meal versus sums of molar concentrations of GSLs present in the meal for four time intervals between GSL and ITC analyses. Also shown are the x = y line (dashed), the trendline (solid) and slope, and the R^2 values.

Factors influencing GSL abundance and ITC quantities liberated

Plots of the abundances of ITCs liberated by hydrolysis of plant tissue samples and the GSLs extracted from the same tissues for six GSL/ITC homologues are shown in Figure 3. Several points are apparent from inspection of Figure 3. First, the position of the trendlines indicates that in most cases, the molar amount of ITC produced by hydrolysis is lower than the amount of the corresponding GSL present in the plant tissues (theoretically, one mole of GSL will produce one mole of ITC). There are several possible reasons for this, including incomplete hydrolysis of GSLs, decomposition, volatilisation or reaction of the produced ITCs and the formation of non-ITC hydrolysis products. Continuation of the hydrolysis procedure for extended periods (to 96 h) resulted in no decrease of ITC concentration, suggesting that decomposition was not occurring (data not shown). Opening of the sealed hydrolysis vessel during the procedure also did not affect the ITC concentration (data not shown), suggesting that quantities of ITCs present in the flask headspace were not significant. Analysis by GC-MS of randomly selected hydrolysed samples revealed

that significant quantities of organic non-ITC hydrolysis products were not produced in the procedure used.

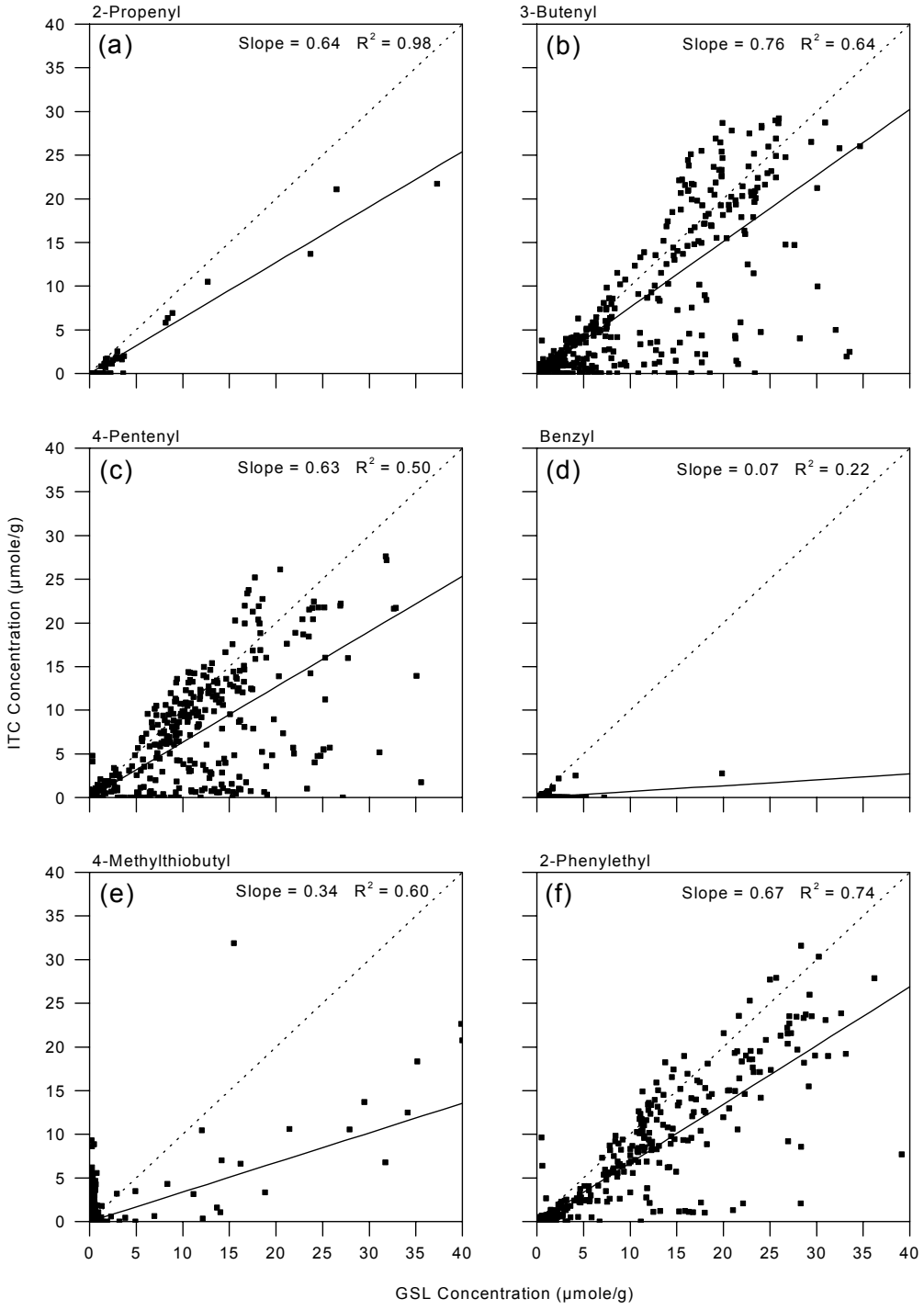

Figure 3. Molar concentrations of ITCs produced by hydrolysis of a *Brassica* meal versus molar concentrations of GSLs present in the meal for six ITC/GSL homologues. Also shown are the x = y line (dashed), the trendline (solid) and slope, and the R^2 values.

It is possible that the lower molar amount of ITC produced relative to GSL is due to incomplete hydrolysis, and was unaffected by extending the time period of hydrolysis. In plant tissue, GSLs and myrosinase are stored in separate intact cells (*2*), and come

into contact when cells are damaged to effect the hydrolysis reaction. The shaking method used in this study is fairly gentle and may not be causing all of the GSL present in the plant tissues to come into contact with myrosinase in order to be hydrolysed. GSLs are reported to occur in cell vacuoles, and they are probably distributed in a variety of cell types (*16*), so it is possible that some GSLs are in cells that are not damaged by the hydrolysis procedure used here, but are recovered in the GSL extraction procedure used.

Another possible reason for the lower molar amount of ITC produced relative to GSL lies in their reactivity with proteins and amino acids. ITCs interact irreversibly with sulfhydryl groups, disulfide bonds and amines (*2*), and may react with such functional groups present in the proteins of the same plants from which the ITCs themselves are produced.

A second point clear in Figure 3 is that the trendlines (ie. the ratios of ITCs to GSLs) are different for different homologues. The slope of the trendline for 3-butenyl of 0.76 was the highest of the six homologues, indicating that the biofumigation potential of this compound as determined by GSL quantification most closely matches the actual amount of toxic ITC produced by hydrolysis of the GSL. The slopes for 2-propenyl, 4-pentenyl and 2-phenylethyl were all slightly lower (in the range 0.63-0.67) suggesting a lower correlation between biofumigation potential measured by GSL concentration and toxic ITC production. The slope for 4-methylthiobutyl (0.34) suggests that the actual biofumigant effect of this compound in soil would be much lower than that predicted from the GSL concentration in plant tissue. Only 26 of the 570 samples yielded benzyl on hydrolysis, so this homologue is not considered further.

There are several possible explanations for the apparent differences in the efficiency of hydrolysis for different GSL homologues. ITCs in different parts of plants may be exposed to different amounts of proteins and/or amino acids, with which they may react. There may be differences in the activity of myrosinase, the enzyme responsible for hydrolysing GSLs to ITCs, towards different GSLs. Different GSLs may form different amounts of other hydrolysis products, including nitriles and thiocyanates, as well as isothiocyanates. Further research is necessary to identify the causes of the difference in efficiency of ITC release between different GSLs.

Another point apparent from inspection of Figure 3 is the variability in the relationship between GSL and ITC values. While there are relatively few samples in which the ITC value is higher than the GSL value, a considerably larger number exhibit significantly lower (a factor of five or greater) ITC values than GSL values. Several factors were examined to determine whether they had an impact on the variation between GSL and ITC values (aside from tissue storage time as discussed earlier): GSL/ITC composition of the tissue (single component v multi-component), tissue type (root v shoot) and growing season (spring v autumn). No significant GSL/ITC variability was observed for growing season, however, the absolute amounts of GSLs/ITCs in spring crops were approximately double those in autumn crops.
Comparison of root v shoot tissues revealed an apparently lower efficiency of formation of ITCs from GSLs in shoot tissue than from root tissue for the 3-butenyl and 4-pentenyl homologues. This may be due to reasons discussed earlier, including GSLs being present in different cell types in roots and shoots, or the presence of

higher levels of proteins or amino acids in shoot than in root material, that react with the liberated ITCs.

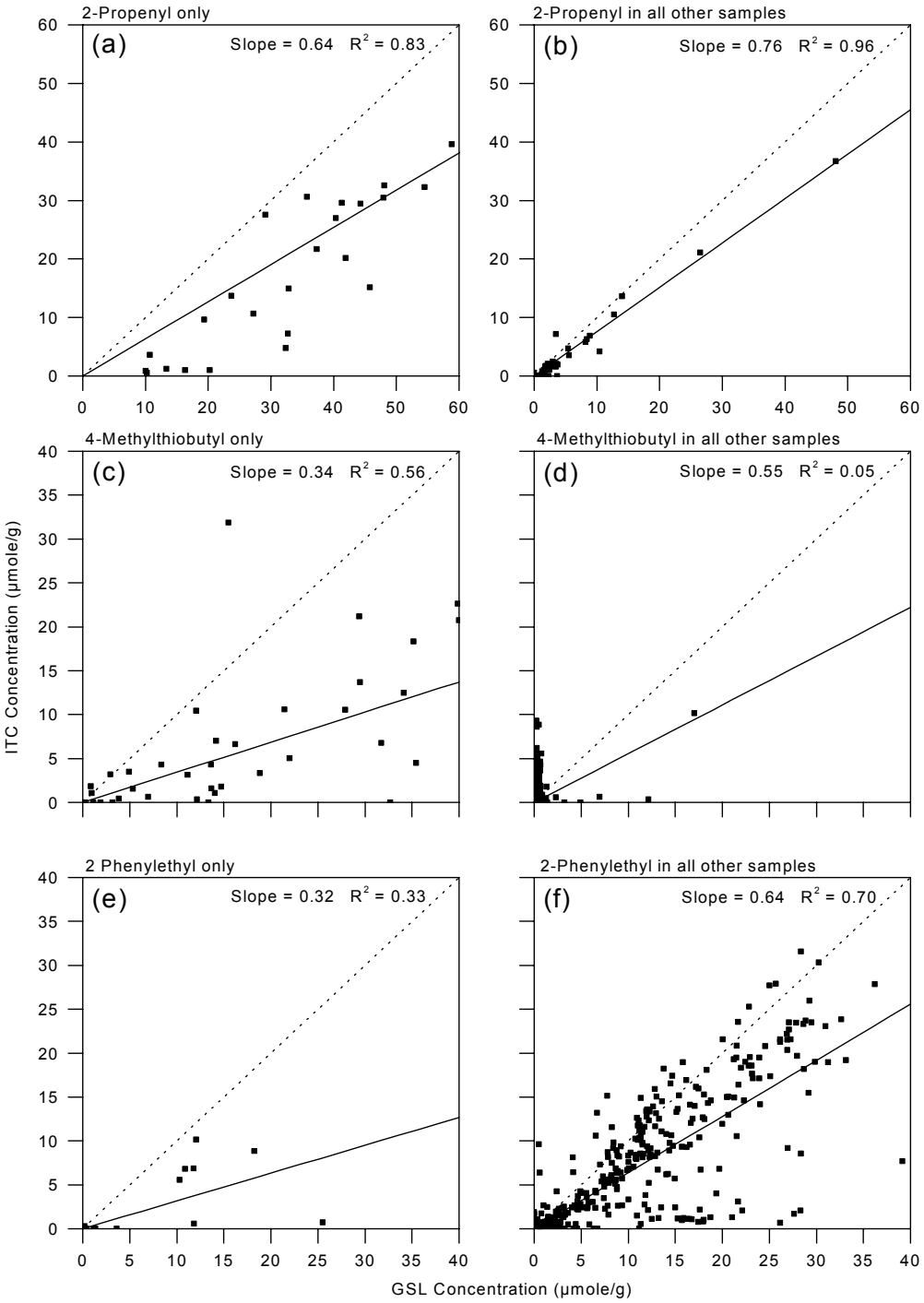

Figure 4. Molar concentrations of ITCs produced by hydrolysis of a *Brassica* meal versus molar concentrations of GSLs present in the meal for three ITC/GSL homologues, differentiated into samples containing > 95% of a single GSL and samples containing mixtures of GSLs. Also shown are the x = y line (dashed), the trendline (solid) and slope, and the R^2 values.

To compare GSL/ITC values for a given compound in single component and multi-component tissue samples, samples were identified in which a single compound comprised greater than 95% of the GSL/ITC concentration. Three of the six

compounds, *viz* 2-propenyl, 4-methylthiobutyl and 2-phenylethyl GSL, accounted for greater than 95% of the total GSLs present in some (approx. 5%) of the tissue samples examined. ITC v GSL concentrations for these tissue samples, and for the same compound in tissues containing a mixture of GSLs/ITCs, for each of these three compounds were plotted (Figure 4). For all three homologues, the slope of the trendline for the homologue in samples containing only (ie. > 95%) that homologue was lower than the slope of the trendline for the homologue in samples containing more than one homologue (2-propenyl 0.64 v 0.76 respectively, 4-methylthiobutyl 0.34 v 0.55, 2-phenylethyl 0.32 v 0.64). This suggests that the hydrolysis is less efficient in samples with only one GSL homologue, regardless of what the actual homologue is. However, due to the small number of samples containing only one homologue, it is unclear whether this observation represents a real trend (Figure 4).

Differences between the molar amounts of ITCs released by hydrolysis and the molar amounts of GSLs present in the plant tissue may reflect factors inherent to some plants that restrict the availability of some GSLs for hydrolysis. This may partly explain some of the anecdotal reports from growers of inconsistencies in the actual biofumigation effect of some plants as observed in the field, although variation in the level of tissue disruption in the soil incorporation process may also be a significant factor.

The variability between concentrations of GSLs in tissues and ITCs liberated from the same tissues has implications for the assessment of the biofumigation potential of *Brassica* tissues. To date, the biofumigation potential of *Brassica* has been determined by quantitative analysis of the GSL concentrations in the plant tissue (*13, 17*). However, there are numerous methods for analysing the individual GSL concentrations in plant tissue (*18*) and these may have varying degrees of efficiency of extraction of GSLs from the plant tissue.

If hydrolysis of GSLs in any given *Brassica* species is less 'efficient' than others (ie. the percentage of GSL present that is converted to ITC is lower) then the actual biofumigation action of the plant may be lower than that predicted on the basis of GSL abundance alone. Further investigation is required to identify the reasons for this variability.

The hydrolysis procedure described here is a simple and rapid method of degrading GSLs in plant tissue to liberate ITCs. Quantitation of the liberated ITCs provides an additional method of assessing the biofumigant capability of plants, aside from the more commonly used method of determining the GSLs present in the plant tissue. Analysis of biofumigant plant tissue using both techniques employed here may provide a more accurate method of assessing biofumigation potential than by GSL analysis alone. It should be noted, however, that this study utilised only freeze-dried plant material, as many of the samples were grown in different parts of Australia and New Zealand for a variety of purposes and fresh samples were unable to be obtained in many cases. This study has not attempted to compare hydrolysis of freeze-dried and fresh tissue samples, an important factor in assessing actual biofumigation potential.

Acknowledgements

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SECTION 2

Maximising isothiocyanate release into soil

Background

It has long been known from laboratory studies that disruption of *Brassica* tissue was needed to bring GSLs into contact with myrosinase enzyme to result in the release of ITCs. Conventional wisdom tended to assume that 'incorporation' of brassicas to achieve ITC release in the field could simply be rotary hoeing of whole plants.

However, studies by colleagues Matthew Morra of the University of Idaho and John Kirkegaard of CSIRO Plant Industry in Canberra, carried out while Matt Morra was on a GRDC Visiting Scientist Fellowship in Australia in 2000/01, cast new light on this issue. Their work was a watershed in stimulating consideration of new approaches to how ITC production and release into soil could be maximised in the field. This aspect became a significant part of the work in this project.

Because of its importance, their paper is included in this report.

Briefly, the Morra and Kirkegaard work showed two key aspects:

i) the importance of disrupting tissue at the cellular level to maximise the formation of ITCs. This was revealed by the vast increase in ITC release following freezing and thawing of *Brassica* tissue, as opposed to just cutting it.
ii) the importance of moisture additional to that derived only from the plant tissue in enhancing the amount of ITC, and in reactivating ITC release from tissue after time.

At the time that the Morra and Kirkegaard studies were being carried out, and during the first year of this project in 2000, this project's team was working on analytical methods for measuring ITCs in soil. Preliminary field sampling of soil around *Brassica* roots was carried out during spring 2000 in the BQMulch and Fumus plots grown in winter-spring 2000.

The plots grown in 2001 were the primary 'test bed' of radical changes to methods used for treating the plants and either incorporating them, or developing techniques for maximising the transport of ITCs into the soil.

Plots grown in 2002 were used to validate the key techniques developed in 2001 in the loam soil at Busselton, WA and to extend the measurement of ITCs in soil after treating brassicas in various ways to the coarse sandy soils typical of the Swan coastal plain near Perth, WA.

Isothiocyanate release from soil-incorporated *Brassica* tissues

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[*NB: While this work was not directly part of this HAL project, it was undertaken during the early stages of the project. The close collaborative association between John Kirkegaard, John Matthiessen and Matthew Morra developed in previous years, and which was a logical extension of a joint Matthiessen/Kirkegaard HRDC project (VG97050) that preceded this one, was ongoing. The research reported in this paper was a watershed in showing how a major improvement could be achieved in ITC release from* Brassica *tissue and in changing our approach to work towards achieving such levels under practical field conditions, which became a core part of this project. It is included in this report to give that important background and for completeness. –* ***John Matthiessen****.*]

Abstract

Isothiocyanates (ITCs) released from glucosinolates in Brassicaceous residues are thought to suppress soil-borne plant pests, however little is known about ITC formation in soil. We conducted field and laboratory studies to determine the concentration and pattern of ITC production in soil following incorporation of rapeseed (*Brassica napus* L.*)* and Indian mustard (*Brassica juncea* Czern. & Coss*)*. After tissue incorporation, ITC was extracted from soil with methanol, derivatized and quantified using HPLC. Maximum ITC concentrations near 1.0 nmol g^{-1} soil were measured immediately after tissue incorporation, with little production detected after 4 d. Only 1% or less of the ITC predicted from tissue glucosinolate concentrations was measured in soil amended with tissues of high glucosinolate rapeseed or mustard varieties. Tissue disruption at the cellular level afforded by freezing and thawing the tissues, resulted in maximum ITC concentrations ranging from 40 to 75 nmol ITC g^{-1} soil, thus increasing ITC release efficiencies to 14 and 26 %. Our work indicates that soil-borne pest suppression is likely to be improved by choosing a high glucosinolate-containing variety of rapeseed or mustard and providing adequate moisture to increase ITC release and soil retention. However, the greatest improvements in the use of *Brassica* biofumigants to control soil-borne plant pests will be achieved by focusing on methods to increase cell disruption thereby maximising glucosinolate hydrolysis and ITC release.

Introduction

Concerns associated with the use of synthetic pesticides encourage the development of alternative strategies for pest management. Glucosinolate-containing plants in the Brassicaceae family represent a potential source of allelochemic control for a variety of soil-borne pests (Fahey et al., 2001). Numerous studies have demonstrated the broad biocidal activity of glucosinolate hydrolysis products (Brown and Morra, 1997). Enzymatic hydrolysis of glucosinolates by myrosinase (thioglucoside glucohydrolase E.C. 3.2.3.1) produces ITCs, oxazolidinethiones, ionic thiocyanate (SCN^-) and organic cyanides in pathways controlled by glucosinolate chemistry and reaction conditions (Larsen, 1981). ITCs are generally regarded as the most toxic products of glucosinolate hydrolysis.

ITCs interact with protein (Kawakishi and Kaneko, 1985) and are thus general biocides inhibiting a wide variety of plant pests (Brown and Morra, 1997). They are used as commercial pesticides in either pure form or produced in soil from an amended precursor (Ware, 2000). Observed pest suppression by glucosinolate-containing plant tissues, a process termed biofumigation (Angus et al., 1994), is assumed to result from ITCs and possibly other allelochemicals produced from glucosinolates within the tissues.

Recent research has focussed on a systematic approach to improving the biofumigation potential of Brassicaceous amendments by identifying high glucosinolate species and cultivars with appropriate agronomic attributes (Fig. 1). This has led to a better understanding of the types, concentrations and distribution of the glucosinolates in different species, cultivars and plant parts (Kirkegaard et al., 1998). Maximum potential ITC release upon tissue disruption can be estimated when identities and concentrations of glucosinolates in plant tissues are known along with the respective amount of biomass (Fig.1). The relative toxicity of different glucosinolate hydrolysis products is also important in maximising the likelihood of effective pest suppression (Sarwar et al. 1998; Borek et al., 1998).

Research indicates however, that ITC formation from incorporated fresh B. napus tissues is less than 5 % of what is predicted by assuming complete conversion of glucosinolate to ITC (Gardiner et al., 1999). Likewise, only a small fraction of the glucosinolate from soil-incorporated B. juncea leaves was measured as propenyl ITC in headspace gas samples (Bending and Lincoln, 1999). This low efficiency of glucosinolate conversion to ITC has led to absolute amounts of isothiocyanate considerably less than those recommended for synthetic fumigants (Brown et al., 1991).

This has raised questions about the role of ITCs in biofumigation, leading Bending and Lincoln (1999) to suggest that other volatile S compounds were likely to be as important in pest control as ITCs. Improving the efficacy of biofumigant crops and making the link between glucosinolate content of the plant and pest suppression requires a more fundamental understanding of the pattern of release of ITCs in soil and the factors influencing release efficiency (Fig. 1).

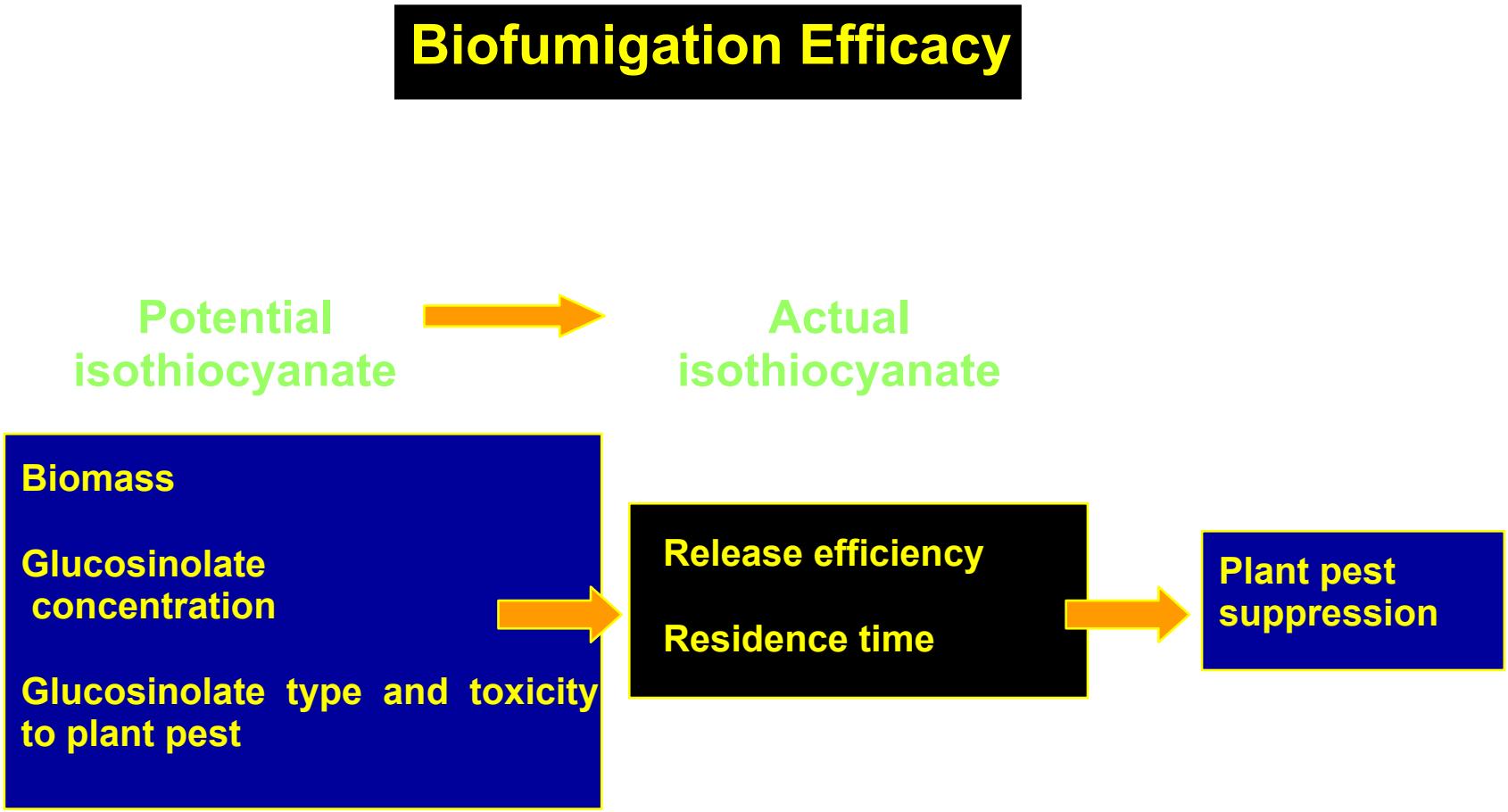

Figure 1.- Theoretical approach in optimizing the use of glucosinolate-containing plants as sources of isothiocyanates for controlling soil-borne plant pests.

Our objectives were to determine the pattern of release of ITCs in soil following incorporation of two common *Brassica* biofumigant crop species and to identify the factors influencing the efficiency of ITC release. Factors considered included the type and concentration of glucosinolates in the incorporated tissues, soil water content and the degree of tissue disruption during incorporation.

Materials and methods

Two field experiments were carried out at the Ginninderra Experiment Station near Canberra, Australia (35°12' S; 149°06' E: 600 m a.s.l.). The soil has a fine sandy loam texture in the surface horizon and is a Yellow podzolic (GN 3.85, Northcote et al., 1971). The surface 10 cm has a pH of 5.9, EC of 85 μS cm^{-1} and a CEC of 64 meq kg^{-1}. It contains 14 g total carbon, 1.1 g total N and 100 mg total S kg^{-1} soil.

Field experiment 1 - ITC release by two commercial biofumigant crops

Two commercial biofumigant crops, FUMUS (*B. juncea*) and BQMulch (*B. napus/B. campestris*) were selected for high biomass and high tissue glucosinolate content. For simplicity, BQMulch will be consistently referred to as the high glucosinolate *B. napus* variety. The crops were sown on April 28, 2000 in individual plots of 6 m x 2 m arranged in a randomised complete block design with 4 blocks. The crops were sown at 5 kg ha^{-1} with starter fertiliser (20 kg N, 18 kg P and 16 kg S ha^{-1}) and later top-dressed with 50 kg N ha^{-1} as urea to ensure vigorous growth. Unsown fallow plots were included in the experiment as controls for ITC measurement.

On October 2, when the plants were in the early flowering stage, 0.4-m^2 quadrats of plants including roots down to 0.15 m were removed from each plot for biomass and glucosinolate analysis. The plants were taken immediately to the laboratory where they were washed, separated into root and shoot and sub-sampled for glucosinolate concentrations and biomass. Following sampling, the biofumigant crops were incorporated into the soil to a depth of 10 cm using two passes of a rotary hoe followed by one pass of a rubber-tyre roller to consolidate the soil prior to sampling. The soil was sampled for ITC 24 and 72 h after incorporation using the method described below.

Field experiment 2 - ITC release from shoots of biofumigant crops differing in glucosinolate type and concentration

The two high glucosinolate commercial biofumigants used in the first experiment and two additional low glucosinolate breeding lines CSIRO-651 (*B. juncea*) (CSIRO Plant Industry) and H103d (*B. napus*) (Wrightson Seeds) were used in the second experiment. Each crop was sown in plots 6 m x 2 m in a completely randomised block design with four blocks adjacent to Experiment 1. An unsown fallow plot was maintained free of plants adjacent to each sown plot so that shoot tissues could be cut and transferred to the fallow plots for incorporation. On October 10, when the *B. juncea* lines were in full flower and the *B. napus* lines were in early flower, plant quadrats for biomass and glucosinolate analysis were removed as in Experiment 1. The remaining shoot material

was cut at ground level and placed evenly by hand onto the adjacent fallow plots at a rate of 7 kg wet biomass m^{-2}. The shoot material was then incorporated as in Experiment 1. Soil was sampled from the 0- to 10-cm layer 2, 24, 48 and 72 h after incorporation and ITCs were measured using the method described below. Rain (11.2 mm) fell on the site between the 48- and 72-h sampling times.

Field sampling and ITC extraction

Soil from each plot was sampled to a depth of 10 cm using a 32-mm internal diameter sampling tube at 10 random locations in each plot. The 10 samples from each plot were placed in a sealed plastic bag and mixed prior to the removal of a 10-g subsample (avoiding plant tissues) for ITC analysis. ITCs were extracted by adding the 10-g soil subsample from each bag to 10 ml of cold methanol in a polypropylene centrifuge tube. The sample was shaken at room temperature for 1 h and centrifuged for 10 min. A 4-ml aliquot of the methanol extract was removed from the centrifuge tube with a 5-ml glass syringe. The methanol extract was passed through a 0.2-µm syringe filter (25 mm Millex-GN, non-sterile, nylon) to obtain approximately 2 ml of clear filtrate. This filtrate was used in an ITC derivatization procedure (see below).

Laboratory experiment to determine the effect of soil water content and tissue disruption on ITC release from B. juncea leaves

A laboratory experiment was conducted using surface soil (0-10 cm) collected from the same field site as above. A leafy vegetable mustard (*B. juncea*) known to contain high concentrations of leaf glucosinolate was obtained from the Asian Vegetable Research and Development Center, Taiwan and grown in the glasshouse in pots containing a peat/sand mix with complete fertiliser to ensure healthy growth. Several plants were grown to provide a large number of uniform leaves that could be utilised in the experiment. On the day of soil incorporation, leaf disks 37 mm in diameter were removed from opposite sides of the midrib of leaves selected from plants that were at a similar phenological stage. Half of the disks were frozen at -19^{o}C to provide a treatment to investigate the impact on ITC release of maximum tissue disruption (upon thawing). One leaf disk, frozen or fresh, was placed in the bottom of a 100-ml screw-top bottle and covered with 10 g of dried, sieved (2 mm) soil collected from the field site. Sufficient water was then added to the soil in half of the bottles to produce a moisture content equivalent to that present at soil suctions of –32 kPa. A larger volume of water was added to soil in the remaining bottles to produce waterlogged soils with a water film visible on the soil surface. Additional frozen leaf discs were retained for glucosinolate analysis. The bottles were stored tightly capped at 19-22^{o}C and three replicate bottles of each of the four treatments (fresh, -32 kPa; frozen, –32 kPa; fresh, waterlogged; frozen, waterlogged) were sampled at 2, 24, 48, 96 and 120 h for ITC concentration. To measure soil ITC concentrations, 10 ml of methanol was added directly to the bottles, the leaf tissue was carefully removed from the soil within 15 s and the remaining soil was shaken for 1 h prior to performing ITC analysis as described below.

Tissue glucosinolate analysis

All shoot and root tissue samples were freeze-dried, ground using a Wiley mill with a 1-mm screen, weighed and stored in sealed bottles at -20^{o}C prior to glucosinolate analysis. Glucosinolates from 300 mg of freeze-dried tissue were extracted and transformed to desulphoglucosinolates according to the method of Magrath et al. (1993) with modifications according to Kirkegaard and Sarwar (1998). The desulphoglucosinolates were then separated and quantified using the HPLC method described in detail by Kirkegaard and Sarwar (1998). Peaks were identified using pure standards either purchased (2-propenyl glucosinolate, Sigma, St Louis, MO USA; benzyl glucosinolate, Canola Council of Canada, Winnipeg, Canada) or kindly provided by Dr. R. Mithen, John Innes Centre, Norwich U.K. and Dr. R. Wallsgrove, Rothamsted U.K.

Soil ITC analysis

Our procedure for isothiocyanate measurement was based on the reaction of ITCs with vicinal dithiols to produce cyclic condensation products amenable to spectroscopic analysis. 1,2-Benzenedithiol (BDT) reacts readily and quantitatively with all but tertiary ITCs. The cyclic condensation product 1,3-benzodithiole-2-thione is stable and amenable to ultraviolet spectrometric detection at 365 nm (Zhang et al., 1992; Zhang et al., 1996).

ITCs were derivatised by adding a 600-µL subsample of the methanol extract containing ITC to 1-mL Chromacol HPLC autosampler vials (8 x 40 mm) containing 600 µL of 100 mM K_2HPO_4 buffer (pH 8.6) and 200 µL of a 35 mM 1,2-benzenedithiol/1% mercaptoethanol solution. Vials were capped (Chromacol, teflon-lined caps) and inverted several times to mix the contents. The samples were incubated at 65^{o}C in a water bath for 1 h, and following removal, stored in the freezer prior to HPLC analysis.

ITCs were quantified using an HPLC with a Waters 600 System Controller, 717 autosampler and 486 UV/Vis detector set at 365 nm. The HPLC was equipped with a Waters 3.9 x 150 mm Nova-Pak C18 (4 µm) column and precolumn. The pump was operated isocratically at 1 ml min^{-1} using 90% methanol as an eluent. The injection volume was adjusted from 10 to 50 µl (based on sample ITC concentration) and the run time was 3 min. A calibration curve was constructed using derivatized phenylethyl isothiocyanate as an external standard.

Calculating ITC release efficiency

The efficiency of ITC release in different treatments was determined as the maximum ITC concentration detected in the soil as a proportion of the potential ITC present in the incorporated plant tissue (estimated from glucosinolate concentration and biomass assumming stoichiometric conversion). A soil bulk density of 1.4 g cm^{-3} was assumed in all calculations.

Table 1. Type, concentration and total amount of ITC-liberating glucosinolates in the tissues of incorporated *Brassica* biofumigant crops, and the amount and efficiency of ITC release in the soil following incorporation of the entire plant. Standard errors for each respective mean are included in parentheses.

Species	Biomass (g m^{-2})	Glucosinolate (μmol g^{-1} tissue)[a]					Potential ITC (nmol g^{-1} soil)[b]	Actual ITC (nmol g^{-1} soil)[c]	Release efficiency (%)[d]
		2-Propenyl	3-Butenyl	4-Pentenyl	2-Pheneylethyl	Total			
B. juncea	Shoot								
	800 (38)	16 (1)	0	0	0	16 (1.0)	89 (6.0)		
	Root								
	134 (4)	10 (1)	0	0	14 (1.5)	24 (1.5)	24 (2.0)		
	Total						112 (7.0)	1.2 (0.09)	0.9 (0.08)
B. napus	Shoot								
	544 (122)	0	8 (0.5)	12 (0.5)	0	20 (0.5)	75 (15.0)		
	Root								
	123 (11)	0	5 (0.5)	4 (0.1)	6 (0.5)	15 (0.5)	13 (1.5)		
	Total						88 (13.0)	0.8 (0.2)	1.0 (0.08)

[a] Concentration of ITC-liberating glucosinolates in freeze-dried tissue.
[b] Shoot, root and total plant ITC-liberating glucosinolates expressed in nmol g^{-1} of soil assuming incorporation to 10 cm and a soil bulk density of 1.4 g cm^{-3}.
[c] Maximum soil ITC concentration detected during the experiment.
[d] Percentage of the potential plant ITC incorporated that was detected in soil (Actual ITC ÷ Potential ITC x 100).

Table 2. Type, concentration and total amount of ITC-liberating glucosinolates in the incorporated shoot tissues of high and low glucosinolate-containing biofumigant crops, and the amount and efficiency of release of ITCs in the soil following incorporation of the shoot tissues only. Standard errors for each respective mean are included in parentheses.

Species/ glucosinolate level	Shoot biomass (g m^{-2})	Shoot biomass (w/w%)	Glucosinolate (µmole g^{-1} tissue)[a]				Potential ITC (nmol g^{-1} soil)[b]	Actual ITC (nmol g^{-1} soil)[c]	Release efficiency (%)[d]
			2-Propenyl	3-Butenyl	4-Pentenyl	Total			
B. juncea									
Low	1211 (25)	0.86	1.5 (0.5)	2.0 (0.50)	0	3.5 (1.0)	29.8 (8.8)	0.5 (0.20)	1.6 (0.20)
high	999 (23)	0.71	20.1 (3.3)	0	0	20.1 (3.3)	145.0 (27.1)	0.9 (0.20)	0.6 (0.10)
B. napus									
Low	879 (32)	0.63	0	0.4 (0.07)	0.2 (0.03)	0.7 (0.1)	4.3 (0.8)	0.2 (0.05)	4.2 (1.90)
high	798 (34)	0.57	0	8.7 (1.00)	6.4 (0.30)	15.2 (1.2)	86.1 (7.1)	0.7 (0.03)	0.8 (0.04)

[a] Concentration of ITC-liberating glucosinolates in freeze-dried tissue.
[b] Shoot, root and total plant ITC-liberating glucosinolates expressed in nmol g^{-1} of soil assuming incorporation to 10 cm and a soil bulk density of 1.4 g cm^{-3}.
[c] Maximum soil ITC concentration detected during the experiment.
[d] Percentage of the potential plant ITC incorporated that was detected in soil (Actual ITC ÷ Potential ITC x 100).

$$\text{Release efficiency} = \frac{\text{Maximum soil ITC concentration (nmol g}^{-1}\text{ soil)}}{\text{Total ITC-liberating glucosinolate in incorporated plant material (nmol g}^{-1}\text{ soil)}} \times 100$$

Results

Field experiment 1 - ITC release by two commercial biofumigant crops

B. juncea contained 2-propenyl glucosinolate in the shoots and 2-propenyl and 2 phenylethyl glucosinolate in the roots (Table 1). *B. napus* contained 3-butenyl and 4-pentenyl glucosinolate in the shoots and 3-butenyl, 4-pentenyl and 2-phenylethyl glucosinolate in the roots. More ITC was potentially available from *B. juncea* tissues incorporated into the soil (112 nmol g^{-1} soil) than *B. napus* tissues (88 nmol g^{-1} soil). However, measured soil isothiocyanate concentrations were near 1 nmol g^{-1} soil thus indicating a release efficiency for both species of approximately 1 % (Table 1). ITC concentrations were highest at 24 h, dropping to less than half of the maximum in 72 h (Fig. 2).

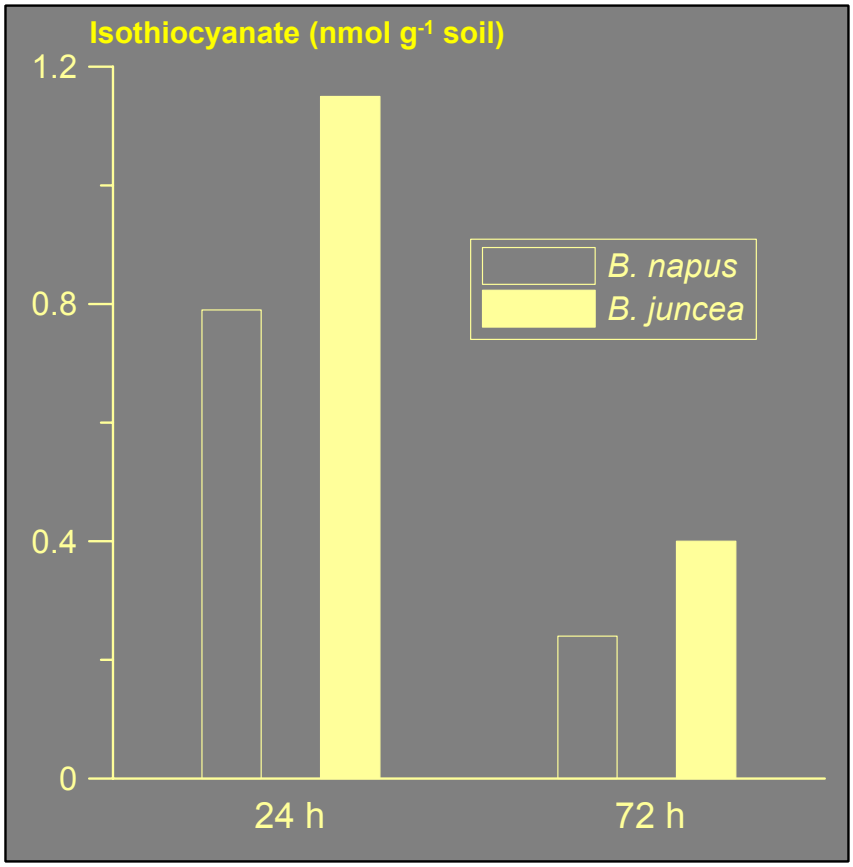

Figure 2. Extractable ITC concentrations determined in soil after the field incorporation of high glucosinolate *B. napus* and *B. juncea* biofumigant crops. Standard errors are shown for each respective mean.

Field experiment 2 - ITC release from shoots of biofumigant crops differing in glucosinolate type and concentration

2-Propenyl and 3-butenyl were the dominant glucosinolates in the low glucosinolate *B. juncea* (Table 2). The high glucosinolate *B. juncea* contained 2-propenyl glucosinolate in a concentration almost six times that of the low glucosinolate variety. Both *B. napus* varieties contained 3-butenyl and 4-pentenyl glucosinolate with the high glucosinolate variety having over 20 times the glucosinolate concentration of the low variety. Total glucosinolate contributed to the top 10 cm of soil following tissue incorporation could potentially produce about 30 and 145 nmol ITC g^{-1} soil for the low and high *B. juncea* varieties, respectively. Potential isothiocyanate from incorporated *B. napus* tissues was estimated as 4 and 86 nmol isothiocyanate g^{-1} soil for the low and high glucosinolate varieties, respectively (Table 2).

Trends showed higher isothiocyanate concentrations in those soils to which high glucosinolate-containing tissues were amended, but the difference was only significant ($P \leq 0.05$) for *B. napus* (Table 2). Maximum ITC concentrations measured in soil extracts did not exceed 1.0 nmol g^{-1} soil for all four varieties. Release efficiency of ITC from precursor glucosinolates ranged from 0.64 % for high glucosinolate *B. juncea* to 4.23 % for the low glucosinolate *B. napus*. ITC release efficiency was higher in each respective species for the lower glucosinolate variety (Table 2).

Maximum ITC release was measured at the first sampling 2 h after tissue incorporation (Fig. 3). ITC concentrations decreased after the initial sampling and reached a minimum at 48 h. The rain (11.2 mm) that fell between the 48- and 72-h sampling may have caused the observed increase in soil ITC that was detected at 72 h. The increase was especially large for the high glucosinolate *B. napus* variety and the low glucosinolate *B. juncea* variety resulting in ITC concentrations almost as high as those determined at 2 h (Fig. 3).

Laboratory experiment to determine the effect of soil water content and tissue disruption on ITC release from B. juncea leaves

Fresh B. juncea leaf tissues added to soil produced extractable ITC concentrations less than 1 nmol g^{-1} soil during the 120-h incubation (Fig. 4). This compares to a potential ITC concentration of 284.4 nmol g^{-1} soil as predicted assuming complete conversion of 2-propenyl glucosinolate (39.1 µmol g^{-1} tissue) in the added tissue (0.072 g) to propenyl ITC (Table 3).

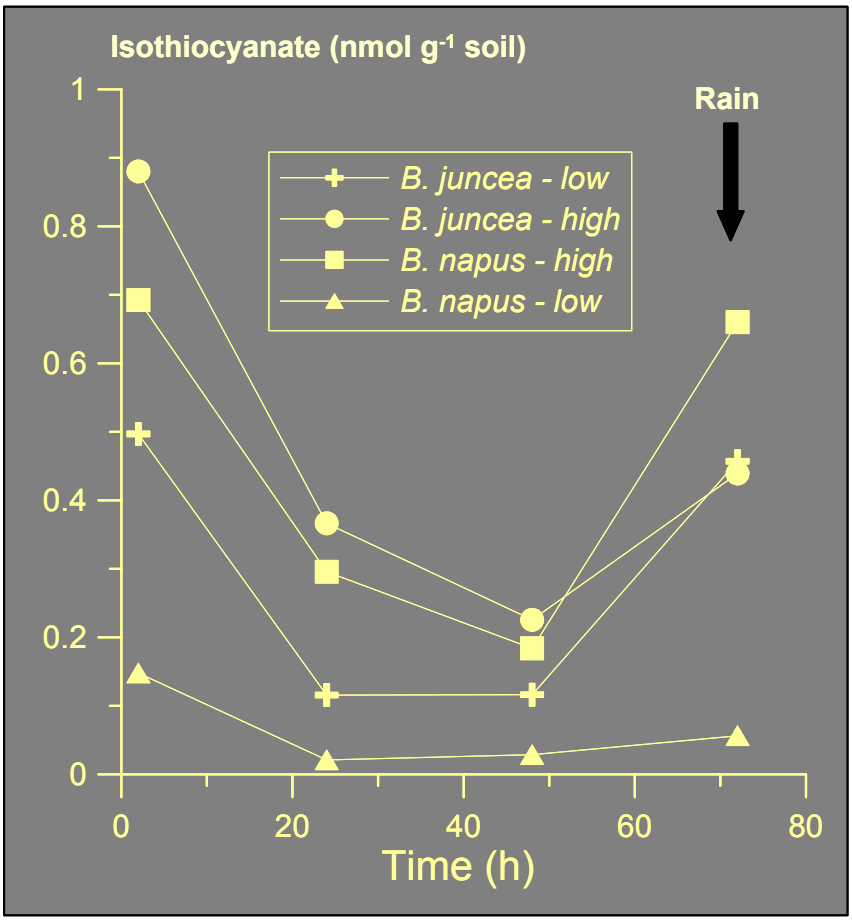

Figure 3. Extractable ITC concentrations determined in soil after the field incorporation of B. napus and B. juncea shoot tissues with low and high glucosinolate concentrations. Standard errors are shown for each respective mean.

Table 3. Maximum extractable concentrations and calculated release efficiencies of ITC produced in 10 g soil amended with 0.072 g (dry weight) of fresh or frozen B. juncea leaf tissue. Standard errors for each respective mean are included in parentheses.

Tissue treatment	Soil water	Maximum soil ITC (nmol g^{-1} soil)	Release efficiency (%)[a]
Fresh	-32 kPa	0.092 (0.034)	0.03
Fresh	waterlogged	0.193 (0.049)	0.07
Frozen	-32 kPa	38.9 (9.100)	13.7
Frozen	waterlogged	75.0 (22.00)	26.4

[a] Release efficiencies calculated using a glucosinolate concentration of 39.1 µmol g^{-1} of tissue (dry weight).

In contrast, freezing the tissues prior to incubation produced a large flush in ITC formation that was measured during the initial 2-h sampling. This resulted in an increase in release efficiency from 0.032 % for the –32 kPa, fresh and 0.068 % for the waterlogged, fresh treatments to 14 % for the –32 kPa, frozen treatment and 26 % for the frozen, waterlogged treatment (Table 3). Extractable ITC concentrations from the frozen tissues quickly dropped

to near 1 nmol ITC g^{-1} soil at 24 h and beyond. Trends for greater ITC formation with wetter soil appear in both the fresh and frozen treatments, but differences between waterlogged and –32 kPa treatments in each respective pair of samples are not significant ($P > 0.05$).

Discussion

A flush in ITC occurred immediately after tissue incorporation into soil because cell membranes were broken during plow down. ITC production from leaf disks incorporated into 10-g soil samples was more constant since the disks were cut prior to soil incorporation. Freezing caused extensive cell membrane disruption and thus permitted greater contact between glucosinolates and the enzyme myrosinase, the enzyme that catalyzes glucosinolate hydrolysis to ITC. The flush in ITC from frozen tissue correspondingly was much more dramatic (Fig. 4). The drop in the extractable ITC concentration after this initial sampling is likely to have occurred because the ITC functional group reacts with nucleophilic functional groups present in soil organic carbon (Borek et al., 1995). The secondary release of ITC that occurred at 72 h in the field experiment with *B. juncea* and *B. napus* shoots may have been a function of precipitation and the associated increase in soil moisture (Fig. 3). This is consistent with laboratory experiments involving *B. juncea* tissues in which we observed a trend for higher ITC concentrations in wetter soils (Fig. 4). Additional moisture could conceivably increase extractable ITC by increasing the amount of water available for glucosinolate hydrolysis, promoting tissue degradation and leaching more ITC out of the tissue. In addition, increased soil moisture content would also serve to decrease the loss of volatile ITCs.

It is difficult to compare the current results on timing of ITC release with previous studies due to experimental differences. Gardiner et al. (1999) extracted ITC from field soils to which two *B. napus* varieties were incorporated and showed that maximum ITC concentrations occurred at 30 h dropping off by 75% of this maximum at 72 h. Bending and Lincoln (1999) reported maximum ITC concentrations in headspace samples above a sandy loam soil with added *B. juncea* tissues at 96 h, although measurements were not initiated until 48 h. Analysis of residual glucosinolate within the *B. juncea* tissues showed that most of the glucosinolate was gone in 6 d and additional ITC production was therefore unlikely.

Collectively our data along with previous investigations indicate that most of the ITC will be released within the first 4 d after tissue incorporation. The exact timing of this release will vary based on soil chemical and physical characteristics, temperature and moisture. ITC concentrations decreased rapidly and thus any pest control will most likely occur as a result of this initial flush although ITC release during plant growth may play a role. Rapid dissipation is consistent with previously reported half-lives of 20 to 60 h for propenyl ITC in six soils having different physical and chemical characteristics (Borek et al., 1995).

Maximum extractable ITC concentrations as determined here in field experiments with *B. napus* and *B. juncea* were near 1 nmol g^{-1} soil. Gardiner et al. (1999) also measured maximum ITC concentrations of 1 nmol g^{-1} soil in field experiments involving a plowdown of *B. napus*. Similarly, Bending and Lincoln (1999) noted that extractable ITC concentrations in laboratory soils with added *B. juncea* tissue were below their analytical detection limit of 2 nmol g^{-1} soil.

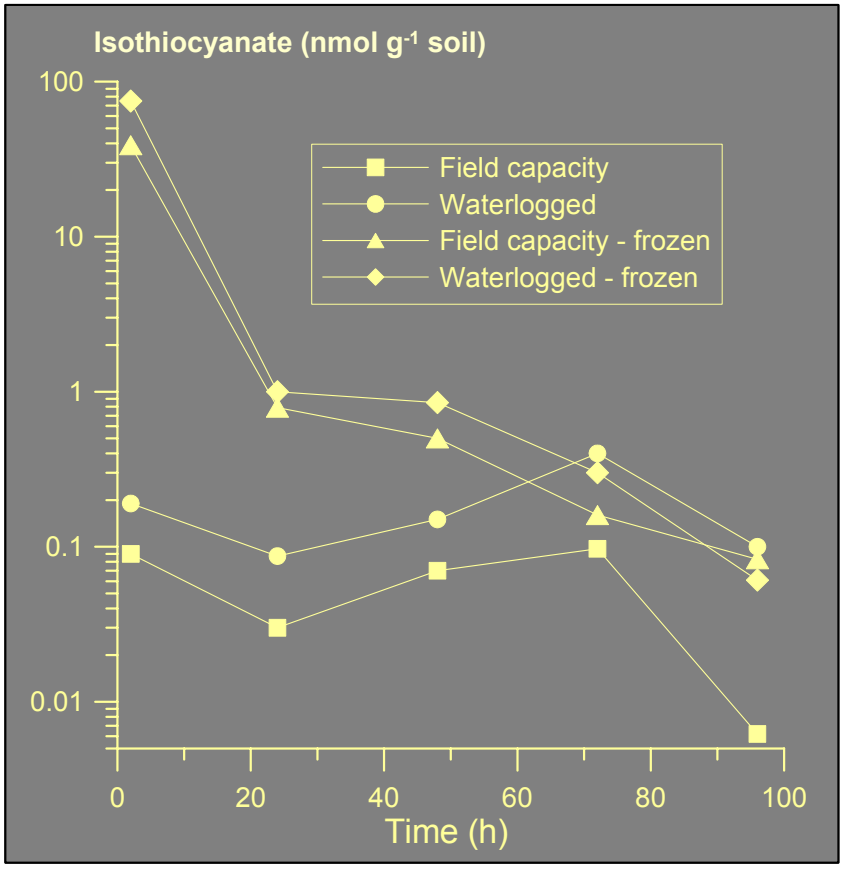

Figure 4.- Extractable ITC concentrations determined in soils of two different moisture contents after the laboratory incorporation of fresh or frozen *B. juncea* tissues.

In contrast to our data showing that shoot glucosinolates release a substantial proportion of the total measured ITC (Tables 1 and 2), Gardiner et al. (1999) reported that *B. napus* roots produced nearly all of the detected ITC. This is a consequence of the different *B. napus* varieties and their respective root and shoot glucosinolate concentrations. The roots of varieties used by Gardiner et al. (1999) contained two to nearly three times the glucosinolate concentration (14 and 21 µmol g^{-1} tissue) of the shoots (<8.0 µmol g^{-1}). In contrast, we used a high glucosinolate *B. napus* variety that contained more glucosinolate in the shoots (20 µmol g^{-1} tissue) than the roots (15 µmol g^{-1} tissue) (Table 1).

Although the exact concentration of ITC necessary for pest control will vary with the ITC, soil and plant pest it is helpful to use the recommended rate of commercially available methyl isothiocyanate application as a reference point for gauging potential efficacy. Calculated values for soil sterilization using methyl isothiocyanate range from 517 to 1294 nmol g^{-1} soil (Brown et al., 1991). More specific work indicates that methyl isothiocyanate addition of 182 nmol g^{-1} soil is necessary to obtain an LC_{90} for larvae of the black vine weevil (*Otiorhynchus sulcatus* (F.)) (Borek et al., 1997). Thus although pest suppression is possible at ITC concentrations near 1 nmol g^{-1} soil, increased concentrations are most likely necessary for effective pest control.

Such an increase in ITC formation seems possible given the low percentage of glucosinolates actually released as ITCs in soil. Release efficiencies of ITC from tissues of high glucosinolate varieties coarsely incorporated into field or laboratory soils were 1% or less

(Tables 1 and 2, Fig. 4). In marked contrast, more thorough tissue disruption at a cellular level afforded by freezing the tissues increased release efficiencies to 14 and 26 % (Table 3). The resulting maximum ITC concentrations of 40 to 75 nmol ITC g^{-1} soil are much nearer the application rates expected to have a pesticidal effect. Pest suppression is even more likely given the fact that the reported values are commercial ITC application rates, not extractable concentrations, and complete soil sterilization may not be necessary for pest suppression.

If we assume that ITCs contribute significantly to the pesticidal activity of *Brassica* tissues, our work indicates that soil-borne pest suppression will be improved by choosing a high glucosinolate-containing variety and providing adequate moisture to promote ITC release and soil retention. However, the major factor limiting potential pest suppression is ITC release from the tissues (Fig. 1). The greatest improvements in the use of *Brassica* biofumigants to control soil-borne plant pests will be achieved by developing methods to increase cell disruption and thereby increase ITC release. Both physical and chemical methods are currently being explored as possible ways to increase ITC release from *Brassica* tissues.

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Methods

Measuring ITCs in soil

To develop the method for measuring ITCs in soil, soil was sampled by shaking it off the roots of brassicas and prepared in the way described below. Subsequently, the method was as described for soils sampled in any part of a site where brassicas had been grown (and the same if sampling for methyl ITC after metham sodium application).

Soil (approx 250 g) was collected from a depth of 0-20 cm, mixed in a plastic bag, sieved (2 mm) and a subsample (40 g) weighed into a 50 ml centrifuge tube. Sufficient ethyl acetate was added such that the solvent surface was 10 mm above the soil surface, and the tubes capped for transport to the laboratory.

The ethyl acetate was decanted and replaced with a similar volume. Tubes were shaken on an orbital shaking table for 20 min at 200 rpm, after which the ethyl acetate was decanted. This procedure was repeated once more and the three extracts combined. Methyl isothiocyanate (1 ml, 100 ppm in ethyl acetate) was added as a normalisation standard and the solvent gently evaporated under a stream of air to a volume of 5 ml. The samples were dried and filtered through a plug of anhydrous magnesium sulfate (approx 4 cm) in a pasteur pipette into a 2ml vial for analysis using gas chromatography (GC).

Samples were analysed using a Hewlett Packard 6890 GC equipped with a flame photometric detector (FPD) in sulfur mode (394 nm). A 30 m × 0.32 mm i.d. WCOT fused silica capillary column coated with a 0.25 μm methylsilicone stationary phase (HP-1, Hewlett Packard) was used. The GC oven was programmed from 50-220°C at 8°C min^{-1}. Samples for analysis were injected splitless using a HP 7683 auto sampler at an oven temperature of 50°C. Helium was used as the carrier gas at a linear velocity of 19 cm s^{-1}.

Treating and incorporating brassicas

During the growth of the large area plots at Busselton in 2000, and before the highly revealing results of the key Morra and Kirkegaard study had become available, discussions with Keith Taylor had centred around methods of treating the plants and trying alternatives to simply rotary hoe incorporation of whole plants. The first alternative tried was to use a hay mower-conditioner to attempt to break up the plant tops more than might be the case with straight rotary hoeing.

In the event, this was not highly successful, particularly for the tall, stemmy Fumus mustard. Nevertheless, it was the start of a reappraisal of what 'incorporation' should really mean. Once Morra and Kirkegaard revealed the importance of cell level tissue disruption, and we had successfully developed methods for measuring ITCs in soil, the stage was set for a major change in the approach to treating the plants and incorporation.

In 2001, a contractor who carries out weed and cover crop mulching in vineyards was engaged to mulch the brassicas. In most of the area in both the BQMulch and Fumus plots, the pulverised plant material was immediately rotary hoed into the soil. This was considered to be the 'best-bet' approach and seemingly the most appropriate practical technique likely to give best breakdown. Some strips were treated differently to gauge the efficacy of some other incorporation options by measurement of ITCs in the soil.

The treatments in 2001were:

1. Mulch, rotary hoe immediately.
2. Mulch, leave on surface.
3. Rotary hoe whole plants.
4. Mow (cut off at ground level), rotary hoe in after 7 days.
5. Mow, leave on surface to dry and rotary hoe in 7 days later.
6. Mow, remove top material to bare area of soil on edge of paddock & separately rotary hoe the separated tops (treatment 6a) and the remaining roots (treatment 6b), in immediately.
7. Mow, remove top material to bare area of soil on edge of paddock & let dry and separately rotary hoe tops (treatment 7a) and roots (treatment 7b) in 7 days later.

The purpose of delaying rotary hoeing in treatments 4 and 5 was to test the effect of delays that a farmer may experience through factors such as machinery breakdown or pressures of other farm operations.

The purpose of treatment 6 was to gauge the amount of soil ITC that was derived from roots and tops, with the overlaying of a sub-treatment of a delay to rotary hoeing for the reasons outlined above for treatments 4 and 5.

The effect of watering was tested by adding water to 30 cm diameter x 30 lengths of PVC pipe embedded in the different treatments. Levels of simulated irrigation or rainfall were 13 mm or 42 mm (1 and 3 L water added, respectively). Water was added immediately, and after 2, 7 and 14 days.

Soil samples at the 5-15 cm depth range were taken in the various treatments at different intervals and analysed for ITCs as described above.

In 2002, plots of BQMulch and Fumus were grown on loam at Busselton and sand at Medina on the outskirts of Perth. Similar sets of treatments were applied as the main ones in 2001 (mulch and rotary hoe in immediately, mulch and rotary hoe in three days later (instead of seven days in 2001) rotary hoe whole plants in, mulch and leave on surface and water soon after mulching, mulch and leave on surface and water three days later).

Treatments in 2002 were:

1. Mulch, rotary hoe immediately.
2. Mulch, leave on surface.
3. Rotary hoe whole plants.
4. Use rotary hoe above ground to chop plants, then rotary hoe conventionally.
5. Mulch and rotary hoe in 3 days later

The separation of above-ground foliage from roots was not included, but the use of the rotary hoe above ground to first chop the plant tops and then rotary hoe in the conventional way was added to test how useful a strategy this might be for a farmer who did not have a mulcher. The treatments where irrigation was not immediately after plant treatment was aimed at determining how much ITC release into soil might be expected if there was a delay in irrigating after mulching.

Treatments in 2002 at both sites included different amounts of simulated irrigation in some of the plots (notably the Fumus mulched and left on the surface, as this had shown the highest levels of ITCs flushing into the soil with added water in 2001 (see below)). In the most complete irrigation simulation (Fumus mulched and left on the surface) watering to simulate 0, 10, 20, 30 and 40 mm of irrigation was carried out. In other treatments it was 0 or 20 mm. The times of irrigation were 0, 1, 2, 3, 8, 72, 168 (7d) and 336 (14 d) hours after treating the plants.

The mulching machines used at both sites in 2002 were different from that used in 2001, having 'L'-shaped cutting blades rather than the solid club-like hammers on the rapidly-rotating horizontal axis. This proved to be a deficiency, as it did not result in the plant material being pulverised into a mush that freely oozed large volumes of liquid. Rather, the blades tended to chop the plant material into small fragments. It was a practical lesson indicated by observations at the time of treatment, and confirmed by much lower levels of ITCs detected in soil in 2002 (detailed below).

Methyl ITC in soil after metham sodium application

In order to assess the concentrations of ITCs in soil relative to the methyl ITC (MITC) concentration after a typical application of 500 L of metham sodium/ha, an area of the same type of loam soils that was treated with rotary hoe-incorporated metham sodium was sampled at different depths for a lengthy period after application.

Results and discussion

Measuring ITCs in soil

Table 1 gives the results of the first measurements of ITCs in soil shaken from the roots of growing plants. There was a correlation between the ITCs detected in soil and those in the plants, notably and not surprisingly, the roots. The results confirmed that ITCs could be detected in soil, validated the field soil sample collection and preparation technique and allowed for fine-tuning of the laboratory procedures preceding analysis on the GC.

Treating and incorporating brassicas

The first three of the following photographs are included as a record of this early attempt at pre-treating the crops rather than just rotary hoeing the whole plants into the soil, and to illustrate the development stage of the brassicas when the operation was carried out in early October 2000.

The use of the hay mower/conditioner did not prove to be very successful in breaking up the above-ground plant tissue, particularly the tall, stemmy Fumus mustard which tended to tangle somewhat or just pass through the machine in long strands. It became apparent in a practical sense that a more vigorous method was required to break the plants up. This became all the more evident and crucial with the subsequent realisation from the Morra and Kirkegaard laboratory results that a very high level of tissue disruption was the key to achieving high levels of ITC release.

The subsequent photographs show the mulching, incorporation, simulated irrigation and soil

sampling operations in 2001. On this occasion, a mulching machine used in vineyards to pulverise the inter-row vegetation was used. It was fitted with club-shaped beaters, which when operated at high speed readily pulverised both types of *Brassica* – the leafy BQMulch fodder rape and the tall, stemmy Fumus mustard.

Although it was unclear at the time, it later became apparent that such mulching machines could have several types of blades or beaters. In 2002, mulching machines were used that had 'L'-shaped knife-edged blades that turned out not to provide the same degree of tissue disruption that the fortuitous use of the club beaters had in 2001. Rather, these cutting rather than beating blades tended to chop the plant material into small fragments instead of pulverising it.

Unfortunately the realisation of this deficiency came too late to change machinery and commitments before all the 2002 plots were treated. Nevertheless, the inter-year comparison had the positive benefit that it revealed the crucial importance of the appropriate type of mulcher.

ITCs in soil and effects of irrigation

2001and 2002 plots

Table 2 gives the full data set from the soil ITC extractions in all the treatments in 2001 for the BQMulch and Fumus plots. Perusal of the data show that some treatments resulted in marked increases in the concentration of ITCs in the soil. The figures that follow show key aspects of the results.

Tables 3 and 4 give the full data sets from the soil chemistry sampling of the wide range of treatments undertaken in the loam soil at Busselton and the sand at Medina. Again, key aspects of the results are shown in subsequent figures.

The tabulated data are included as a detailed record of what was done, as there were many treatments overlaying each other making graphical presentation of all information complex and extensive.

Comparison of the results of 2002 with those from 2001 immediately shows that considerably lower levels of ITCs were measured in soil in 2002. This was attributed principally to the 'chopping' produced by the bladed mulching machine rather than true 'mulching' achieved by the clubbed mulching machine.

2000 plots

Early attempts at pre-treating foliage (Fumus in this case) using a hay mower, 2000 plots.

Mown BQMulch 2000 plots.

Rotary hoeing plants directly in (BQMulch + Fumus plot), with mown strips on right – 2000 plots

2001 plots - Busselton

Mulching and immediate rotary hoe incorporation – BQMulch 2001 plots.

Mulching Fumus mustard. Note spray, which shows extent of pulverisation, and the amount of residue in relation to the initial amount of biomass.

Mulching BQMulch. Note spray and velocity of expelled pulverised tissue.


Rotary hoe incorporation of whole BQMulch plants compared to are area at left mulched first and then rotary hoed (green lines are from wheel tracks as mulcher was offset for vineyard use).

Simulated irrigation cylinders set up on area of Fumus mulched and left on the soil surface.

Sampling soil and simulated irrigation cylinders in an area of BQMulch mulched and rotary hoed, with area mulched and left on the surface to the right centre.

Mulched and rotary hoed are of Fumus, with plants yet to be treated on the right, showing high Fumus biomass.

Mark Shackleton and Ben Warton processing soil samples.

2002 plots – i) Busselton

Mulching BQMulch. Note 'chopping' of tissue and less forceful tissue expulsion from the machine compared to 2001 (above).

Using rotary hoe to chop BQMulch foliage before passing back over to incorporate it into the soil. Mulched and left on surface plot to left; mulched and incorporated plot to left again, with cylinders for simulated irrigation.

Using rotary hoe to chop Fumus foliage before passing back over to incorporate it into the soil. Mulched and left on surface plot to left; mulched and incorporated plot to left again, with cylinders for simulated irrigation.

2002 plots – ii) Medina

Mulching Fumus.

Mulched Fumus. Note chopped nature of the foliage.

Mulching BQMulch. Note chopped nature of foliage and low velocity of expulsion.

Cylinders for simulated irrigation in different treatments. Intensity of work varies in different plots (most sampling was in Fumus mulched and left on the surface).

Table 1. 2000 plots. Average ITC concentrations (plant - µmole/g, soil - nmole/g)

			2-Propenyl			3-Butenyl			4-Pentenyl			Benzyl			2-Phenylethyl		
Variety	Sample	Date	Soil	Root	Shoot	Soil	Root	Shoot	Soil	Root	Shoot	Soil	Root	Shoot	Soil	Root	Shoot
Bqmulch B	1	17-Aug-00		0.82	2.61		5.03	2.35		2.36	1.11		0	0		23.40	0.56
	2	07-Sep-00		0	0		20.20	10.90		11.40	7.30		0	0		16.30	0
	3	27-Sep-00		0	0.31		23.20	13.70		12.10	6.91		0	0		13.20	0
	3b	04-Oct-00	0.16	0		0.47	26.10		0.23	15.20		0	0		0.90	13.30	
	4	17-Oct-00	0.08	0.27	0	0.70	32.50	11.40	0.45	16.00	5.67	0	0	0	1.63	11.40	0
	5	08-Nov-00	0	0	0	0.31	35.00	13.90	0.23	16.00	7.26	0	0	0	0.25	8.47	0
	6	30-Nov-00		0	0		38.70	23.70		15.90	10.10		0	0		6.92	0.57
	7	20-Dec-00		0	0		45.70	36.20		19.50	16.30		0	0.15		7.81	0.58
	8	10-Jan-01		0	0		29.80	40.90		14.30	19.20		0	0		7.78	0.46
	9	28-Feb-01		0	0		5.43	8.86		2.26	1.52		0	0		4.03	0
Bqmulch C	1	17-Aug-00		0.82	2.61		5.03	2.35		2.36	1.11		0	0		23.40	0.56
	2	07-Sep-00		0.48	0.45		12.3	10.30		0.71	0		0	0		29.80	0.37
	3	27-Sep-00		0.83	0.76		12.3	11.90		0.21	0		0	0		22.70	0.37
	3b	04-Oct-00	0.28	1.09		0.34	25.1		0	0.80		0	0		1.34	28.40	
	4	17-Oct-00	0	0.96	1.06	0.18	24.8	12.40	0	0.21	0	0	0	0	1.29	23.10	0
	5	08-Nov-00	0.15	0.74	0.69	0.30	10.4	5.10	0	0	0	0	0	0	0.57	9.94	0
	6	30-Nov-00		1.16	1.44		9.92	10.20		0.54	2.99		0	0		12.10	0.68
	7	20-Dec-00		1.90	1.95		22.9	16.80		2.37	2.74		0	0		16.30	0.64
	8	10-Jan-01		1.49	1.28		22.9	14.00		1.73	3.99		0	0		14.20	0.27
	9	28-Feb-01		0.60	1.30		8.42	12.00		0.54	4.78		0	0		5.54	0
Fumus	1	17-Aug-00		5.99	19.90		1.08	1.45		0.39	0.42		0	0		24.30	2.74
	2	07-Sep-00		15.50	43.50		0.27	0.76		0	0		0	0		20.10	1.35
	3	27-Sep-00		7.84	54.70		0	1.50		0	0		0	0		6.79	0.66
	3b	04-Oct-00	1.50	10.50		0	0		0	0		0	0		2.28	6.16	
	4	17-Oct-00	0.69	8.46	34.70	0	0	0	0	0	0	0	0	0	1.29	4.27	0
	5	08-Nov-00	1.01	11.00	12.80	0	0.35	0	0	0	0	0	0	0	0.73	4.85	0
	6	30-Nov-00		1.57	43.60		0	0		0	0		0	0		4.05	0
	7	20-Dec-00		0.45	22.30		0	0		0	0		0	0		2.30	0
	8	10-Jan-01		0	68.90		0	0		0	0		0	0		0	0
	9	28-Feb-01															

Table 2. Oct 2001 soil extractions from WA *Brassica* plots - loam soil Busselton WA

			Date	Water	Time (h)	Time (h)	ITC Concentrations (nmole/g) (mean of 3 samples per cylinder)										
No	Variety	Treatment	Sampled	added (L)	Wet	Sampled	iso-Propyl	2-Propenyl	sec-Butyl	3-Butenyl	4-Pentenyl	3MeThiopropyl	Benzyl	4-MeThiobutyl	2-Phenylethyl	5-MeThiopentyl	Total
1	BQ	1Mulched+RH	3-Oct			0	0.000	0.145	0.000	2.988	1.133	0.000	0.000	0.000	1.251	0.000	5.517
2	BQ	1Mulched+RH	3-Oct			2	0.000	0.157	0.000	3.769	1.512	0.000	0.000	0.000	1.175	0.000	6.613
3	BQ	1Mulched+RH	3-Oct			24	0.000	0.000	0.000	0.502	0.226	0.000	0.000	0.000	0.491	0.000	1.219
4	BQ	1Mulched+RH	3-Oct			48	0.000	0.000	0.000	0.324	0.113	0.000	0.000	0.000	0.162	0.000	0.599
5	BQ	1Mulched+RH	10-Oct			168	0.000	0.000	0.000	0.188	0.000	0.000	0.000	0.000	0.166	0.000	0.353
6	BQ	1Mulched+RH	17-Oct			336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7																	
8	BQ	2Mulched,Surface	3-Oct			0	0.000	0.000	0.000	0.883	0.209	0.000	0.000	0.000	0.339	0.000	1.432
9	BQ	2Mulched,Surface	3-Oct			2	0.000	0.000	0.000	0.492	0.092	0.000	0.000	0.000	0.276	0.000	0.859
10	BQ	2Mulched,Surface	3-Oct			24	0.000	0.000	0.000	0.198	0.069	0.000	0.000	0.000	0.240	0.000	0.506
11	BQ	2Mulched,Surface	3-Oct			48	0.000	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.119	0.000	0.241
12	BQ	2Mulched,Surface	10-Oct			168	0.000	0.000	0.000	0.098	0.000	0.000	0.000	0.000	0.238	0.000	0.336
13	BQ	2Mulched,Surface	17-Oct			336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14																	
15	BQ	3RH only whole plants	3-Oct			0	0.000	0.000	0.000	0.827	0.314	0.000	0.000	0.000	0.539	0.000	1.680
16	BQ	3RH only whole plants	3-Oct			2	0.000	0.000	0.000	0.534	0.079	0.000	0.000	0.000	0.404	0.000	1.017
17	BQ	3RH only whole plants	3-Oct			24	0.000	0.000	0.000	0.212	0.000	0.000	0.000	0.000	0.055	0.000	0.267
18	BQ	3RH only whole plants	3-Oct			48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
19	BQ	3RH only whole plants	10-Oct			168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	BQ	3RH only whole plants	17-Oct			336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21																	
22	BQ	5Mowed,Surface+RH@7d(10-Oct)	3-Oct			2	0.000	0.000	0.000	0.159	0.065	0.000	0.000	0.000	0.157	0.000	0.381
23	BQ	5Mowed,Surface+RH@7d(10-Oct)	3-Oct			24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.106	0.000	0.106
24	BQ	5Mowed,Surface+RH@7d(10-Oct)	10-Oct			168+2	0.000	0.000	0.000	0.699	0.318	0.000	0.000	0.000	0.335	0.000	1.352
25	BQ	5Mowed,Surface+RH@7d(10-Oct)	10-Oct			168+24	0.000	0.000	0.000	0.378	0.126	0.000	0.000	0.000	0.199	0.000	0.704
26	BQ	5Mowed,Surface+RH@7d(10-Oct)	17-Oct			336	0.000	0.000	0.000	0.000	0.055	0.000	0.000	0.000	0.000	0.000	0.055
27																	
28	BQ	6aMowed,Tops-moved+RH	3-Oct			0	0.000	0.000	0.000	0.664	0.180	0.000	0.000	0.000	0.000	0.000	0.843
29	BQ	6aMowed,Tops-moved+RH	3-Oct			2	0.000	0.000	0.000	0.404	0.000	0.000	0.000	0.000	0.000	0.000	0.404
30	BQ	6aMowed,Tops-moved+RH	3-Oct			24	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.083
31	BQ	6aMowed,Tops-moved+RH	10-Oct			168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
32	BQ	6aMowed,Tops-moved+RH	17-Oct			336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

33																	
34	BQ	6b	Roots only RH	3-Oct			0	0.000	0.000	0.000	0.396	0.127	0.000	0.000	0.000	0.228	0.000	0.750
35	BQ	6b	Roots only RH	3-Oct			2	0.000	0.000	0.000	0.249	0.057	0.000	0.000	0.000	0.263	0.000	0.569
36	BQ	6b	Roots only RH	3-Oct			24	0.000	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.191	0.000	0.313
37	BQ	6b	Roots only RH	10-Oct			168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
38	BQ	6b	Roots only RH	17-Oct			336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
39																		
40	BQ	7	Mowed,Tops-moved+RH@7d(10-Oct)	3-Oct			2	0.000	0.000	0.000	0.330	0.185	0.000	0.000	0.000	0.000	0.000	0.514
41	BQ	7	Mowed,Tops-moved+RH@7d(10-Oct)	3-Oct			24	0.000	0.000	0.000	0.247	0.052	0.000	0.000	0.000	0.000	0.000	0.299
42	BQ	7	Mowed,Tops-moved+RH@7d(10-Oct)	10-Oct			168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
43	BQ	7	Mowed,Tops-moved+RH@7d(10-Oct)	17-Oct			336	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
44																		
45	BQ	1+1L	Mulched+RH + 1L water	10-Oct	1	168	2	0.000	0.000	0.000	0.118	0.000	0.000	0.000	0.000	0.052	0.000	0.170
46	BQ	1+1L	Mulched+RH + 1L water	10-Oct	1	168	24	0.000	0.000	0.000	0.168	0.000	0.000	0.000	0.000	0.120	0.000	0.288
47	BQ	1+1L	Mulched+RH + 1L water	17-Oct	1	336	2	0.000	0.000	0.000	0.179	0.000	0.000	0.000	0.000	0.000	0.000	0.179
48	BQ	1+1L	Mulched+RH + 1L water	17-Oct	1	336	24	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.120
49																		
50	BQ	1+3L	Mulched+RH + 3L water	3-Oct	3	24	2	0.000	0.063	0.000	3.601	1.698	0.000	0.000	0.000	0.951	0.172	6.485
51	BQ	1+3L	Mulched+RH + 3L water	3-Oct	3	24	24	0.000	0.000	0.000	0.504	0.268	0.000	0.000	0.000	0.307	0.000	1.079
52	BQ	1+3L	Mulched+RH + 3L water	3-Oct	3	48	2	0.000	0.373	0.000	7.399	2.208	0.000	0.000	0.132	1.370	0.169	11.652
53	BQ	1+3L	Mulched+RH + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	0.413	0.094	0.000	0.000	0.000	0.151	0.000	0.657
54	BQ	1+3L	Mulched+RH + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.191	0.000	0.000	0.000	0.000	0.164	0.000	0.355
55	BQ	1+3L	Mulched+RH + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.269	0.000	0.000	0.000	0.000	0.000	0.000	0.269
56	BQ	1+3L	Mulched+RH + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.080
57																		
58	BQ	2+3L	Mulched,Surface + 3L water	3-Oct	3	0	2	0.000	0.297	0.000	10.255	5.554	0.000	0.000	0.000	3.856	0.244	20.206
59	BQ	2+3L	Mulched,Surface + 3L water	3-Oct	3	0	24	0.000	0.000	0.000	1.277	0.643	0.000	0.000	0.000	0.547	0.000	2.467
60	BQ	2+3L	Mulched,Surface + 3L water	3-Oct	3	48	2	0.000	0.000	0.000	0.119	0.000	0.000	0.000	0.000	0.323	0.000	0.442
61	BQ	2+3L	Mulched,Surface + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	0.566	0.361	0.000	0.000	0.000	0.940	0.000	1.867
62	BQ	2+3L	Mulched,Surface + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.179	0.000	0.179
63	BQ	2+3L	Mulched,Surface + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.080
64	BQ	2+3L	Mulched,Surface + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.139	0.000	0.139
65																		
66	BQ	3+1L	RH only whole plants + 1L water	10-Oct	1	168	2	0.000	0.000	0.000	0.178	0.000	0.000	0.000	0.000	0.247	0.000	0.425
67	BQ	3+1L	RH only whole plants + 1L water	10-Oct	1	168	24	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.000	0.292	0.000	0.442

68	BQ	3+1L	RH only whole plants + 1L water	17-Oct	1	336	2	0.108	0.000	0.000	0.073	0.000	0.000	0.000	0.000	0.000	0.000	0.181
69	BQ	3+1L	RH only whole plants + 1L water	17-Oct	1	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
70																		
71	BQ	3+3L	RH only whole plants + 3L water	3-Oct	3	48	2	0.000	0.000	0.000	0.258	0.304	0.000	0.000	0.000	0.409	0.000	0.970
72	BQ	3+3L	RH only whole plants + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	3.794	2.788	0.000	0.000	0.000	0.494	0.000	7.077
73	BQ	3+3L	RH only whole plants + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.479	0.092	0.000	0.000	0.000	1.002	0.000	1.573
74	BQ	3+3L	RH only whole plants + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.235	0.000	0.000	0.000	0.000	0.000	0.000	0.235
75	BQ	3+3L	RH only whole plants + 3L water	17-Oct	3	336	24	0.091	0.000	0.000	0.139	0.000	0.000	0.000	0.000	0.000	0.000	0.231
76																		
77	BQ	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	3-Oct	3	0	2	0.000	0.098	0.000	1.167	0.526	0.000	0.000	0.000	0.000	0.000	1.791
78	BQ	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	3-Oct	3	0	24	0.055	0.000	0.000	0.378	0.192	0.000	0.000	0.000	0.000	0.000	0.626
79	BQ	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	10-Oct	3	168	2	0.000	0.217	0.000	0.674	0.342	0.000	0.000	0.000	1.317	0.000	2.550
80	BQ	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	10-Oct	3	168	24	0.056	0.000	0.000	0.449	0.200	0.000	0.000	0.000	0.293	0.000	0.998
81	BQ	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.205	0.000	0.000	0.000	0.000	0.408	0.000	0.613
82	BQ	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
83																		
84	BQ	6a+3L	Mowed,Tops-moved+RH + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	0.514	0.000	0.000	0.000	0.000	0.000	0.000	0.514
85	BQ	6a+3L	Mowed,Tops-moved+RH + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.210	0.000	0.000	0.000	0.000	0.000	0.000	0.210
86	BQ	6a+3L	Mowed,Tops-moved+RH + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
87	BQ	6a+3L	Mowed,Tops-moved+RH + 3L water	17-Oct	3	336	24	0.270	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333
88																		
89	BQ	6b+3L	Roots only RH + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.132	0.000	0.132
90	BQ	6b+3L	Roots only RH + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.045
91	BQ	6b+3L	Roots only RH + 3L water	17-Oct	3	336	2	0.000	0.247	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.247
92	BQ	6b+3L	Roots only RH + 3L water	17-Oct	3	336	24	0.209	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.292
93																		
94	BQ	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	0.549	0.277	0.000	0.000	0.000	0.000	0.000	0.826
95	BQ	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	10-Oct	3	168	24	0.065	0.000	0.000	0.525	0.278	0.000	0.000	0.000	0.000	0.000	0.867
96	BQ	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	17-Oct	3	336	2	0.066	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.113
97	BQ	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	17-Oct	3	336	24	0.092	0.000	0.000	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.157
98																		
99	Fumus	1	Mulched+RH	3-Oct			0	0.000	7.347	0.000	0.782	0.000	0.000	0.000	0.000	2.443	0.000	10.572
100	Fumus	1	Mulched+RH	3-Oct			2	0.000	7.826	0.000	0.724	0.000	0.000	0.000	0.000	2.146	0.000	10.697
101	Fumus	1	Mulched+RH	3-Oct			24	0.000	2.702	0.000	0.208	0.000	0.000	0.000	0.000	0.904	0.000	3.814
102	Fumus	1	Mulched+RH	3-Oct			48	0.000	0.721	0.000	0.000	0.000	0.000	0.000	0.000	0.427	0.000	1.148

103	Fumus	1	Mulched+RH	10-Oct	168	0.000	0.294	0.000	0.000	0.000	0.000	0.000	0.000	0.339	0.000	0.633
104	Fumus	1	Mulched+RH	17-Oct	336	0.000	0.339	0.000	0.000	0.000	0.000	0.000	0.000	0.249	0.000	0.588
105																
106	Fumus	2	Mulched,Surface	3-Oct	0	0.000	1.492	0.000	0.161	0.000	0.000	0.000	0.000	0.967	0.000	2.620
107	Fumus	2	Mulched,Surface	3-Oct	2	0.000	0.393	0.000	0.000	0.000	0.000	0.000	0.000	0.406	0.000	0.799
108	Fumus	2	Mulched,Surface	3-Oct	24	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.329	0.000	0.529
109	Fumus	2	Mulched,Surface	3-Oct	48	0.000	0.199	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.448
110	Fumus	2	Mulched,Surface	10-Oct	168	0.000	0.193	0.000	0.000	0.000	0.000	0.000	0.000	0.236	0.000	0.429
111	Fumus	2	Mulched,Surface	17-Oct	336	0.000	0.113	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.000	0.179
112																
113	Fumus	3	RH only whole plants	3-Oct	0	0.000	1.872	0.000	0.132	0.000	0.000	0.000	0.000	1.226	0.000	3.230
114	Fumus	3	RH only whole plants	3-Oct	2	0.000	0.769	0.000	0.000	0.000	0.000	0.000	0.000	0.628	0.000	1.397
115	Fumus	3	RH only whole plants	3-Oct	24	0.000	0.385	0.000	0.000	0.000	0.000	0.000	0.000	0.338	0.000	0.723
116	Fumus	3	RH only whole plants	3-Oct	48	0.000	0.425	0.000	0.000	0.000	0.000	0.000	0.000	0.268	0.000	0.694
117	Fumus	3	RH only whole plants	10-Oct	168	0.000	0.135	0.000	0.000	0.000	0.000	0.000	0.000	0.123	0.000	0.258
118	Fumus	3	RH only whole plants	17-Oct	336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.167
119																
120	Fumus	5	Mowed,Surface+RH@7d(10-Oct)	3-Oct	2	0.000	0.312	0.000	0.000	0.000	0.000	0.000	0.000	0.526	0.000	0.837
121	Fumus	5	Mowed,Surface+RH@7d(10-Oct)	3-Oct	24	0.000	0.152	0.000	0.000	0.000	0.000	0.000	0.000	0.324	0.000	0.476
122	Fumus	5	Mowed,Surface+RH@7d(10-Oct)	10-Oct	168+2	0.000	2.292	0.000	0.000	0.000	0.000	0.000	0.000	0.798	0.000	3.090
123	Fumus	5	Mowed,Surface+RH@7d(10-Oct)	10-Oct	168+24	0.000	0.985	0.000	0.000	0.000	0.000	0.000	0.000	0.398	0.000	1.383
124	Fumus	5	Mowed,Surface+RH@7d(10-Oct)	17-Oct	336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
125																
126	Fumus	6a	Mowed,Tops-moved+RH	3-Oct	0	0.000	1.422	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	1.621
127	Fumus	6a	Mowed,Tops-moved+RH	3-Oct	2	0.000	1.215	0.000	0.000	0.000	0.000	0.000	0.000	0.190	0.000	1.405
128	Fumus	6a	Mowed,Tops-moved+RH	3-Oct	24	0.000	0.339	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.339
129	Fumus	6a	Mowed,Tops-moved+RH	10-Oct	168	0.000	0.116	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.116
130	Fumus	6a	Mowed,Tops-moved+RH	17-Oct	336	0.000	0.134	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.239	0.373
131																
132	Fumus	6b	Roots only RH	3-Oct	0	0.000	0.476	0.000	0.000	0.000	0.000	0.000	0.000	1.018	0.000	1.494
133	Fumus	6b	Roots only RH	3-Oct	2	0.000	0.674	0.000	0.000	0.000	0.000	0.000	0.000	0.513	0.000	1.187
134	Fumus	6b	Roots only RH	3-Oct	24	0.000	0.284	0.000	0.000	0.000	0.000	0.000	0.000	0.345	0.000	0.630
135	Fumus	6b	Roots only RH	10-Oct	168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.041	0.000	0.041
136	Fumus	6b	Roots only RH	17-Oct	336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
137																

138	Fumus	7	Mowed,Tops-moved+RH@7d(10-Oct)	3-Oct			2	0.000	1.607	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.607
139	Fumus	7	Mowed,Tops-moved+RH@7d(10-Oct)	3-Oct			24	0.000	0.666	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.666
140	Fumus	7	Mowed,Tops-moved+RH@7d(10-Oct)	10-Oct			168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
141	Fumus	7	Mowed,Tops-moved+RH@7d(10-Oct)	17-Oct			336	0.000	0.062	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.062
142																		
143	Fumus	1+1L	Mulched+RH + 1L water	10-Oct	1	168	2	0.000	0.767	0.000	0.000	0.000	0.000	0.061	0.000	0.262	0.000	1.090
144	Fumus	1+1L	Mulched+RH + 1L water	10-Oct	1	168	24	0.000	0.367	0.000	0.000	0.000	0.000	0.000	0.000	1.475	0.000	1.843
145	Fumus	1+1L	Mulched+RH + 1L water	17-Oct	1	336	2	0.000	0.596	0.000	0.000	0.000	0.000	0.000	0.000	0.134	0.000	0.730
146	Fumus	1+1L	Mulched+RH + 1L water	17-Oct	1	336	24	0.000	0.370	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.370
147																		
148	Fumus	1+3L	Mulched+RH + 3L water	3-Oct	3	24	2	0.000	7.837	0.000	0.936	0.000	0.000	0.000	0.000	6.687	0.000	15.461
149	Fumus	1+3L	Mulched+RH + 3L water	3-Oct	3	24	24	0.000	1.660	0.000	0.052	0.000	0.000	0.000	0.000	0.968	0.000	2.680
150	Fumus	1+3L	Mulched+RH + 3L water	3-Oct	3	48	2	0.000	3.381	0.000	0.855	0.000	0.000	0.000	0.000	2.598	0.000	6.834
151	Fumus	1+3L	Mulched+RH + 3L water	10-Oct	3	168	2	0.000	0.900	0.000	0.000	0.000	0.000	0.000	0.000	1.200	0.000	2.100
152	Fumus	1+3L	Mulched+RH + 3L water	10-Oct	3	168	24	0.000	0.325	0.000	0.000	0.000	0.000	0.000	0.000	0.176	0.000	0.500
153	Fumus	1+3L	Mulched+RH + 3L water	17-Oct	3	336	2	0.104	0.770	0.000	0.053	0.000	0.000	0.000	0.000	0.351	0.000	1.278
154	Fumus	1+3L	Mulched+RH + 3L water	17-Oct	3	336	24	0.000	0.452	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.452
155																		
156	Fumus	2+3L	Mulched,Surface + 3L water	3-Oct	3	0	2	0.000	68.450	0.000	3.037	0.000	0.000	0.000	0.000	27.199	0.000	98.685
157	Fumus	2+3L	Mulched,Surface + 3L water	3-Oct	3	0	24	0.000	7.576	0.000	0.502	0.000	0.000	0.000	0.000	3.565	0.000	11.643
158	Fumus	2+3L	Mulched,Surface + 3L water	3-Oct	3	48	2	0.000	1.083	0.000	0.140	0.000	0.000	0.000	0.000	2.757	0.000	3.980
159	Fumus	2+3L	Mulched,Surface + 3L water	10-Oct	3	168	2	0.000	0.386	0.000	0.000	0.000	0.000	0.000	0.000	0.388	0.000	0.774
160	Fumus	2+3L	Mulched,Surface + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.108	0.000	0.108
161	Fumus	2+3L	Mulched,Surface + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.126	0.000	0.126
162	Fumus	2+3L	Mulched,Surface + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
163																		
164	Fumus	3+1L	RH only whole plants + 1L water	10-Oct	1	168	2	0.000	0.383	0.000	0.000	0.083	0.000	0.000	0.000	0.225	0.000	0.691
165	Fumus	3+1L	RH only whole plants + 1L water	10-Oct	1	168	24	0.000	0.155	0.000	0.000	0.000	0.000	0.000	0.000	0.120	0.000	0.275
166	Fumus	3+1L	RH only whole plants + 1L water	17-Oct	1	336	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.063
167	Fumus	3+1L	RH only whole plants + 1L water	17-Oct	1	336	24	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.095
168																		
169	Fumus	3+3L	RH only whole plants + 3L water	3-Oct	3	48	2	0.000	9.580	0.000	4.758	0.000	0.000	0.000	0.000	3.639	0.000	17.977
170	Fumus	3+3L	RH only whole plants + 3L water	10-Oct	3	168	2	0.000	1.724	0.000	0.096	0.000	0.000	0.000	0.000	0.631	0.000	2.452
171	Fumus	3+3L	RH only whole plants + 3L water	10-Oct	3	168	24	0.088	0.180	0.000	0.000	0.000	0.000	0.000	0.000	0.229	0.000	0.498
172	Fumus	3+3L	RH only whole plants + 3L water	17-Oct	3	336	2	0.000	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060

173	Fumus	3+3L	RH only whole plants + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
174																		
175	Fumus	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	3-Oct	3	0	2	0.000	7.631	0.000	0.145	0.000	0.000	0.000	0.000	0.374	0.000	8.150
176	Fumus	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	3-Oct	3	0	24	0.125	0.766	0.000	0.116	0.000	0.000	0.000	0.000	0.156	0.000	1.164
177	Fumus	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	10-Oct	3	168	2	0.000	5.778	0.000	0.000	0.000	0.000	0.000	0.000	2.288	0.000	8.066
178	Fumus	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	10-Oct	3	168	24	0.059	0.460	0.000	0.000	0.000	0.000	0.000	0.000	0.501	0.000	1.019
179	Fumus	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	17-Oct	3	336	2	0.000	0.053	0.000	0.566	0.204	0.000	0.000	0.000	0.466	0.000	1.289
180	Fumus	5+3L	Mowed,Surface+RH@7d(10-Oct) + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
181																		
182	Fumus	6a+3L	Mowed,Tops-moved+RH + 3L water	10-Oct	3	168	2	0.000	0.138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138
183	Fumus	6a+3L	Mowed,Tops-moved+RH + 3L water	10-Oct	3	168	24	0.000	0.261	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.261
184	Fumus	6a+3L	Mowed,Tops-moved+RH + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
185	Fumus	6a+3L	Mowed,Tops-moved+RH + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
186																		
187	Fumus	6b+3L	Roots only RH + 3L water	10-Oct	3	168	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.609	0.000	0.609
188	Fumus	6b+3L	Roots only RH + 3L water	10-Oct	3	168	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.173	0.000	0.173
189	Fumus	6b+3L	Roots only RH + 3L water	17-Oct	3	336	2	0.181	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.181
190	Fumus	6b+3L	Roots only RH + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
191																		
192	Fumus	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	10-Oct	3	168	2	0.085	2.418	0.000	0.067	0.000	0.000	0.000	0.000	0.266	0.000	2.836
193	Fumus	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	10-Oct	3	168	24	0.000	0.892	0.000	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.950
194	Fumus	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	17-Oct	3	336	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
195	Fumus	7+3L	Mowed,Tops-moved+RH@7d(10-Oct) + 3L water	17-Oct	3	336	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 3. Oct 2002 soil extractions from WA *Brassica* plots – sand soil Medina WA

				Average concentrations (3 reps) (nmole/g) (only for main ITCs detected)							
Variety/Sample ID	Day	hr	mm	iso-Propyl	2-Propenyl	3-Butenyl	4-Pentenyl	4-MeThiobutyl	2-Phenylethyl	5-MeThiopentyl	Total
Blank_D3_0h_0mm	3	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Blank_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BM_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.000	0.139	0.025	0.165
BM_D1_2h_0mm	1	2	0	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.059
BM_D1_2h_20mm	1	2	20	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.087
BM_D1_24h_0mm	1	24	0	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.073
BM_D1_24h_20mm	1	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BM_D3_0h_0mm	3	0	0	0.000	0.281	0.000	0.064	0.000	0.177	0.024	0.546
BM_D3_2h_0mm	3	2	0	0.000	0.564	0.000	0.000	0.033	0.257	0.000	0.854
BM_D3_2h_20mm	3	2	20	0.000	0.170	0.197	0.082	0.000	0.252	0.000	0.700
BM_D3_24h_0mm	3	24	0	0.000	0.000	0.000	0.047	0.017	0.150	0.000	0.215
BM_D3_24h_20mm	3	24	20	0.000	0.000	0.000	0.044	0.059	0.197	0.000	0.300
BM_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.034
BM_D8_2h_0mm	8	2	0	0.000	0.000	0.000	0.000	0.000	0.049	0.000	0.049
BM_D8_2h_20mm	8	2	20	0.000	0.000	0.082	0.000	0.000	0.150	0.000	0.231
BM_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.026	0.140	0.017	0.183
BM_D8_24h_20mm	8	24	20	0.000	0.000	0.099	0.000	0.070	0.248	0.000	0.417
BM_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.180	0.091	0.000	0.081	0.000	0.352
BM_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.058	0.063	0.041	0.092	0.022	0.275
BMR_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.104	0.227	0.000	0.331
BMR_D1_2h_0mm	1	2	0	0.000	0.000	0.726	0.378	0.097	0.670	0.000	1.871
BMR_D1_2h_20mm	1	2	20	0.000	0.000	0.634	0.436	0.121	0.714	0.000	1.905
BMR_D1_24h_0mm	1	24	0	0.000	0.000	0.245	0.000	0.036	0.278	0.000	0.558
BMR_D1_24h_20mm	1	24	20	0.000	0.000	0.181	0.000	0.034	0.192	0.000	0.408
BMR_D3_0h_0mm	3	0	0	0.000	0.000	0.257	0.000	0.028	0.253	0.000	0.538
BMR_D3_2h_0mm	3	2	0	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.056
BMR_D3_2h_20mm	3	2	20	0.000	0.000	0.605	0.284	0.135	0.461	0.000	1.486

BMR_D3_24h_0mm	3	24	0	0.000	0.000	0.131	0.000	0.000	0.093	0.000	0.225
BMR_D3_24h_20mm	3	24	20	0.000	0.000	0.046	0.000	0.055	0.129	0.000	0.229
BMR_D8_0h_0mm	8	0	0	0.000	0.000	0.102	0.000	0.000	0.067	0.000	0.169
BMR_D8_2h_0mm	8	2	0	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.036
BMR_D8_2h_20mm	8	2	20	0.000	0.000	0.227	0.000	0.029	0.212	0.000	0.469
BMR_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BMR_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.035	0.051	0.000	0.086
BMR_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.189	0.056	0.000	0.086	0.000	0.331
BMR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.048	0.000	0.000	0.035	0.000	0.084
BMR3_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BMR3_D1_2h_0mm	1	2	0	0.000	0.000	0.832	0.597	0.102	0.568	0.028	2.127
BMR3_D1_2h_20mm	1	2	20	0.000	0.124	0.882	0.591	0.112	0.462	0.000	2.171
BMR3_D1_24h_0mm	1	24	0	0.000	0.000	0.515	0.255	0.058	0.218	0.051	1.096
BMR3_D1_24h_20mm	1	24	20	0.000	0.000	0.368	0.203	0.025	0.205	0.000	0.801
BMR3_D3_0h_0mm	3	0	0	0.000	0.000	0.306	0.138	0.035	0.328	0.000	0.807
BMR3_D3_2h_0mm	3	2	0	0.000	0.000	0.049	0.000	0.000	0.156	0.000	0.205
BMR3_D3_2h_20mm	3	2	20	0.000	0.000	0.272	0.062	0.021	0.253	0.000	0.608
BMR3_D3_24h_0mm	3	24	0	0.000	0.000	0.182	0.000	0.029	0.196	0.000	0.407
BMR3_D3_24h_20mm	3	24	20	0.000	0.000	0.207	0.041	0.049	0.172	0.000	0.470
BMR3_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.035
BMR3_D8_2h_0mm	8	2	0	0.000	0.000	0.072	0.000	0.000	0.119	0.000	0.191
BMR3_D8_2h_20mm	8	2	20	0.000	0.000	0.141	0.080	0.024	0.177	0.000	0.422
BMR3_D8_24h_0mm	8	24	0	0.000	0.000	0.226	0.044	0.046	0.227	0.000	0.543
BMR3_D8_24h_20mm	8	24	20	0.000	0.000	0.071	0.044	0.000	0.036	0.000	0.152
BMR3_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.047	0.000	0.000	0.034	0.000	0.081
BMR3_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.118	0.084	0.000	0.106	0.019	0.327
BRR_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.056	0.033	0.280	0.000	0.368
BRR_D1_2h_0mm	1	2	0	0.000	0.000	0.850	0.547	0.088	0.512	0.000	1.996
BRR_D1_2h_20mm	1	2	20	0.000	0.000	1.170	0.717	0.128	0.686	0.086	2.787
BRR_D1_24h_0mm	1	24	0	0.000	0.000	0.263	0.088	0.059	0.222	0.000	0.633
BRR_D1_24h_20mm	1	24	20	0.000	0.000	0.496	0.254	0.034	0.099	0.027	0.910

BRR_D3_0h_0mm	3	0	0	0.000	0.000	0.440	0.279	0.000	0.211	0.000	0.930
BRR_D3_2h_0mm	3	2	0	0.000	0.000	0.295	0.191	0.102	0.232	0.000	0.820
BRR_D3_2h_20mm	3	2	20	0.000	0.000	0.331	0.000	0.068	0.173	0.000	0.572
BRR_D3_24h_0mm	3	24	0	0.000	0.000	0.172	0.099	0.000	0.032	0.000	0.304
BRR_D3_24h_20mm	3	24	20	0.000	0.000	0.167	0.055	0.000	0.161	0.000	0.383
BRR_D8_0h_0mm	8	0	0	0.000	0.000	0.142	0.000	0.000	0.095	0.000	0.237
BRR_D8_2h_0mm	8	2	0	0.000	0.000	0.127	0.000	0.000	0.000	0.000	0.127
BRR_D8_2h_20mm	8	2	20	0.000	0.000	0.154	0.046	0.000	0.041	0.000	0.242
BRR_D8_24h_0mm	8	24	0	0.000	0.000	0.073	0.000	0.000	0.039	0.000	0.111
BRR_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BRR_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.117	0.000	0.000	0.000	0.000	0.117
BRR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.119	0.000	0.000	0.000	0.000	0.119
FM_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_1h_0mm	1	1	0	0.000	0.084	0.000	0.000	0.000	0.000	0.000	0.084
FM_D1_3h_0mm	1	3	0	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.089
FM_D1_8h_0mm	1	8	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_24h_0mm	1	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_336h_0mm	1	336	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_1h_10mm	1	1	10	0.000	2.547	0.000	0.000	0.000	0.084	0.000	2.632
FM_D1_3h_10mm	1	3	10	0.000	0.155	0.000	0.000	0.000	0.000	0.000	0.155
FM_D1_8h_10mm	1	8	10	0.000	0.146	0.000	0.000	0.000	0.000	0.000	0.146
FM_D1_24h_10mm	1	24	10	0.000	0.119	0.000	0.000	0.000	0.053	0.000	0.172
FM_D1_72h_10mm	1	72	10	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.072
FM_D1_168h_10mm	1	168	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_336h_10mm	1	336	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_1h_20mm	1	1	20	0.000	0.601	0.000	0.000	0.000	0.252	0.000	0.853
FM_D1_3h_20mm	1	3	20	0.000	0.844	0.000	0.000	0.000	0.236	0.000	1.080
FM_D1_8h_20mm	1	8	20	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.091
FM_D1_24h_20mm	1	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_72h_20mm	1	72	20	0.000	0.069	0.000	0.000	0.000	0.061	0.000	0.130
FM_D1_168h_20mm	1	168	20	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.032
FM_D1_336h_20mm	1	336	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

FM_D1_1h_30mm	1	1	30	0.000	0.305	0.000	0.000	0.000	0.000	0.000	0.305
FM_D1_3h_30mm	1	3	30	0.000	0.393	0.000	0.000	0.000	0.000	0.000	0.393
FM_D1_8h_30mm	1	8	30	0.000	0.386	0.000	0.000	0.000	0.077	0.000	0.464
FM_D1_24h_30mm	1	24	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_72h_30mm	1	72	30	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.089
FM_D1_168h_30mm	1	168	30	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.034
FM_D1_336h_30mm	1	336	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1_1h_40mm	1	1	40	0.000	1.016	0.000	0.000	0.000	0.056	0.000	1.072
FM_D1_3h_40mm	1	3	40	0.000	0.526	0.000	0.000	0.000	0.130	0.000	0.656
FM_D1_8h_40mm	1	8	40	0.000	0.604	0.000	0.000	0.000	0.086	0.000	0.690
FM_D1_24h_40mm	1	24	40	0.000	0.165	0.000	0.000	0.000	0.074	0.000	0.239
FM_D1_72h_40mm	1	72	40	0.000	0.075	0.000	0.000	0.000	0.181	0.000	0.256
FM_D1_168h_40mm	1	168	40	0.000	0.000	0.000	0.000	0.000	0.096	0.000	0.096
FM_D1_336h_40mm	1	336	40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_0h_0mm	3	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_1h_0mm	3	1	0	0.000	0.000	0.000	0.000	0.000	0.149	0.000	0.149
FM_D3_3h_0mm	3	3	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_8h_0mm	3	8	0	0.000	0.000	0.000	0.000	0.000	0.123	0.000	0.123
FM_D3_24h_0mm	3	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_72h_0mm	3	72	0	0.000	0.000	0.000	0.000	0.000	0.101	0.000	0.101
FM_D3_168h_0mm	3	168	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_336h_0mm	3	336	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_1h_10mm	3	1	10	0.000	0.310	0.000	0.000	0.000	0.055	0.000	0.364
FM_D3_3h_10mm	3	3	10	0.000	0.245	0.000	0.000	0.000	0.045	0.000	0.291
FM_D3_8h_10mm	3	8	10	0.000	0.299	0.000	0.000	0.000	0.163	0.000	0.462
FM_D3_24h_10mm	3	24	10	0.000	0.084	0.000	0.000	0.000	0.155	0.000	0.239
FM_D3_72h_10mm	3	72	10	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.086
FM_D3_168h_10mm	3	168	10	0.000	0.000	0.000	0.000	0.000	0.139	0.000	0.139
FM_D3_336h_10mm	3	336	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_1h_20mm	3	1	20	0.000	0.379	0.000	0.000	0.000	0.000	0.000	0.379
FM_D3_3h_20mm	3	3	20	0.000	0.180	0.000	0.000	0.000	0.264	0.000	0.444
FM_D3_8h_20mm	3	8	20	0.000	0.156	0.000	0.000	0.000	0.202	0.000	0.358
FM_D3_24h_20mm	3	24	20	0.000	0.067	0.000	0.000	0.000	0.072	0.000	0.139

FM_D3_72h_20mm	3	72	20	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.111
FM_D3_168h_20mm	3	168	20	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.077
FM_D3_336h_20mm	3	336	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_1h_30mm	3	1	30	0.000	0.687	0.000	0.000	0.000	0.129	0.000	0.816
FM_D3_3h_30mm	3	3	30	0.000	0.328	0.000	0.000	0.000	0.118	0.000	0.446
FM_D3_8h_30mm	3	8	30	0.000	0.089	0.000	0.000	0.000	0.203	0.000	0.293
FM_D3_24h_30mm	3	24	30	0.000	0.064	0.000	0.000	0.000	0.049	0.000	0.112
FM_D3_72h_30mm	3	72	30	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.067
FM_D3_168h_30mm	3	168	30	0.000	0.000	0.000	0.000	0.000	0.088	0.000	0.088
FM_D3_336h_30mm	3	336	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D3_1h_40mm	3	1	40	0.000	0.521	0.000	0.000	0.000	0.096	0.000	0.617
FM_D3_3h_40mm	3	3	40	0.000	0.469	0.000	0.000	0.000	0.000	0.000	0.469
FM_D3_8h_40mm	3	8	40	0.000	0.115	0.000	0.000	0.000	0.155	0.000	0.270
FM_D3_24h_40mm	3	24	40	0.000	0.205	0.000	0.000	0.000	0.263	0.000	0.468
FM_D3_72h_40mm	3	72	40	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.083
FM_D3_168h_40mm	3	168	40	0.000	0.000	0.000	0.000	0.000	0.094	0.000	0.094
FM_D3_336h_40mm	3	336	40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.155	0.000	0.155
FM_D8_1h_0mm	8	1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_3h_0mm	8	3	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_8h_0mm	8	8	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_72h_0mm	8	72	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_168h_0mm	8	168	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_336h_0mm	8	336	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_1h_10mm	8	1	10	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.063
FM_D8_3h_10mm	8	3	10	0.000	0.149	0.000	0.000	0.000	0.056	0.000	0.204
FM_D8_8h_10mm	8	8	10	0.000	0.089	0.000	0.000	0.000	0.096	0.000	0.185
FM_D8_24h_10mm	8	24	10	0.000	0.084	0.000	0.000	0.000	0.193	0.000	0.277
FM_D8_72h_10mm	8	72	10	0.000	0.085	0.000	0.000	0.000	0.154	0.000	0.240
FM_D8_168h_10mm	8	168	10	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.063
FM_D8_336h_10mm	8	336	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_1h_20mm	8	1	20	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.069

FM_D8_3h_20mm	8	3	20	0.000	0.205	0.000	0.000	0.000	0.117	0.000	0.322
FM_D8_8h_20mm	8	8	20	0.000	0.056	0.000	0.000	0.000	0.109	0.000	0.165
FM_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_72h_20mm	8	72	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_168h_20mm	8	168	20	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.043
FM_D8_336h_20mm	8	336	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_1h_30mm	8	1	30	0.000	0.307	0.000	0.000	0.000	0.154	0.000	0.461
FM_D8_3h_30mm	8	3	30	0.000	0.362	0.000	0.000	0.000	0.149	0.000	0.511
FM_D8_8h_30mm	8	8	30	0.000	0.174	0.000	0.000	0.000	0.125	0.000	0.298
FM_D8_24h_30mm	8	24	30	0.000	0.000	0.000	0.000	0.000	0.153	0.000	0.153
FM_D8_72h_30mm	8	72	30	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.034
FM_D8_168h_30mm	8	168	30	0.000	0.000	0.000	0.000	0.000	0.061	0.000	0.061
FM_D8_336h_30mm	8	336	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_1h_40mm	8	1	40	0.000	0.293	0.000	0.000	0.000	0.078	0.000	0.370
FM_D8_3h_40mm	8	3	40	0.000	0.113	0.000	0.000	0.000	0.082	0.000	0.196
FM_D8_8h_40mm	8	8	40	0.000	0.117	0.000	0.000	0.000	0.111	0.000	0.228
FM_D8_24h_40mm	8	24	40	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.033
FM_D8_72h_40mm	8	72	40	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.025
FM_D8_168h_40mm	8	168	40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D8_336h_40mm	8	336	40	0.000	0.097	0.000	0.000	0.000	0.000	0.000	0.097
FM_D1+D8_1h_10mm	1+8	1	10	0.000	0.062	0.000	0.000	0.000	0.074	0.000	0.136
FM_D1+D8_3h_10mm	1+8	3	10	0.000	0.156	0.000	0.000	0.000	0.094	0.000	0.251
FM_D1+D8_8h_10mm	1+8	8	10	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.086
FM_D1+D8_24h_10mm	1+8	24	10	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.083
FM_D1+D8_72h_10mm	1+8	72	10	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.037
FM_D1+D8_168h_10mm	1+8	168	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1+D8_336h_10mm	1+8	336	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1+D8_1h_20mm	1+8	1	20	0.000	0.060	0.000	0.000	0.000	0.122	0.000	0.182
FM_D1+D8_3h_20mm	1+8	3	20	0.000	0.128	0.000	0.000	0.000	0.000	0.000	0.128
FM_D1+D8_8h_20mm	1+8	8	20	0.000	0.000	0.000	0.000	0.000	0.088	0.000	0.088
FM_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.000	0.000	0.000	0.096	0.000	0.096
FM_D1+D8_72h_20mm	1+8	72	20	0.000	0.000	0.000	0.000	0.000	0.051	0.000	0.051
FM_D1+D8_168h_20mm	1+8	168	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

FM_D1+D8_336h_20mm	1+8	336	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1+D8_1h_30mm	1+8	1	30	0.000	0.143	0.000	0.000	0.000	0.073	0.000	0.216
FM_D1+D8_3h_30mm	1+8	3	30	0.000	0.151	0.000	0.000	0.000	0.134	0.000	0.286
FM_D1+D8_8h_30mm	1+8	8	30	0.000	0.000	0.000	0.000	0.000	0.082	0.000	0.082
FM_D1+D8_24h_30mm	1+8	24	30	0.000	0.000	0.000	0.000	0.000	0.096	0.000	0.096
FM_D1+D8_72h_30mm	1+8	72	30	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.035
FM_D1+D8_168h_30mm	1+8	168	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1+D8_336h_30mm	1+8	336	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1+D8_1h_40mm	1+8	1	40	0.000	0.180	0.000	0.000	0.000	0.043	0.000	0.223
FM_D1+D8_3h_40mm	1+8	3	40	0.000	0.256	0.000	0.000	0.000	0.046	0.000	0.302
FM_D1+D8_8h_40mm	1+8	8	40	0.000	0.089	0.000	0.000	0.000	0.000	0.000	0.089
FM_D1+D8_24h_40mm	1+8	24	40	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.100
FM_D1+D8_72h_40mm	1+8	72	40	0.000	0.000	0.000	0.000	0.000	0.079	0.000	0.079
FM_D1+D8_168h_40mm	1+8	168	40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FM_D1+D8_336h_40mm	1+8	336	40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR_D1_2h_0mm	1	2	0	0.000	1.929	0.000	0.000	0.000	0.405	0.000	2.334
FMR_D1_2h_20mm	1	2	20	0.000	2.282	0.000	0.000	0.000	0.558	0.000	2.840
FMR_D1_24h_0mm	1	24	0	0.000	0.324	0.000	0.000	0.000	0.218	0.000	0.541
FMR_D1_24h_20mm	1	24	20	0.000	0.875	0.000	0.000	0.000	0.156	0.000	1.031
FMR_D3_0h_0mm	3	0	0	0.000	0.000	0.000	0.000	0.000	0.170	0.000	0.170
FMR_D3_2h_0mm	3	2	0	0.000	0.331	0.000	0.000	0.000	0.252	0.000	0.582
FMR_D3_2h_20mm	3	2	20	0.000	0.353	0.000	0.000	0.000	0.207	0.000	0.561
FMR_D3_24h_0mm	3	24	0	0.000	0.000	0.000	0.000	0.000	0.120	0.000	0.120
FMR_D3_24h_20mm	3	24	20	0.000	0.090	0.000	0.000	0.000	0.233	0.000	0.323
FMR_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.067
FMR_D8_2h_0mm	8	2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR_D8_2h_20mm	8	2	20	0.000	0.064	0.000	0.000	0.000	0.051	0.000	0.115
FMR_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.031

FMR3_D1_0h_0mm	1	0	0	0.000	0.097	0.000	0.000	0.000	0.062	0.000	0.159
FMR3_D1_2h_0mm	1	2	0	0.000	1.480	0.000	0.000	0.000	0.282	0.000	1.762
FMR3_D1_2h_20mm	1	2	20	0.000	2.659	0.000	0.000	0.000	0.444	0.000	3.103
FMR3_D1_24h_0mm	1	24	0	0.000	0.370	0.160	0.000	0.000	0.177	0.000	0.707
FMR3_D1_24h_20mm	1	24	20	0.000	0.491	0.000	0.000	0.000	0.217	0.000	0.708
FMR3_D3_0h_0mm	3	0	0	0.000	0.050	0.043	0.000	0.000	0.085	0.000	0.178
FMR3_D3_2h_0mm	3	2	0	0.000	0.000	0.000	0.000	0.000	0.103	0.000	0.103
FMR3_D3_2h_20mm	3	2	20	0.000	0.137	0.000	0.000	0.000	0.205	0.000	0.341
FMR3_D3_24h_0mm	3	24	0	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.077
FMR3_D3_24h_20mm	3	24	20	0.000	0.055	0.000	0.000	0.000	0.114	0.000	0.169
FMR3_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR3_D8_2h_0mm	8	2	0	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.033
FMR3_D8_2h_20mm	8	2	20	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.052
FMR3_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR3_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR3_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FMR3_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FRR_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.057
FRR_D1_2h_0mm	1	2	0	0.000	1.617	0.000	0.000	0.000	0.418	0.000	2.035
FRR_D1_2h_20mm	1	2	20	0.000	1.885	0.000	0.000	0.000	0.381	0.000	2.266
FRR_D1_24h_0mm	1	24	0	0.000	0.557	0.000	0.000	0.000	0.175	0.000	0.732
FRR_D1_24h_20mm	1	24	20	0.000	0.754	0.000	0.000	0.000	0.076	0.000	0.830
FRR_D3_0h_0mm	3	0	0	0.000	0.343	0.000	0.000	0.000	0.125	0.000	0.468
FRR_D3_2h_0mm	3	2	0	0.000	0.652	0.000	0.000	0.000	0.210	0.000	0.863
FRR_D3_2h_20mm	3	2	20	0.000	1.070	0.000	0.000	0.000	0.382	0.000	1.452
FRR_D3_24h_0mm	3	24	0	0.000	0.259	0.000	0.000	0.000	0.113	0.000	0.372
FRR_D3_24h_20mm	3	24	20	0.000	0.301	0.000	0.000	0.000	0.147	0.000	0.447
FRR_D8_0h_0mm	8	0	0	0.000	0.141	0.000	0.000	0.000	0.000	0.000	0.141
FRR_D8_2h_0mm	8	2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FRR_D8_2h_20mm	8	2	20	0.000	0.223	0.000	0.000	0.000	0.000	0.000	0.223
FRR_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

FRR_D8_24h_20mm	8	24	20	0.000	0.076	0.000	0.000	0.000	0.000	0.000	0.076
FRR_D1+D8_2h_20mm	1+8	2	20	0.000	0.086	0.000	0.000	0.000	0.000	0.000	0.086
FRR_D1+D8_24h_20mm	1+8	24	20	0.000	0.123	0.000	0.000	0.000	0.024	0.000	0.147

Legend:

Brassica plot treatments and sample legend for Busselton and Medina - October 2002				
		Day water	Hours after	Water
Variety	Treatment	added	Treatment (h)	added (mm)
(B): Bqmulch	(M) : Mulched only	(D1)	0	0
(F): Fumus	(MR) : Mulched & Rotary hoed in	(D3)	1	10
	(MR3) : Mulched & Rotary hoed in 3 days later	(D8)	2	20
	(RR) : Rotary hoed above ground then Rotary hoed in	(D1 + D8)	3	30
			8	40
			72	
			168	
			336	

Table 3. Oct 2002 soil extractions from WA *Brassica* plots – loam soil Busselton WA

				Average concentrations (3 reps) (nmole/g) (only for main ITCs detected)							
Variety/Sample ID	Day	hr	mm	iso-Propyl	2-Propenyl	3-Butenyl	4-Pentenyl	4-MeThiobutyl	2-Phenylethyl	5-MeThiopentyl	Total
Blank_D1_0h_0mm	1	0	0	0.121	0.000	0.000	0.000	0.000	0.000	0.000	0.121
Blank_D3_0h_0mm	3	0	0	0.000	0.000	0.216	0.149	0.000	0.172	0.000	0.537
Blank_D8_0h_0mm	8	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BM_D1_0h_0mm	1	0	0	0.000	0.000	0.000	0.000	0.000	0.112	0.000	0.112
BM_D1_0-1h_0mm	1	0-1	0	0.000	0.000	0.226	0.097	0.000	0.428	0.000	0.752
BM_D1_2h_0mm	1	2	0	0.198	0.000	0.748	0.283	0.000	0.363	0.174	1.766
BM_D1_2h_20mm	1	2	20	0.000	0.000	0.622	0.283	0.000	0.369	0.000	1.275
BM_D1_24h_0mm	1	24	0	0.000	0.000	0.278	0.113	0.000	0.293	0.000	0.685
BM_D1_24h_20mm	1	24	20	0.000	0.000	0.175	0.131	0.000	0.167	0.000	0.473
BM_D3_0h_0mm	3	0	0	0.000	0.000	0.238	0.177	0.000	0.479	0.000	0.894
BM_D3_2h_0mm	3	2	0	0.000	0.000	0.316	0.195	0.000	0.451	0.000	0.962
BM_D3_2h_20mm	3	2	20	0.000	0.000	0.400	0.258	0.000	0.529	0.000	1.187
BM_D3_24h_0mm	3	24	0	0.000	0.000	0.498	0.162	0.000	0.896	0.026	1.582
BM_D3_24h_20mm	3	24	20	0.000	0.000	0.213	0.099	0.000	0.248	0.000	0.561
BM_D8_0h_0mm	8	0	0	0.000	0.000	0.158	0.100	0.000	0.302	0.000	0.560
BM_D8_2h_0mm	8	2	0	0.000	0.000	0.280	0.141	0.000	0.272	0.000	0.693
BM_D8_2h_20mm	8	2	20	0.000	0.000	0.272	0.175	0.000	0.297	0.000	0.744
BM_D8_24h_0mm	8	24	0	0.000	0.000	0.442	0.245	0.000	0.605	0.000	1.292
BM_D8_24h_20mm	8	24	20	0.000	0.000	0.238	0.185	0.000	0.237	0.000	0.661
BM_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.141	0.051	0.000	0.191	0.000	0.384
BM_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.049	0.000	0.000	0.123	0.000	0.172
BMR_D1_0h_0mm	1	0	0	0.000	0.000	0.041	0.058	0.000	0.219	0.000	0.319
BMR_D1_0-1h_0mm	1	0-1	0	0.000	0.188	2.329	0.693	0.000	0.698	0.000	3.908
BMR_D1_2h_0mm	1	2	0	0.000	0.000	1.855	0.726	0.000	0.810	0.000	3.391
BMR_D1_2h_20mm	1	2	20	0.092	0.123	2.423	0.931	0.044	1.174	0.079	4.866
BMR_D1_24h_0mm	1	24	0	0.000	0.000	0.751	0.257	0.000	0.377	0.000	1.385
BMR_D1_24h_20mm	1	24	20	0.000	0.000	1.157	0.495	0.000	0.582	0.000	2.233

BMR_D3_0h_0mm	3	0	0	0.000	0.000	0.900	0.296	0.000	0.377	0.000	1.574
BMR_D3_2h_0mm	3	2	0	0.000	0.000	0.968	0.433	0.000	0.498	0.000	1.900
BMR_D3_2h_20mm	3	2	20	0.000	0.000	1.114	0.522	0.000	0.697	0.000	2.332
BMR_D3_24h_0mm	3	24	0	0.066	0.000	0.590	0.326	0.000	0.248	0.024	1.255
BMR_D3_24h_20mm	3	24	20	0.000	0.000	1.005	0.325	0.000	0.654	0.000	1.984
BMR_D8_0h_0mm	8	0	0	0.059	0.000	0.759	0.361	0.000	0.235	0.016	1.430
BMR_D8_2h_0mm	8	2	0	0.000	0.000	0.095	0.000	0.000	0.111	0.000	0.206
BMR_D8_2h_20mm	8	2	20	0.000	0.000	0.823	0.336	0.000	0.289	0.000	1.449
BMR_D8_24h_0mm	8	24	0	0.000	0.000	0.275	0.078	0.000	0.138	0.000	0.491
BMR_D8_24h_20mm	8	24	20	0.000	0.000	0.333	0.141	0.000	0.116	0.000	0.590
BMR_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.543	0.070	0.000	0.294	0.000	0.908
BMR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.364	0.124	0.000	0.302	0.000	0.791
BMR3_D1_0h_0mm	1	0	0	0.000	0.000	0.218	0.104	0.000	0.441	0.000	0.763
BMR3_D1_0-1h_0mm	1	0-1	0	0.000	0.000	0.536	0.272	0.000	0.551	0.000	1.360
BMR3_D1_2h_0mm	1	2	0	0.102	0.113	1.174	0.565	0.000	0.674	0.154	2.781
BMR3_D1_2h_20mm	1	2	20	0.000	0.124	2.155	0.941	0.000	0.831	0.000	4.051
BMR3_D1_24h_0mm	1	24	0	0.000	0.000	0.877	0.574	0.000	0.668	0.000	2.119
BMR3_D1_24h_20mm	1	24	20	0.000	0.000	0.538	0.228	0.000	0.281	0.000	1.048
BRR_D1_0h_0mm	1	0	0	0.000	0.000	0.154	0.138	0.000	0.329	0.000	0.622
BRR_D1_0-1h_0mm	1	0-1	0	0.052	0.103	2.044	0.784	0.000	0.716	0.000	3.699
BRR_D1_2h_0mm	1	2	0	0.000	0.000	1.943	0.788	0.000	0.716	0.000	3.447
BRR_D1_2h_20mm	1	2	20	0.000	0.000	1.176	0.452	0.000	0.571	0.000	2.200
BRR_D1_24h_0mm	1	24	0	0.066	0.000	1.082	0.470	0.000	0.527	0.024	2.168
BRR_D1_24h_20mm	1	24	20	0.000	0.000	0.785	0.301	0.000	0.357	0.000	1.443
BRR_D3_0h_0mm	3	0	0	0.000	0.000	0.507	0.314	0.000	0.414	0.000	1.235
BRR_D3_2h_0mm	3	2	0	0.000	0.068	1.420	0.426	0.000	0.440	0.000	2.355
BRR_D3_2h_20mm	3	2	20	0.000	0.000	1.338	0.657	0.000	0.391	0.000	2.385
BRR_D3_24h_0mm	3	24	0	0.000	0.000	0.605	0.232	0.000	0.170	0.000	1.008
BRR_D3_24h_20mm	3	24	20	0.000	0.000	0.744	0.337	0.000	0.246	0.000	1.327
BRR_D8_0h_0mm	8	0	0	0.073	0.042	1.124	0.562	0.000	0.288	0.024	2.113
BRR_D8_2h_0mm	8	2	0	0.000	0.000	0.951	0.269	0.000	0.178	0.000	1.399

BRR_D8_2h_20mm	8	2	20	0.000	0.000	0.624	0.208	0.000	0.164	0.192	1.189
BRR_D8_24h_0mm	8	24	0	0.000	0.000	0.366	0.127	0.000	0.146	0.000	0.639
BRR_D8_24h_20mm	8	24	20	0.000	0.000	0.895	0.395	0.000	0.282	0.000	1.572
BRR_D1+D8_2h_20mm	1+8	2	20	0.000	0.000	0.463	0.174	0.000	0.248	0.000	0.884
BRR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	1.289	0.215	0.000	0.147	0.000	1.651
FM_D1_0h_0mm	1	0	0	0.000	0.226	0.000	0.000	0.000	0.226	0.000	0.452
FM_D1_1h_0mm	1	1	0	0.000	0.348	0.000	0.000	0.000	0.267	0.000	0.615
FM_D1_3h_0mm	1	3	0	0.000	0.640	0.000	0.000	0.000	0.386	0.000	1.025
FM_D1_24h_0mm	1	24	0	0.000	0.197	0.000	0.000	0.000	0.274	0.000	0.471
FM_D1_0-1h_0mm	1	0-1	0	0.000	0.384	0.000	0.000	0.000	0.275	0.000	0.660
FM_D1_1h_10mm	1	1	10	0.000	0.387	0.000	0.000	0.037	0.275	0.000	0.699
FM_D1_3h_10mm	1	3	10	0.000	0.420	0.000	0.000	0.000	0.243	0.000	0.663
FM_D1_24h_10mm	1	24	10	0.000	0.331	0.000	0.000	0.000	0.344	0.000	0.675
FM_D1_72h_10mm	1	72	10	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.080
FM_D1_168h_10mm	1	168	10	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.091
FM_D1_1h_20mm	1	1	20	0.000	0.226	0.000	0.000	0.000	0.165	0.000	0.392
FM_D1_3h_20mm	1	3	20	0.000	0.301	0.000	0.000	0.000	0.159	0.000	0.460
FM_D1_24h_20mm	1	24	20	0.000	0.210	0.000	0.000	0.000	0.214	0.000	0.424
FM_D1_72h_20mm	1	72	20	0.000	0.038	0.000	0.000	0.000	0.077	0.000	0.115
FM_D1_168h_20mm	1	168	20	0.000	0.000	0.000	0.000	0.000	0.119	0.000	0.119
FM_D1_1h_30mm	1	1	30	0.050	0.722	0.000	0.000	0.000	0.302	0.027	1.100
FM_D1_3h_30mm	1	3	30	0.000	0.589	0.000	0.000	0.000	0.340	0.000	0.928
FM_D1_24h_30mm	1	24	30	0.000	0.170	0.000	0.000	0.000	0.228	0.000	0.398
FM_D1_72h_30mm	1	72	30	0.000	0.000	0.000	0.000	0.000	0.157	0.000	0.157
FM_D1_168h_30mm	1	168	30	0.000	0.000	0.000	0.000	0.000	0.131	0.000	0.131
FM_D1_1h_40mm	1	1	40	0.000	1.915	0.092	0.000	0.000	2.588	0.000	4.595
FM_D1_3h_40mm	1	3	40	0.000	0.540	0.000	0.000	0.000	0.318	0.000	0.858
FM_D1_24h_40mm	1	24	40	0.000	0.321	0.000	0.000	0.000	0.572	0.000	0.894
FM_D1_72h_40mm	1	72	40	0.000	0.171	0.000	0.000	0.000	0.418	0.000	0.589
FM_D1_168h_40mm	1	168	40	0.000	0.000	0.000	0.000	0.000	0.149	0.000	0.149
FM_D3_0h_0mm	3	0	0	0.000	0.302	0.000	0.000	0.000	0.632	0.000	0.934
FM_D3_1h_0mm	3	1	0	0.000	0.207	0.000	0.000	0.000	0.251	0.000	0.458

FM_D3_3h_0mm	3	3	0	0.000	0.098	0.000	0.000	0.000	0.280	0.000	0.378
FM_D3_24h_0mm	3	24	0	0.000	0.000	0.094	0.049	0.000	0.241	0.000	0.383
FM_D3_144h_0mm	3	144	0	0.000	0.000	0.000	0.000	0.000	0.088	0.000	0.088
FM_D3_1h_10mm	3	1	10	0.000	0.163	0.000	0.000	0.000	0.326	0.000	0.489
FM_D3_3h_10mm	3	3	10	0.000	0.214	0.000	0.000	0.000	0.454	0.000	0.668
FM_D3_24h_10mm	3	24	10	0.000	0.000	0.000	0.000	0.000	0.160	0.000	0.160
FM_D3_144h_10mm	3	144	10	0.000	0.000	0.000	0.000	0.000	0.384	0.000	0.384
FM_D3_1h_20mm	3	1	20	0.092	0.224	0.000	0.000	0.000	0.431	0.059	0.805
FM_D3_3h_20mm	3	3	20	0.000	0.297	0.067	0.000	0.000	0.297	0.000	0.660
FM_D3_24h_20mm	3	24	20	0.000	0.000	0.000	0.000	0.000	0.326	0.000	0.326
FM_D3_144h_20mm	3	144	20	0.000	0.000	0.000	0.000	0.000	0.438	0.000	0.438
FM_D3_1h_30mm	3	1	30	0.000	0.505	0.000	0.000	0.000	0.549	0.000	1.053
FM_D3_3h_30mm	3	3	30	0.000	0.379	0.000	0.000	0.000	0.324	0.000	0.703
FM_D3_24h_30mm	3	24	30	0.000	0.090	0.000	0.000	0.000	0.461	0.000	0.550
FM_D3_144h_30mm	3	144	30	0.000	0.000	0.000	0.000	0.000	0.253	0.000	0.253
FM_D3_1h_40mm	3	1	40	0.000	0.428	0.000	0.000	0.000	0.506	0.000	0.935
FM_D3_3h_40mm	3	3	40	0.000	0.498	0.000	0.000	0.000	0.444	0.000	0.942
FM_D3_24h_40mm	3	24	40	0.000	0.071	0.000	0.000	0.000	0.327	0.000	0.398
FM_D3_144h_40mm	3	144	40	0.000	0.000	0.000	0.000	0.000	0.238	0.000	0.238
FM_D8_0h_0mm	8	0	0	0.000	0.457	0.000	0.000	0.000	0.194	0.000	0.651
FM_D8_1h_0mm	8	1	0	0.000	0.056	0.000	0.000	0.000	0.257	0.000	0.313
FM_D8_3h_0mm	8	3	0	0.000	0.000	0.000	0.000	0.000	0.191	0.000	0.191
FM_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.080
FM_D8_1h_10mm	8	1	10	0.000	0.055	0.000	0.000	0.000	0.327	0.000	0.382
FM_D8_3h_10mm	8	3	10	0.000	0.000	0.000	0.000	0.000	0.199	0.000	0.199
FM_D8_24h_10mm	8	24	10	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.071
FM_D8_1h_20mm	8	1	20	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.200
FM_D8_3h_20mm	8	3	20	0.000	0.295	0.000	0.000	0.000	0.311	0.000	0.606
FM_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.000	0.124	0.000	0.124
FM_D8_1h_30mm	8	1	30	0.000	0.000	0.000	0.000	0.000	0.426	0.000	0.426
FM_D8_3h_30mm	8	3	30	0.000	0.087	0.000	0.000	0.000	0.112	0.000	0.199
FM_D8_24h_30mm	8	24	30	0.042	0.000	0.000	0.000	0.000	0.161	0.024	0.227
FM_D8_1h_40mm	8	1	40	0.000	0.187	0.000	0.000	0.000	0.388	0.000	0.575

FM_D8_3h_40mm	8	3	40	0.000	0.080	0.000	0.000	0.000	0.200	0.000	0.280
FM_D8_24h_40mm	8	24	40	0.000	0.000	0.000	0.000	0.000	0.162	0.000	0.162
FM_D1+D8_1h_10mm	1+8	1	10	0.000	0.000	0.000	0.000	0.000	0.480	0.000	0.480
FM_D1+D8_3h_10mm	1+8	3	10	0.000	0.000	0.000	0.000	0.000	0.173	0.000	0.173
FM_D1+D8_24h_10mm	1+8	24	10	0.000	0.000	0.000	0.000	0.000	0.061	0.000	0.061
FM_D1+D8_1h_20mm	1+8	1	20	0.000	0.000	0.000	0.000	0.000	0.249	0.000	0.249
FM_D1+D8_3h_20mm	1+8	3	20	0.000	0.000	0.000	0.000	0.000	0.216	0.000	0.216
FM_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.000	0.000	0.000	0.124	0.000	0.124
FM_D1+D8_1h_30mm	1+8	1	30	0.000	0.081	0.000	0.000	0.000	0.402	0.000	0.483
FM_D1+D8_3h_30mm	1+8	3	30	0.000	0.156	0.000	0.000	0.000	0.197	0.000	0.353
FM_D1+D8_24h_30mm	1+8	24	30	0.000	0.000	0.000	0.000	0.000	0.132	0.000	0.132
FM_D1+D8_1h_40mm	1+8	1	40	0.000	0.168	0.000	0.000	0.000	0.695	0.000	0.863
FM_D1+D8_3h_40mm	1+8	3	40	0.000	0.081	0.000	0.000	0.000	0.224	0.000	0.305
FM_D1+D8_24h_40mm	1+8	24	40	0.000	0.000	0.000	0.000	0.000	0.068	0.000	0.068
FMR_D1_0h_0mm	1	0	0	0.049	0.201	0.000	0.000	0.000	0.153	0.000	0.402
FMR_D1_0-1h_0mm	1	0-1	0	0.000	1.576	0.000	0.000	0.024	0.548	0.000	2.148
FMR_D1_2h_0mm	1	2	0	0.000	1.704	0.123	0.000	0.061	0.533	0.000	2.421
FMR_D1_2h_20mm	1	2	20	0.000	1.513	0.151	0.052	0.084	0.653	0.000	2.452
FMR_D1_24h_0mm	1	24	0	0.194	1.741	0.096	0.000	0.031	0.401	0.148	2.611
FMR_D1_24h_20mm	1	24	20	0.000	0.703	0.000	0.000	0.019	0.408	0.000	1.130
FMR_D3_0h_0mm	3	0	0	0.000	0.785	0.000	0.000	0.027	0.401	0.000	1.213
FMR_D3_2h_0mm	3	2	0	0.000	1.001	0.055	0.000	0.000	0.400	0.000	1.456
FMR_D3_2h_20mm	3	2	20	0.000	2.489	0.123	0.000	0.101	2.256	0.000	4.969
FMR_D3_24h_0mm	3	24	0	0.000	0.255	0.000	0.000	0.000	0.226	0.000	0.481
FMR_D3_24h_20mm	3	24	20	0.000	0.378	0.000	0.000	0.000	0.273	0.000	0.651
FMR_D8_0h_0mm	8	0	0	0.000	0.070	0.000	0.000	0.000	0.222	0.000	0.292
FMR_D8_2h_0mm	8	2	0	0.000	0.075	0.000	0.000	0.000	0.088	0.000	0.163
FMR_D8_2h_20mm	8	2	20	0.000	0.000	0.000	0.000	0.000	0.074	0.000	0.074
FMR_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.033
FMR_D8_24h_20mm	8	24	20	0.000	0.072	0.000	0.000	0.000	0.068	0.000	0.139
FMR_D1+D8_2h_20mm	1+8	2	20	0.051	0.051	0.000	0.000	0.000	0.090	0.000	0.192
FMR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.000	0.000	0.000	0.095	0.000	0.095

FMR3_D1_0h_0mm	1	0	0	0.000	0.410	0.000	0.000	0.000	0.639	0.000	1.048
FMR3_D1_0-1h_0mm	1	0-1	0	0.000	2.268	0.000	0.000	0.000	0.725	0.000	2.993
FMR3_D1_2h_0mm	1	2	0	0.000	1.919	0.000	0.000	0.000	0.617	0.000	2.536
FMR3_D1_2h_20mm	1	2	20	0.000	2.896	0.060	0.000	0.000	0.631	0.000	3.587
FMR3_D1_24h_0mm	1	24	0	0.000	0.468	0.000	0.000	0.000	0.244	0.000	0.712
FMR3_D1_24h_20mm	1	24	20	0.000	0.376	0.000	0.000	0.000	0.428	0.000	0.803
FRR_D1_0h_0mm	1	0	0	0.063	0.570	0.000	0.000	0.000	0.346	0.000	0.979
FRR_D1_0-1h_0mm	1	0-1	0	0.119	2.960	0.264	0.078	0.000	0.789	0.120	4.330
FRR_D1_2h_0mm	1	2	0	0.000	2.328	0.117	0.000	0.000	0.484	0.000	2.929
FRR_D1_2h_20mm	1	2	20	0.000	2.532	0.317	0.075	0.000	0.949	0.000	3.873
FRR_D1_24h_0mm	1	24	0	0.325	2.883	0.295	0.080	0.000	0.534	0.251	4.368
FRR_D1_24h_20mm	1	24	20	0.000	1.224	0.751	0.071	0.000	0.669	0.000	2.714
FRR_D3_0h_0mm	3	0	0	0.000	0.454	0.000	0.000	0.000	0.436	0.000	0.890
FRR_D3_2h_0mm	3	2	0	0.000	1.001	0.078	0.000	0.000	0.334	0.000	1.413
FRR_D3_2h_20mm	3	2	20	0.000	1.032	0.099	0.041	0.000	0.399	0.000	1.571
FRR_D3_24h_0mm	3	24	0	0.053	0.759	0.078	0.043	0.000	0.194	0.000	1.128
FRR_D3_24h_20mm	3	24	20	0.000	0.978	0.119	0.057	0.000	0.353	0.000	1.507
FRR_D8_0h_0mm	8	0	0	0.000	0.128	0.000	0.000	0.000	0.071	0.000	0.198
FRR_D8_2h_0mm	8	2	0	0.000	0.130	0.000	0.000	0.000	0.138	0.000	0.268
FRR_D8_2h_20mm	8	2	20	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.031
FRR_D8_24h_0mm	8	24	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FRR_D8_24h_20mm	8	24	20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FRR_D1+D8_2h_20mm	1+8	2	20	0.077	0.000	0.000	0.000	0.000	0.039	0.035	0.151
FRR_D1+D8_24h_20mm	1+8	24	20	0.000	0.000	0.000	0.000	0.000	0.103	0.000	0.103

Figure 1 shows the total ITC concentration in soil for the BQMulch and Fumus plots that were rotary hoed into the soil as whole plants, mulched and left on the surface, and mulched and immediately rotary hoed into the soil. It is immediately apparent that mulching the tops of the plants before rotary hoe incorporation substantially increased the concentration of ITCs in the soil, particularly for the mustard. The increased release of ITCs was also associated with a prolonging of their presence in the soil.

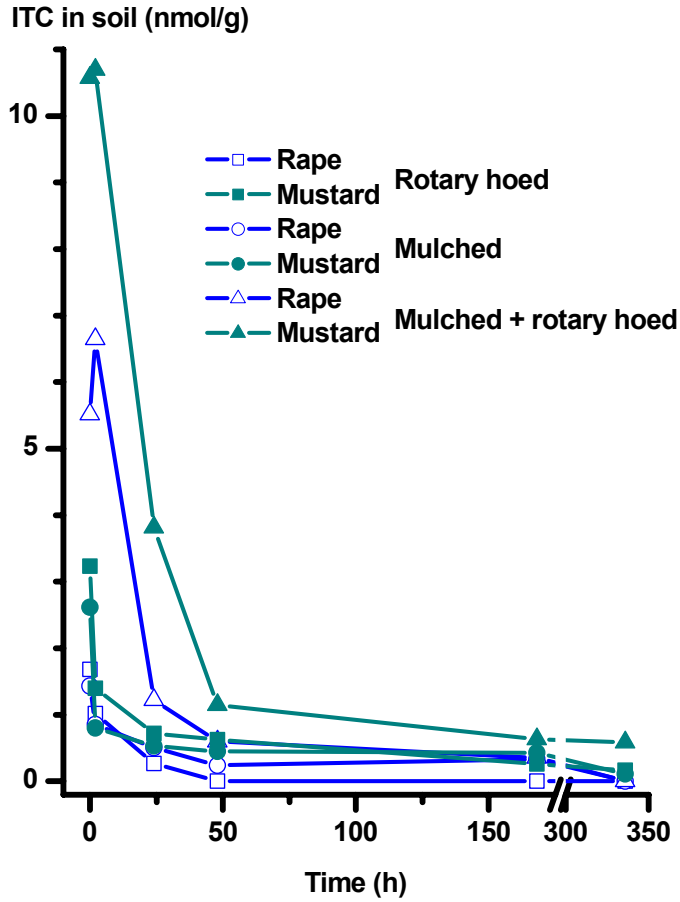

Figure 1. ITC concentration in soil - 2001 plots. Treatments without irrigation. Note y-axis scale in relation to that of following figures.

Figure 2 shows the concentration of total ITCs in soil after whole plants were rotary hoed into the soil and there was heavy watering (3 L/cylinder, or 42 mm equivalent) after two or seven days. In the case of the BQMulch fodder rape it appeared that the watering after seven days caused a reactivation of ITC release, which declined within the following 24 hours. The watering caused a near doubling of the ITC concentration in soil (compare Figs 1 and 2; note y-axis scale difference).

In contrast, the ITC release was greater in Fumus mustard when watering was carried out two days after incorporation, suggesting a lesser capacity for longer-term reactivation of ITC release by watering for the mustard than for the fodder rape.

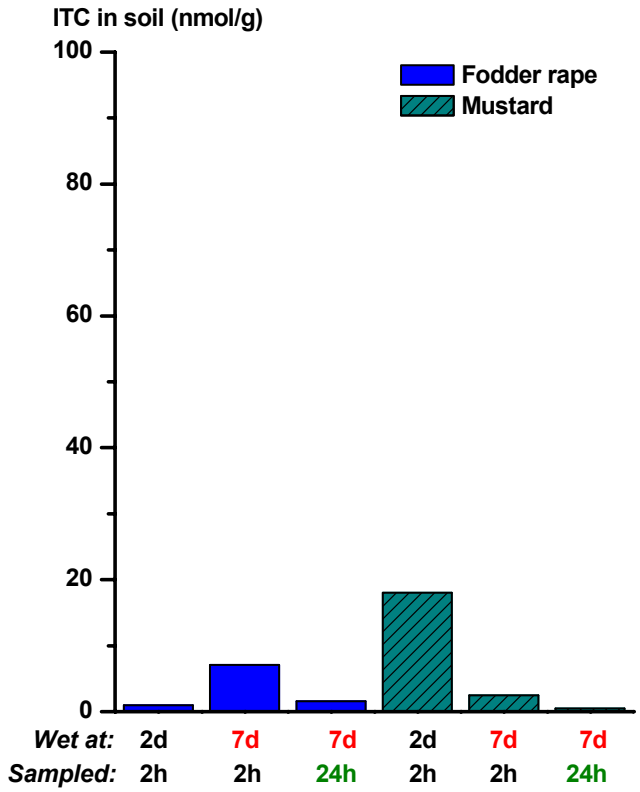

Figure 2. Concentration of ITCs in soil for BQMulch fodder rape and Fumus mustard when whole plants were rotary hoed into the soil and 3 L water was added at the times after incorporation shown.

Figure 3 shows the soil total ITC concentration when the brassicas were mulched and rotary hoed into the soil before irrigating at different intervals. When compared to the results shown in Fig. 2, it appears that reactivation of ITC release is more general when the plants are pulverised, rather than the chopping effect achieved with the rotary hoe alone, although the concentration of total ITCs was not increased.

While watering increased the release of ITCs into soil from plant material rotary hoed into the soil, a very much larger effect occurred when the mulched plant material was left on the surface of the ground and watered heavily, particularly for the mustard (Fig. 4). While addition of 1 L water did not produce a similar effect, the result with 3 L water illustrated and reinforced the second principle revealed by Morra and Kirkegaard that excess water is a key ingredient in maximising the release of ITCs, and clearly also their transport into the soil.

Figure 5 shows the concentration of MITC in the soil following metham sodium application. Comparison with Fig. 4 shows that levels of total ITC achieved from heavily watering mulched mustard were well within the range of the MITC concentration achieved from the synthetic ITC precursor metham sodium.

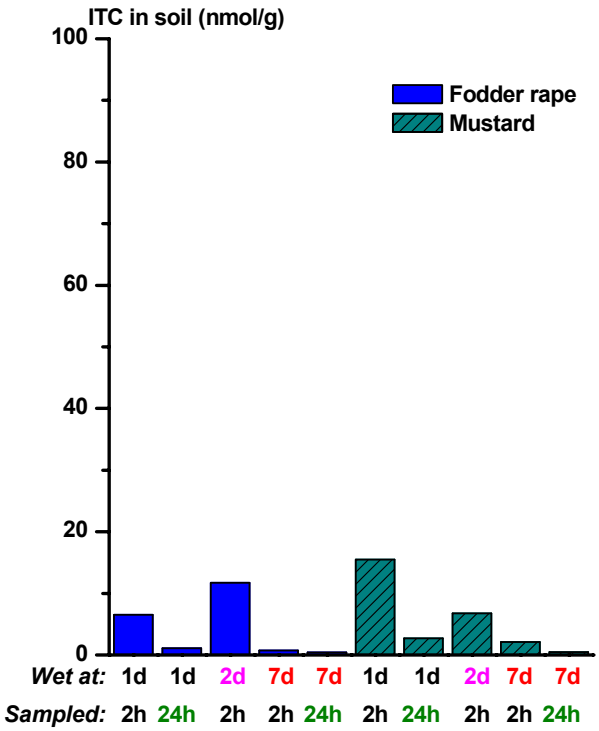

Figure 3. Total ITCs in soil when plant were mulched and immediately rotary hoed in, before watering with 3 L (42 mm) water at the specified times.

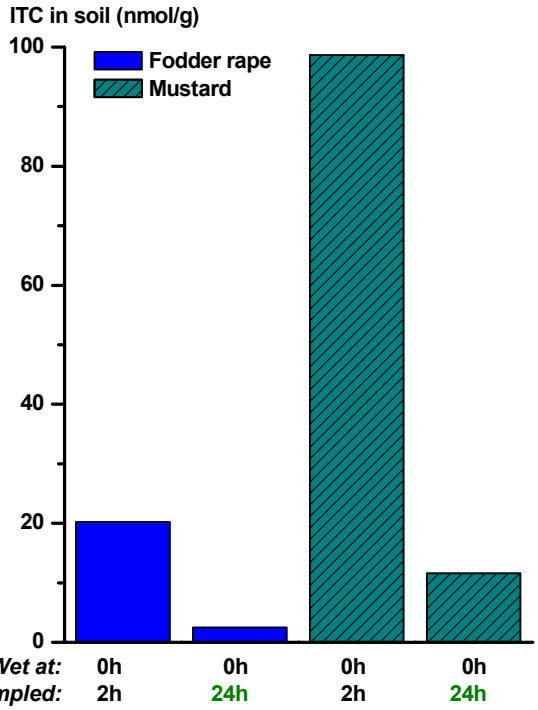

Figure 4. Concentration of total ITCs in soil when the plants were mulched and left on the surface of the ground and watered heavily (3 L – 42 mm) immediately or after 24h.

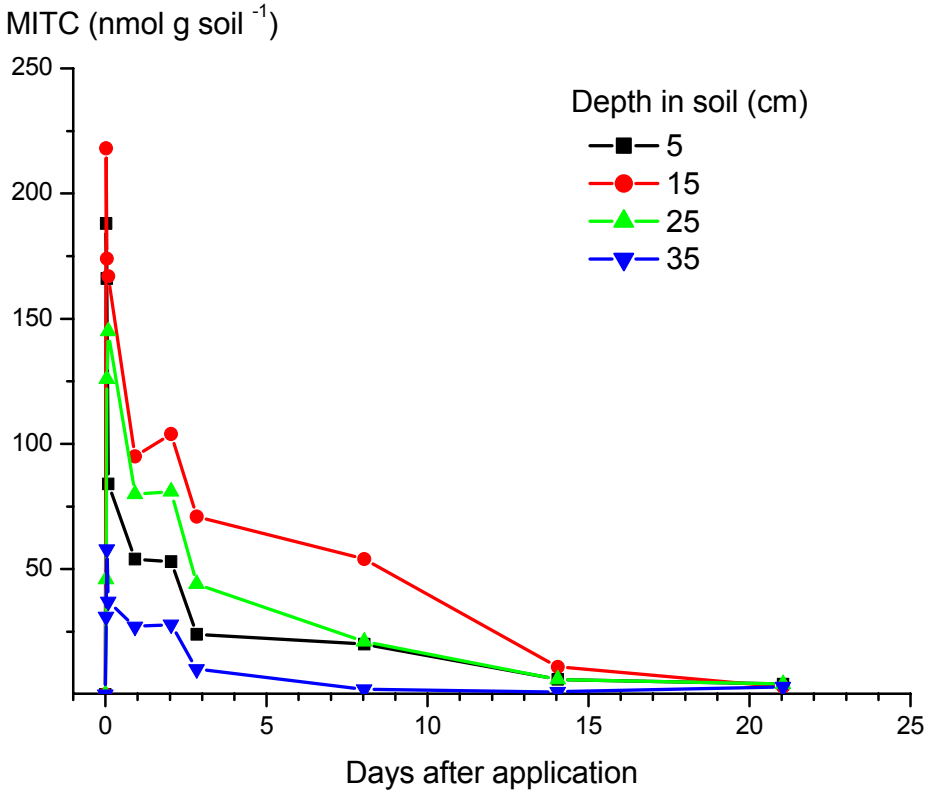

Figure 5. Concentration of MITC in loam soil after application of 500 L/ha of metham sodium by boom spray with immediate rotary hoe incorporation.

Figure 6 shows the total ITCs in the sandy soil at Medina after applying 20 mm water at different intervals after plant treatment (see bottom of Table 2 above for treatment legend). The top part of the figure shows the ITC concentration 2 h after watering, while the bottom section shows it 24 h after watering.

In summary, it is clear that only relatively low concentrations of ITCs were detected in all treatments. This is believed to be primarily the result of the poor tissue pulverisation in 2002, probably compounded by the lesser amount of water applied (20 mm compared to 42 mm in 2001). Comparison within and between the two sections of Fig. 6 shows that ITCs could be detected primarily within the first 24 h after adding water, and mostly for soil wet soon after treating the plants, but there were lingering small amounts of ITCs detected over a week later.

Figure 7 shows the total ITCs measured in the sand soil at Medina in the soil adjacent to the simulated irrigation cylinder enclosures, where the plant treatment was the same, but no water was added. Comparison within and between Figs. 6 and 7 indicate that the 20 mm watering did not greatly enhance ITC concentration, but it did tend to reactivate release of ITCs. When the 2002 results are compared with those of 2001, the lack of enhancement of release seems likely to be related to the poor plant pulverisation and the lesser amount of water added.

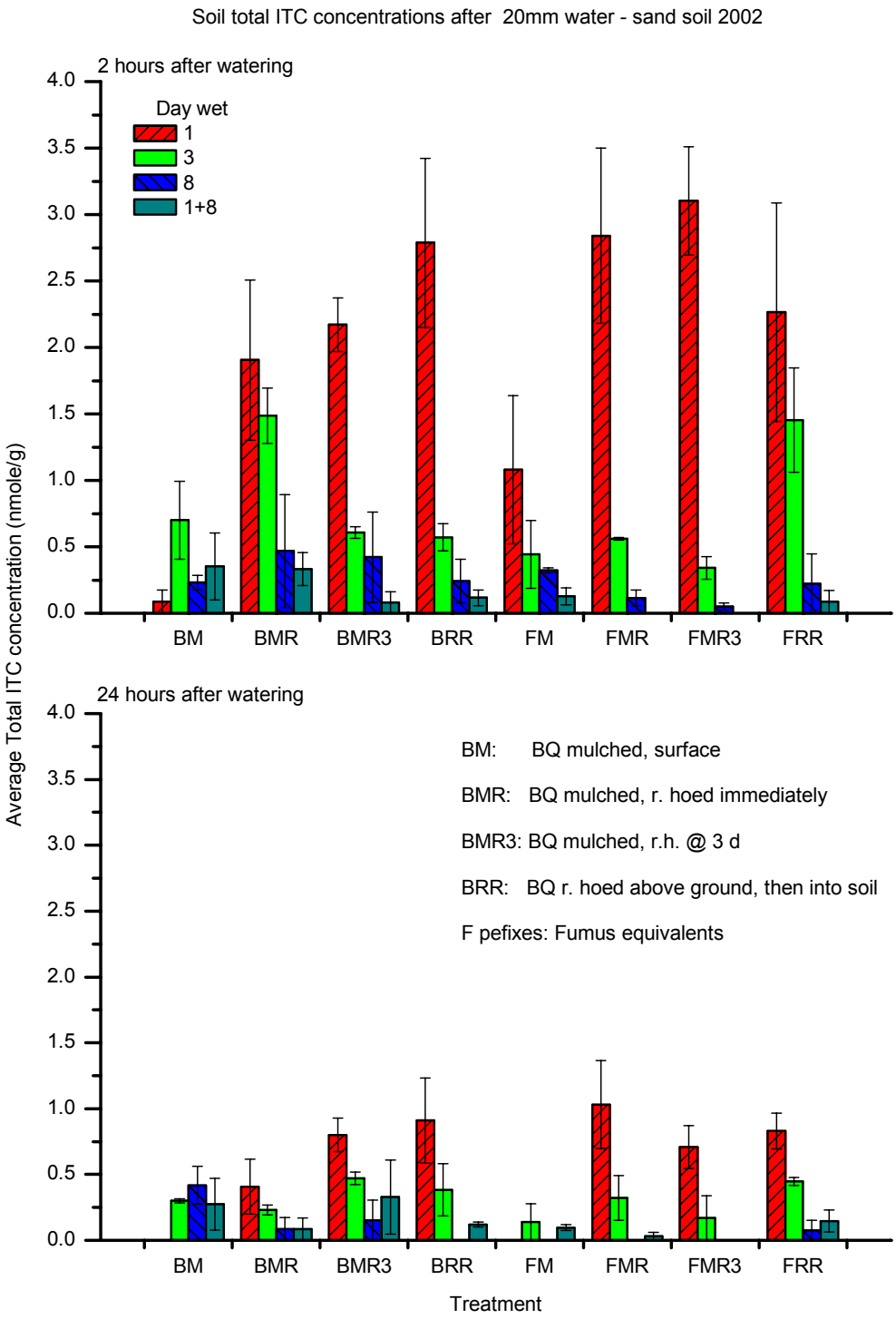

Figure 6. Concentration of total ITCs in sand soil at Medina 2 and 24h after adding water on the designated days for the range of plant treatments.

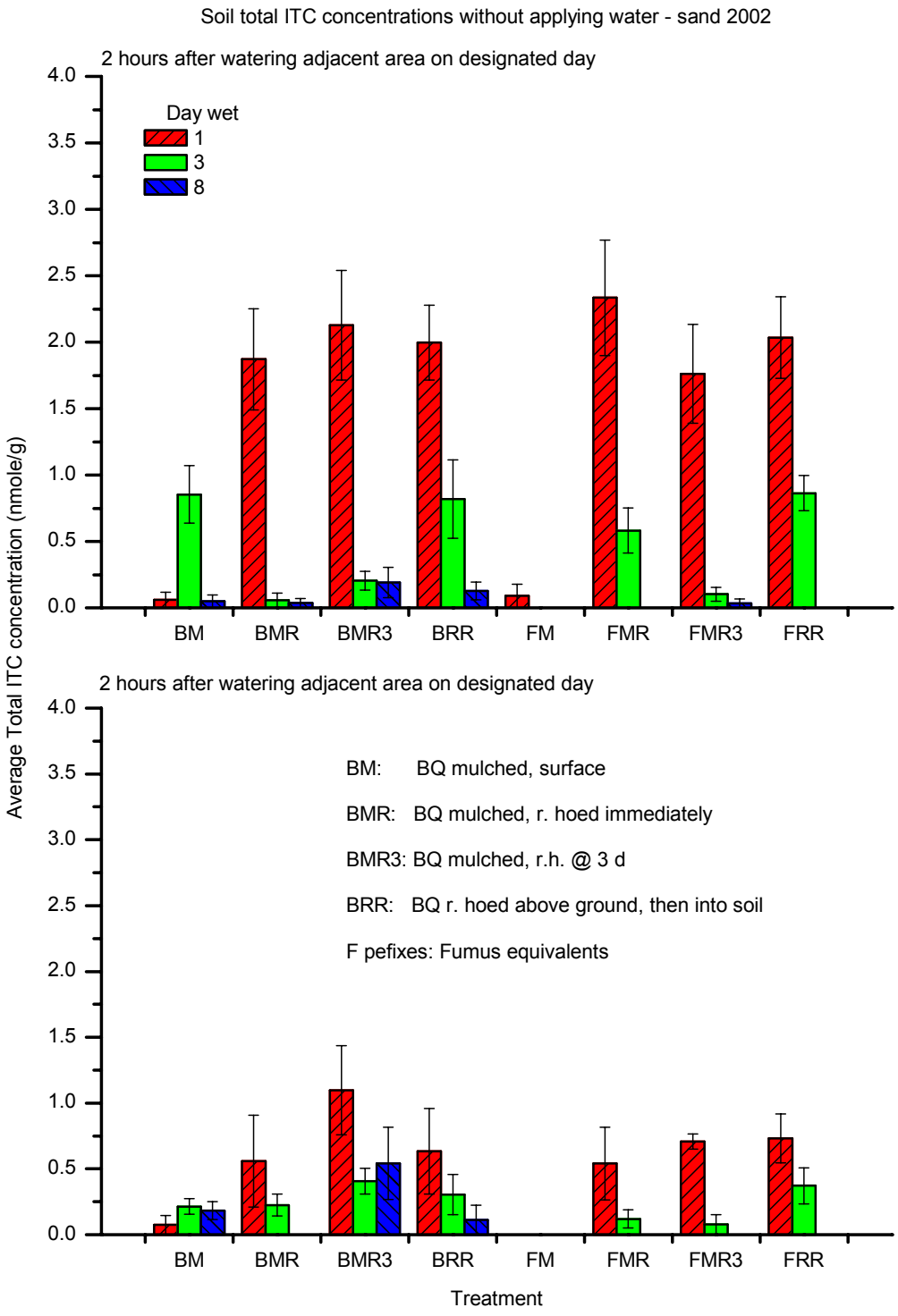

Figure 7. Concentration of total ITCs in non-watered sand soil at Medina 2 and 24h after adding water to the adjacent irrigation cylinders on the designated days for the range of plant treatments.

Figure 8 shows a comparison of the ITC concentrations in the sand at Medina with the loam at Busselton for the same treatments in 2002. Very similar patterns and concentrations are evident. This further supports the conclusion that the pulverisation of the plant tissue was inadequate in 2002 – and reinforces the importance of appropriate mulching as a crucial first step to maximising ITC release. The slightly higher concentration of ITCs in some of the BQMulch plots at Busselton may be a reflection of the greater biomass achieved there.

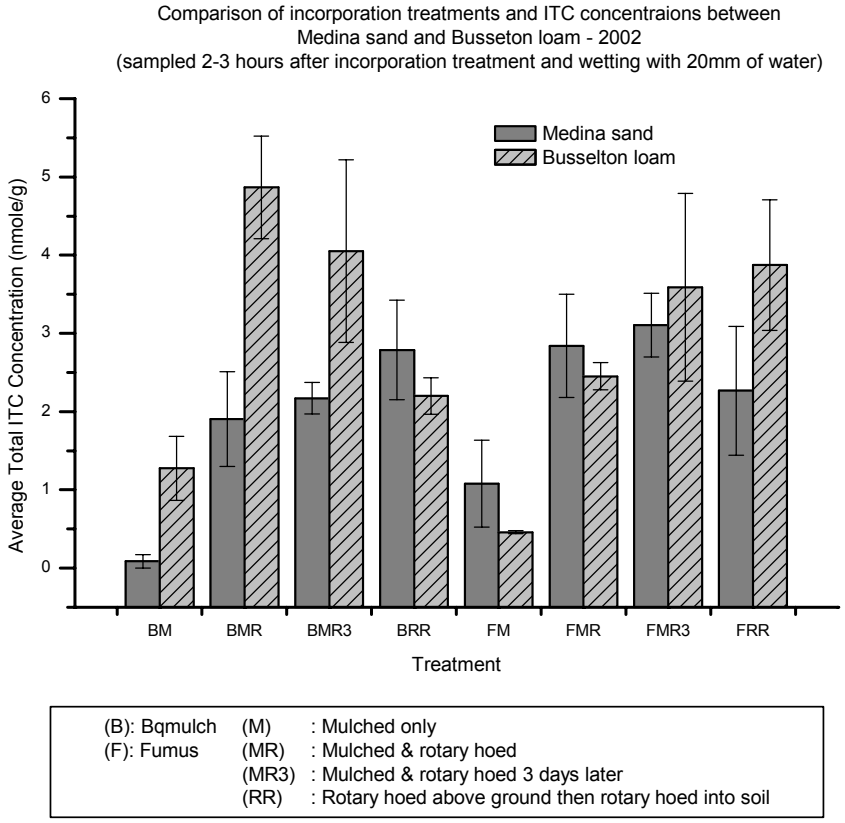

Figure 8. Comparison of total ITC concentration in sandy soil at Medina with that in loam soil at Busselton for various treatments.

SECTION 3

Toxicity and biofumigation effects

Background

Toxicity effects

During the HRDC project that preceded this (VG97050), an insect-based bioassay technique was developed. It used larvae of the relevant soil-dwelling insect whitefringed weevil, *Naupactus leucoloma*. This bioassay methodology enabled measurement of the toxicity of pure ITCs and evaluation of the biofumigation potential of different *Brassica* tissues.

The studies conducted at that time concentrated on *in vitro* tests of the small range of pure ITCs that are commercially available, but which fortunately are also some of the more common ITCs in the mixtures that commonly occur in *Brassica* tissue. The tests were carried out over a range of temperatures (5, 10, 15, 20 ºC), representing the range that might commonly be expected in soils in various growing seasons and locations.

Our bioassay technique tested the vapour toxicity of the various pure compounds. The insects were not treated directly with the test material; rather they were exposed to the vapour in sealed flasks. Similarly, the biofumigation effect of *Brassica* tissue was determined from the toxicity of the vapours evolved from hydrolysing freeze-dried material.

During the course of this project, the extensive series of bioassays was expanded to include *in vivo* studies carried out in the presence of three contrasting soil types from horticultural production areas (a coarse sand, a loam and a high organic matter peat swamp soil). Earlier studies (Matthiessen *et al.* 1996 – *Journal of Economic Entomology* 89: 1372-1378) had shown that different soils had different capacities to sorb methyl isothiocyanate, and thereby dampen its toxic capacity.

In practical terms, depending on the soil type, this would modify the capacity of metham sodium to control soil-borne pests or diseases in the field. Similarly, it seemed likely that biofumigant effects could be modified in the same way. The objective of the laboratory bioassays carried out during this project was to assess this for the same three soils used in the earlier study with methyl isothiocyanate, using the main ITCs of the two chemical families represented in brassicas (aliphatic, or chain, types and aromatic, or ring-structured, types), as well as tissue from the major brassicas being used commercially as 'best bet' biofumigants.

A team at the University of Idaho at Moscow has carried out similar studies to ours but they examined the contact toxicity of various pure ITCs against eggs, also of a weevil, by dipping the eggs into solutions of the test compounds. It is arguable just what is the mode of toxic action of ITCs, especially once molecules with varying solubility, volatility, reactivity and stability are introduced into heterogeneous soil environments. The Idaho studies and ours usefully complement each other to help gauge what may be happening with various ITCs introduced into soil from treatment of biofumigant brassicas.

The Idaho team found that aromatic ITCs are intrinsically substantially more toxic than aliphatic ITCs, but they are very much less volatile and less soluble in water. The question that arises from this contrast is how it plays out in reality. For example, mustards produce

high levels of propenyl ITC (aliphatic) (in their shoots) which is intrinsically less toxic, but more soluble and volatile than the phenylethyl ITC (aromatic), a dominant ITC in fodder rapes (in their roots). These questions are highly complex and present formidable technical challenges to answer. Our objective was to make progress towards understanding aspects of this issue to complement and build on the Idaho team's findings to move closer to a broader understanding of how plant-derived ITCs act to suppress soil-borne pests and diseases and how the positive empirically-observed effects can be enhanced.

Field biofumigation effects

One of the greatest problems in conducting field trials to evaluate the effect of treatments on reducing damage caused by soil-borne pests and diseases is achieving an adequate or sufficiently uniform infestation of the pest or disease to conduct statistically rigorous experimental trials. Usually soil-borne pests and diseases are very patchily distributed and it is difficult to scout or map the highly infested areas because of the cryptic nature of many of the pest organisms (small size, microbial, different stages expressed at different times, low abundance, hidden in soil). Field trials in horticulture are also logistically demanding and costly, both to set up and in terms of lost product from yield suppression or product that has to be discarded. These have all been serious issues throughout research on the capacity of biofumigation to suppress soil-borne pests and diseases.

Discussions with Department of Agriculture, Western Australia (AgWA) colleagues Elaine Davison and Alan McKay provided an opportunity to resolve this impasse. A site at Medina Research Station that had been deliberately uniformly infected with the soil-borne disease *Pythium sulcatum*, the cause of cavity spot disease in carrots had been established for an earlier Horticulture Australia-funded project. Following the completion of that work, the site provided an opportunity to bioassay the effects of biofumigants and the methods used to release the ITCs from plant tissue and incorporate them into the soil, as the final stage of this project.

Methods

Laboratory bioassays

In vitro *bioassays*

Pure isothiocyanates

First instar whitefringed weevil larvae (20 per replicate) were added to 100 ml Erlenmeyer flasks fitted with a cone/screw 24/29 quickfit stopper sealed with vacuum grease. A piece of moistened filter paper (approximately 1cm^2) was placed on the inside of each flask to hold the chemical which was injected onto it via a microsyringe through a 13 mm rubber septum held in the quickfit adapter.

The flask was then put into an incubator at the desired temperature for 24 hours. After 24 hours of exposure the weevils were removed from the flasks and transferred into plastic vials and kept at 15 ºC for a further 72 hours (96 hours after the assay was set up), after which they were counted and mortality was assessed by probit analysis, correcting for control mortality using Abbott's formula.

Brassica tissue

As above, the larvae were placed in the bottom of 100 ml Erlenmeyer flasks fitted with a quickfit stopper. However, in this case the plastic threaded top of the quickfit adapter was replaced with a 6mm rubber stopper with a bent stainless steel pin to act as hook from which a 5.6 x 1.5 cm glass vial (~ 9ml) was suspended. The meal and the appropriate amount of deionised water were added to the glass vial before the system was sealed.

For masses of meal under 50 mg, 50 µl of water is added, thereafter the volume of water added in µl was 1.5 times the mass of *Brassica* material used, i.e. for 100 mg of B*rassica* meal 150 µl water was used.

For assays involving over 100 mg of *Brassica* meal, the method was altered such that the WFW larvae were placed in the suspended glass vial whilst the tissue was put in the bottom of the flask.

In vivo *bioassays*

Pure isothiocyanates

The apparatus and methodology was generally the same as for the *in vitro* bioassays, but with some modification to ensure separation of the small insects (only 1mm long) from the soil. This was necessary, as it proved impossible to readily separate the insects from the soil and determine whether they were dead or alive from the assay treatment or as a result of the handling required to separate them from the soil.

The chemical was injected directly onto 10g soil in the base of the Erlenmeyer flask. The soil was then mixed and the weevils suspended in the glass vial from the rubber stopper, as for the *Brassica* tissue assays when more than 100mg tissue was used.

Brassica *tissue*

These assays were carried out by mixing the *Brassica* tissue with 10 g soil prepared to moisture content 70% of field capacity in the bottom of the flask. The weevils, in the suspended glass vial, were exposed to volatiles from the hydrolising meal for 24 hours and their mortality was assessed after 96 hours.

Field assessments

As part of the AgWA studies on cavity spot disease management the *Pythium* 'nursery' site at Medina R.S. had been cropped with various rotational (non-carrot) plots. These may have impacted on the uniformity of the *Pythium* infection over the area. In preparation for the biofumigation trials, the whole area was sown to a highly *Pythium* sensitive carrot variety in early September 2001. The objective of this crop was to build and make as uniform as possible the *Pythium* inoculum over the area. These carrots were sampled in an intensive grid over the area in early January 2002 to essentially map the *Pythium* level.

The area then remained fallow until preparations for sowing the brassicas and setting up the treatment plots in June 2002. Such fallowing is not detrimental to the *Pythium*.

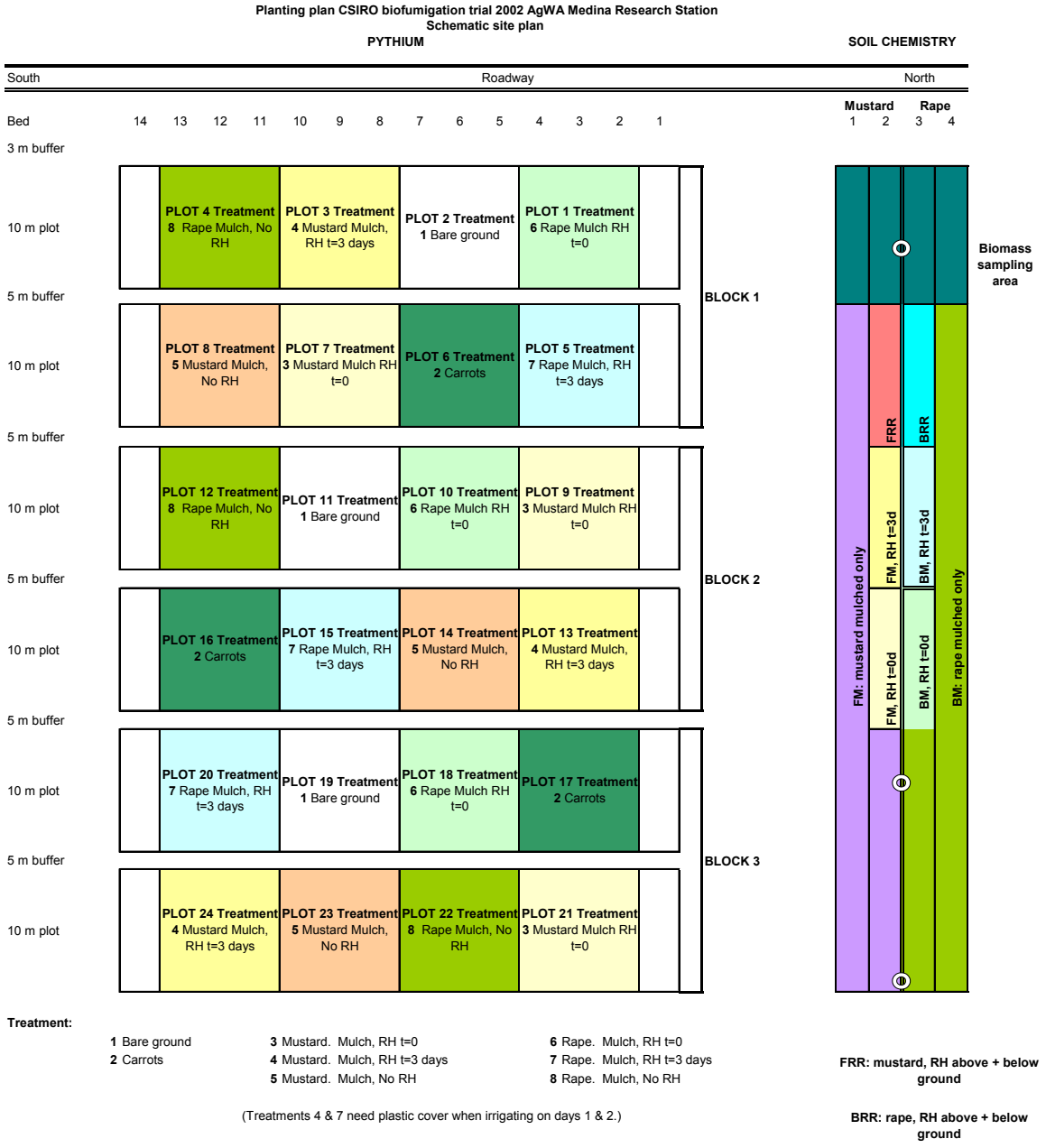

Figure 1. Plot layout and treatment plan for assessment of biofumigation against *Pythium sulcatum* and for measurement of ITCs in soil at Medina Research Centre.

Figure 1 shows schematically the plot layout for the biofumigation study and the separate plots for the accompanying soil chemistry study. A similar intensive soil chemistry study was carried out at Taylor's property on loam soil at Busselton (see details of soil chemistry work in the Component 2 section of this report above).

The experimental design was a randomised complete block of eight treatments and three replications. Each plot was the width of three 1.5m-wide carrot beds and 10m long, with a 5m buffer between each as detailed in Fig. 1. The plots were sown in June 2002, with mulching and associated treatments being carried out on 7 October 2002. Plots that were mulched and the plant material allowed to dry on the surface prior to incorporation were covered with plastic sheet during the daily irrigation on the two days following mulching.

After completing the treatment regime on the brassicas the area was left fallow until February 2003 when it was prepared for sowing to carrots to evaluate treatment effects. A relatively old currently non-commercially preferred *Pythium*-sensitive cultivar was sown over the whole area in March 2003, with the objective of maximising determination of treatment effects. Unfortunately, this coincided with a lengthy period of extremely hot weather. Germination was poor, which may have been related to the severe heat or it may have been a result of a batch of poor seed. The result was that it was clear from early on that yield assessment was unlikely to provide meaningful results. Sampling therefore was directed only at determining the impacts of infection by the disease.

Seedlings were sampled in early April when they were about 2cm tall. After thorough rinsing in water the taproot of 20 plants from each treatment was excised from the top and the lateral hair roots were carefully removed with a scalpel. The taproot was then cut into five pieces which were all plated onto a *Pythium*-selective agar in a single Petri dish and incubated for four days.

A count was then made of the number of root segments that had produced a *Pythium* colony. The proportion of plants producing at least one colony gave a measure of *Pythium* incidence, while the number of root segments from each plant, aggregated over all plants in the sample for a treatment, provided a measure of the severity of the *Pythium* infection.

When the carrots were mature (June 2003), samples were taken and assessed for cavity spot infection and other damage parameters such as forking and stumping and other such deformities associated with *Pythium* lesions destroying seedling taproot tissue. They were also allocated into various marketable categories.

Results and discussion

ITC characteristics

Table 1 summarises key physicochemical characteristics of the two aliphatic (methyl and propenyl), and two aromatic (benzyl and 2-phenylethyl) ITCs used in the bioassays. It also includes contact toxicity information drawn from the work of Borek *et al.* at the University of Idaho.

Although methyl ITC occurs very rarely in brassicas, it is highly relevant as a benchmark because it is the ITC, and active toxin, produced from hydrolysis of metham sodium, the commercial fumigant-like pesticide. Propenyl ITC occurs as the predominant ITC in the shoots of mustards. 2-phenylethyl ITC is common in the roots of fodder rapes. Although benzyl is not a major ITC in many brassicas, it was included because it is one of the few ITCs available commercially and as a second aromatic ITC for comparative purposes.

It can be seen that MITC is essentially 5x more volatile and 4x as soluble in water as propenyl ITC, while the aromatic ITCs have very limited volatility and are very insoluble. In contrast, the aromatic ITCs have a vastly greater contact toxicity than the aliphatic ITCs.

Laboratory bioassays

Table 2 gives the LD_{50} and LD_{95} values and the slope of the dose response line from the probit analyses of the bioassay data for the pure ITCs tested, at each temperature for the *in*

vitro and *in vivo* studies. Also included in Table 2 is the ratio of the LD_{50} and LD_{95} compared to the equivalent value for metham sodium in the same medium, to provide a comparative evaluation of efficacy relative to the commercial pesticide.

Tables 3-5 include also the data for the *Brassica* tissue bioassays. The fodder rape used was the 'C' component of BQMulch, the commercial biofumigant marketed by Wrightson Seeds. BQMulch consists of a mixture of two or three fodder rapes. Component C has the highest concentration of 2-phenylethyl ITC, and it tends to be the most dominant component of the mix in its biomass production.

Table 3 shows the LD ratios based on comparison with the *in vitro* results within each compound, while Table 4 shows the ratio relative to metham sodium *in vitro* and Table 5 shows it relative to MITC *in vitro*.

The vapour toxicity tests carried out as part of this project show that propenyl ITC is only about 0.25 times as toxic as MITC at 10 ºC, while benzyl ITC was 0.5 times as toxic and 2-phenylethyl ITC was only 0.2 times as toxic as MITC at 10 ºC. These ratios contrasted sharply with those of Borek *et al*'s contact tests where propenyl ITC was around 2.5 times more toxic than MITC and benzyl and 2-phenylethyl ITCs were around 75 and 100 times more toxic.

These highly contrasting results raise questions about how ITCs behave in soil, how they achieve their pesticidal effect and just what is the real comparative toxicity or biofumigation potential of each one in the soil environment. These are bigger and more complex questions than could be covered by the scope of this project.

Table 1. Physicochemical characteristics of the two aliphatic and two aromatic ITCs used in the bioassays, and their contact toxicity as determined by bioassay against black vine weevil eggs (Borek *et al.* 1995. *J. Economic Entomology* 88: 1192-1196).

ITC	Molecular weight	Boiling Point (ºC)	Vapour Pressure (mm Hg)	Solubility (g/L H_2O)	Toxicity (LC_{90}) mg/L	LC_{90} ratio (to MITC)
Methyl	73.118	119	23.6	7.6	174.2	1.0
Propenyl	99.156	152	5.3	2.0	73.8	2.4
Benzyl	149.215	242	<1	n.a.	2.3	75.7
2-phenylethyl	163.240	248	n.a.	0.11	1.8	96.8

Figure 2 shows the relative toxicity of each compound or tissue tested in the bioassays, for each soil medium and at each temperature. A log y-axis is used to place all plots on the same scale because of the very large range in the data, but as it makes visualising the information more difficult Tables 2-5 can be referred to for the actual data, while figures following Fig. 5 show more specific aspects of these data plotted on y-axis scales relevant to within-material, rather than between-material comparisons.

Tables 2-5. Summary of all bioassay data. Each table compares different ratios of LD_{50} and LD_{95} values.

Table 2.

Bioassay summary - vapour (LD values - mg/l/24h) (tissue: mg)																				
LD ratio (LDR) values relative to metham (in same medium)																				
ITC	**Medium**	**5deg. C**					**10deg. C**					**15deg. C**					**20deg. C**			
		LD50	**LDR50**	**LD95**	**LDR95**	**slope**	**LD50**	**LDR50**	**LD95**	**LDR95**	**slope**	**LD50**	**LDR50**	**LD95**	**LDR95**	**slope**	**LD50**	**LDR50**	**LD95**	**LDR95**
	Medium			5		5			10		10			15		15			20	
metham	**in vitro**	0.068	1.0000	0.120	1.0000	31.669	0.063	1.0000	0.094	1.0000	51.865	0.050	1.0000	0.067	1.0000	100.547	0.046	1.0000	0.061	1.0000
	sand	0.569		0.653		19.520	0.329		0.434		15.730	0.226		0.367		11.720	0.255		0.323	
	loam	0.651		0.848		8.370	0.387		0.570		9.020	0.322		0.478		10.550	0.274		0.334	
	peat	3.106		4.351		1.320	1.041		1.393		4.670	1.239		1.582		4.790	0.671		0.929	
Aliphatic	Medium			5		5			10		10			15		15			20	
MITC	**in vitro**	0.052	1.3077	0.070	1.7143	91.110	0.036	1.7500	0.052	1.8077	102.110	0.033	1.5152	0.046	1.4565	127.070	0.027	1.7037	0.034	1.7941
	sand	1.006	0.5656	1.287	0.5074	5.854	0.480	0.6854	0.599	0.7245	13.814	0.375	0.6027	0.492	0.7459	14.046	0.237	1.0759	0.292	1.1062
	loam	0.962	0.6767	1.210	0.7008	6.645	0.454	0.8524	0.593	0.9612	11.847	0.340	0.9471	0.440	1.0864	16.407	0.217	1.2627	0.276	1.2101
	peat	2.886	1.0762	3.415	1.2741	3.112	1.290	0.8070	1.653	0.8427	4.523	0.901	1.3751	1.192	1.3272	5.646	0.642	1.0452	0.830	1.1193
	Medium			5		5			10		10			15		15			20	
propenyl	**in vitro**	0.239	0.2845	0.296	0.4054	28.652	0.166	0.3795	0.211	0.4455	36.648	0.153	0.3268	0.213	0.3146	27.400	0.131	0.3511	0.175	0.3486
	sand	1.926	0.2954	2.151	0.3036	7.293	1.226	0.2684	1.716	0.2529	3.357	1.031	0.2192	1.354	0.2710	5.107	0.758	0.3364	0.996	0.3243
	loam	2.011	0.3237	2.465	0.3440	3.622	1.294	0.2991	1.615	0.3529	5.110	1.196	0.2692	1.581	0.3023	4.275	0.836	0.3278	1.024	0.3262
	peat	10.715	0.2899	12.912	0.3370	0.749	4.573	0.2276	5.760	0.2418	1.386	4.309	0.2875	5.583	0.2834	1.292	2.546	0.2636	3.149	0.2950
Aromatic	Medium			5		5			10		10			15		15			20	
benzyl	**in vitro**	0.114	0.5965	0.182	0.6593	24.406	0.072	0.8750	0.097	0.9691	64.535	0.079	0.6329	0.103	0.6505	66.734	0.073	0.6301	0.095	0.6421
	sand	7.984	0.0713	11.635	0.0561	0.450	3.874	0.0849	4.830	0.0899	1.720	3.153	0.0717	3.935	0.0933	2.104	3.125	0.0816	4.153	0.0778
	loam	5.165	0.1260	6.641	0.1277	1.115	3.107	0.1246	4.701	0.1213	1.032	2.794	0.1152	3.517	0.1359	2.274	2.081	0.1317	2.594	0.1288
	peat	68.850	0.0451	91.549	0.0475	0.072	45.004	0.0231	54.871	0.0254	0.167	31.543	0.0393	39.446	0.0401	0.208	21.812	0.0308	27.171	0.0342
	Medium			5		5			10		10			15		15			20	
phenylethyl	**in vitro**	0.146	0.4658	0.236	0.5085	18.230	0.117	0.5385	0.226	0.4159	15.145	0.069	0.7246	0.098	0.6837	57.780	0.077	0.5974	0.096	0.6354
	sand	26.453	0.0215	51.883	0.0126	0.065	9.027	0.0364	21.572	0.0201	0.131	4.712	0.0480	6.148	0.0597	1.145	2.925	0.0872	3.731	0.0866
	loam	26.956	0.0242	56.326	0.0151	0.056	6.612	0.0585	23.825	0.0239	0.096	5.462	0.0590	7.532	0.0635	0.794	2.839	0.0965	3.774	0.0885
	peat	945.393	0.0033	3284.843	0.0013	0.001	82.965	0.0125	207.511	0.0067	0.013	70.142	0.0177	102.477	0.0154	0.051	34.250	0.0196	44.585	0.0208

Table 3.

LD ratio (LDR) values relative to in vitro (within each compound)																				
		5deg. C					10deg. C					15deg. C					20deg. C			
		LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95
	Medium			5	5	5			10	10	10			15	15	15			20	20
metham	**in vitro**	0.068	1.0000	0.120	1.0000	31.669	0.063	1.0000	0.094	1.0000	51.865	0.050	1.0000	0.067	1.0000	100.547	0.046	1.0000	0.061	1.0000
	sand	0.569	0.1195	0.653	0.1838	19.520	0.329	0.1915	0.434	0.2166	15.730	0.226	0.2212	0.367	0.1826	11.720	0.255	0.1804	0.323	0.1889
	loam	0.651	0.1045	0.848	0.1415	8.370	0.387	0.1628	0.570	0.1649	9.020	0.322	0.1553	0.478	0.1402	10.550	0.274	0.1679	0.334	0.1826
	peat	3.106	0.0219	4.351	0.0276	1.320	1.041	0.0605	1.393	0.0675	4.670	1.239	0.0404	1.582	0.0424	4.790	0.671	0.0686	0.929	0.0657
	Medium			5	5	5			10	10	10			15	15	15			20	20
MITC	**in vitro**	0.052	1.0000	0.070	1.0000	91.110	0.036	1.0000	0.052	1.0000	102.110	0.033	1.0000	0.046	1.0000	127.070	0.027	1.0000	0.034	1.0000
	sand	1.006	0.0517	1.287	0.0544	5.854	0.480	0.0750	0.599	0.0868	13.814	0.375	0.0880	0.492	0.0935	14.046	0.237	0.1139	0.292	0.1164
	loam	0.962	0.0541	1.210	0.0579	6.645	0.454	0.0793	0.593	0.0877	11.847	0.340	0.0971	0.440	0.1045	16.407	0.217	0.1244	0.276	0.1232
	peat	2.886	0.0180	3.415	0.0205	3.112	1.290	0.0279	1.653	0.0315	4.523	0.901	0.0366	1.192	0.0386	5.646	0.642	0.0421	0.830	0.0410
	Medium			5	5	5			10	10	10			15	15	15			20	20
propenyl	**in vitro**	0.239	1.0000	0.296	1.0000	28.652	0.166	1.0000	0.211	1.0000	36.648	0.153	1.0000	0.213	1.0000	27.400	0.131	1.0000	0.175	1.0000
	sand	1.926	0.1241	2.151	0.1376	7.293	1.226	0.1354	1.716	0.1230	3.357	1.031	0.1484	1.354	0.1573	5.107	0.758	0.1728	0.996	0.1757
	loam	2.011	0.1188	2.465	0.1201	3.622	1.294	0.1283	1.615	0.1307	5.110	1.196	0.1279	1.581	0.1347	4.275	0.836	0.1567	1.024	0.1709
	peat	10.715	0.0223	12.912	0.0229	0.749	4.573	0.0363	5.760	0.0366	1.386	4.309	0.0355	5.583	0.0382	1.292	2.546	0.0515	3.149	0.0556
	Medium			5	5	5			10	10	10			15	15	15			20	20
Mustard meal	**in vitro**	24.600	1.0000	43.500	1.0000	0.090	10.200	1.0000	21.300	1.0000	0.149	7.900	1.0000	13.000	1.0000	0.320	6.800	1.0000	11.500	1.0000
	sand	50.900	0.4833	71.700	0.6067	0.079	35.000	0.2914	52.800	0.4034	0.092	31.100	0.2540	44.000	0.2955	0.128	31.300	0.2173	38.500	0.2987
	loam	65.100	0.3779	93.800	0.4638	0.057	36.800	0.2772	55.600	0.3831	0.087	28.000	0.2821	41.000	0.3171	0.125	23.700	0.2869	36.500	0.3151
	peat	441.900	0.0557	718.300	0.0606	0.006	143.000	0.0713	224.800	0.0948	0.020	118.400	0.0667	165.900	0.0784	0.350	93.300	0.0729	125.600	0.0916
	Medium			5	5	5			10	10	10			15	15	15			20	20
benzyl	**in vitro**	0.114	1.0000	0.182	1.0000	24.406	0.072	1.0000	0.097	1.0000	64.535	0.079	1.0000	0.103	1.0000	66.734	0.073	1.0000	0.095	1.0000
	sand	7.984	0.0143	11.635	0.0156	0.450	3.874	0.0186	4.830	0.0201	1.720	3.153	0.0251	3.935	0.0262	2.104	3.125	0.0234	4.153	0.0229
	loam	5.165	0.0221	6.641	0.0274	1.115	3.107	0.0232	4.701	0.0206	1.032	2.794	0.0283	3.517	0.0293	2.274	2.081	0.0351	2.594	0.0366
	peat	68.850	0.0017	91.549	0.0020	0.072	45.004	0.0016	54.871	0.0018	0.167	31.543	0.0025	39.446	0.0026	0.208	21.812	0.0033	27.171	0.0035
	Medium			5	5	5			10	10	10			15	15	15			20	20
phenylethyl	**in vitro**	0.146	1.0000	0.236	1.0000	18.230	0.117	1.0000	0.226	1.0000	15.145	0.069	1.0000	0.098	1.0000	57.780	0.077	1.0000	0.096	1.0000
	sand	26.453	0.0055	51.883	0.0045	0.065	9.027	0.0130	21.572	0.0105	0.131	4.712	0.0146	6.148	0.0159	1.145	2.925	0.0263	3.731	0.0257
	loam	26.956	0.0054	56.326	0.0042	0.056	6.612	0.0177	23.825	0.0095	0.096	5.462	0.0126	7.532	0.0130	0.794	2.839	0.0271	3.774	0.0254
	peat	945.393	0.0002	3284.843	0.0001	0.001	82.965	0.0014	207.511	0.0011	0.013	70.142	0.0010	102.477	0.0010	0.051	34.250	0.0022	44.585	0.0022
	Medium			5	5	5			10	10	10			15	15	15			20	20
BQMulchC root	**in vitro**	191.144	1.0000	480.362	1.0000	0.006	49.532	1.0000	102.149	1.0000	0.031	32.817	1.0000	69.985	1.0000	0.044	21.220	1.0000	35.657	1.0000
	sand	652.465	0.2930	912.562	0.5264	0.006	188.487	0.2628	251.452	0.4062	0.026	149.669	0.2193	202.865	0.3450	0.031	118.919	0.1784	145.462	0.2451
	loam	645.614	0.2961	1229.388	0.3907	0.003	142.297	0.3481	197.864	0.5163	0.030	98.137	0.3344	149.989	0.4666	0.032	78.199	0.2714	109.509	0.3256
	peat	na		na		na	na		na		na	na		na		na	na		na	

Table 4.

Bioassay summary - vapour (LD values - mg/l/24h) (tissue: mg)																				
LD ratio (LDR) values relative to metham in vitro																				
		5deg. C					10deg. C					15deg. C					20deg. C			
		LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95
	Medium			5deg					10deg					15deg					20deg	
metham	**in vitro**	0.068	1.0000	0.120	1.0000	31.669	0.063	1.0000	0.094	1.0000	51.865	0.050	1.0000	0.067	1.0000	100.547	0.046	1.0000	0.061	1.0000
	sand	0.569	0.1195	0.653	0.1838	19.520	0.329	0.1915	0.434	0.2166	15.730	0.226	0.2212	0.367	0.1826	11.720	0.255	0.1804	0.323	0.1889
	loam	0.651	0.1045	0.848	0.1415	8.370	0.387	0.1628	0.570	0.1649	9.020	0.322	0.1553	0.478	0.1402	10.550	0.274	0.1679	0.334	0.1826
	peat	3.106	0.0219	4.351	0.0276	1.320	1.041	0.0605	1.393	0.0675	4.670	1.239	0.0404	1.582	0.0424	4.790	0.671	0.0686	0.929	0.0657
	Medium			5					10					15					20	
MITC	**in vitro**	0.052	1.3077	0.070	1.7143	91.110	0.036	1.7500	0.052	1.8077	102.110	0.033	1.5152	0.046	1.4565	127.070	0.027	1.7037	0.034	1.7941
	sand	1.006	0.0676	1.287	0.0932	5.854	0.480	0.1313	0.599	0.1569	13.814	0.375	0.1333	0.492	0.1362	14.046	0.237	0.1941	0.292	0.2089
	loam	0.962	0.0676	1.210	0.0992	6.645	0.454	0.1313	0.593	0.1585	11.847	0.340	0.1471	0.440	0.1523	16.407	0.217	0.2120	0.276	0.2210
	peat	2.886	0.0707	3.415	0.0351	3.112	1.290	0.1388	1.653	0.0569	4.523	0.901	0.0555	1.192	0.0562	5.646	0.642	0.0717	0.830	0.0735
	Medium			5					10					15					20	
propenyl	**in vitro**	0.239	0.2845	0.296	0.4054	28.652	0.166	0.3795	0.211	0.4455	36.648	0.153	0.3268	0.213	0.3146	27.400	0.131	0.3511	0.175	0.3486
	sand	1.926	0.0353	2.151	0.0558	7.293	1.226	0.0514	1.716	0.0548	3.357	1.031	0.0485	1.354	0.0495	5.107	0.758	0.0607	0.996	0.0612
	loam	2.011	0.0338	2.465	0.0487	3.622	1.294	0.0487	1.615	0.0582	5.110	1.196	0.0418	1.581	0.0424	4.275	0.836	0.0550	1.024	0.0596
	peat	10.715	0.0063	12.912	0.0093	0.749	4.573	0.0138	5.760	0.0163	1.386	4.309	0.0116	5.583	0.0120	1.292	2.546	0.0181	3.149	0.0194
	Medium			5					10					15					20	
benzyl	**in vitro**	0.114	0.5965	0.182	0.6593	24.406	0.072	0.8750	0.097	0.9691	64.535	0.079	0.6329	0.103	0.6505	66.734	0.073	0.6301	0.095	0.6421
	sand	7.984	0.0085	11.635	0.0103	0.450	3.874	0.0163	4.830	0.0195	1.720	3.153	0.0159	3.935	0.0170	2.104	3.125	0.0147	4.153	0.0147
	loam	5.165	0.0132	6.641	0.0181	1.115	3.107	0.0203	4.701	0.0200	1.032	2.794	0.0179	3.517	0.0191	2.274	2.081	0.0221	2.594	0.0235
	peat	68.850	0.0010	91.549	0.0013	0.072	45.004	0.0014	54.871	0.0017	0.167	31.543	0.0016	39.446	0.0017	0.208	21.812	0.0021	27.171	0.0022
	Medium			5					10					15					20	
phenylethyl	**in vitro**	0.146	0.4658	0.236	0.5085	18.230	0.117	0.5385	0.226	0.4159	15.145	0.069	0.7246	0.098	0.6837	57.780	0.077	0.5974	0.096	0.6354
	sand	26.453	0.0026	51.883	0.0023	0.065	9.027	0.0070	21.572	0.0044	0.131	4.712	0.0106	6.148	0.0109	1.145	2.925	0.0157	3.731	0.0163
	loam	26.956	0.0025	56.326	0.0021	0.056	6.612	0.0095	23.825	0.0039	0.096	5.462	0.0092	7.532	0.0089	0.794	2.839	0.0162	3.774	0.0162
	peat	945.393	0.0001	3284.843	0.0000	0.001	82.965	0.0008	207.511	0.0005	0.013	70.142	0.0007	102.477	0.0007	0.051	34.250	0.0013	44.585	0.0014

Table 5.

LD ratio (LDR) values relative to MITC in vitro																				
		5deg. C					10deg. C					15deg. C					20deg. C			
		LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95	slope	LD50	LDR50	LD95	LDR95
	Medium			5	5	5			10	10	10			15	15	15			20	20
metham	**in vitro**	0.068	0.7647	0.120	0.5833	31.669	0.063	0.5714	0.094	0.5532	51.865	0.050	0.6600	0.067	0.6866	100.547	0.046	0.5870	0.061	0.5574
	sand	0.569	0.0914	0.653	0.1072	19.520	0.329	0.1094	0.434	0.1198	15.730	0.226	0.1460	0.367	0.1253	11.720	0.255	0.1059	0.323	0.1053
	loam	0.651	0.0799	0.848	0.0825	8.370	0.387	0.0930	0.570	0.0912	9.020	0.322	0.1025	0.478	0.0962	10.550	0.274	0.0985	0.334	0.1018
	peat	3.106	0.0167	4.351	0.0161	1.320	1.041	0.0346	1.393	0.0373	4.670	1.239	0.0266	1.582	0.0291	4.790	0.671	0.0402	0.929	0.0366
	Medium				5	5			10	10	10			15	15	15			20	20
MITC	**in vitro**	0.052	1.0000	0.070	1.0000	91.110	0.036	1.0000	0.052	1.0000	102.110	0.033	1.0000	0.046	1.0000	127.070	0.027	1.0000	0.034	1.0000
	sand	1.006	0.0517	1.287	0.0544	5.854	0.480	0.0750	0.599	0.0868	13.814	0.375	0.0880	0.492	0.0935	14.046	0.237	0.1139	0.292	0.1164
	loam	0.962	0.0541	1.210	0.0579	6.645	0.454	0.0793	0.593	0.0877	11.847	0.340	0.0971	0.440	0.1045	16.407	0.217	0.1244	0.276	0.1232
	peat	2.886	0.0180	3.415	0.0205	3.112	1.290	0.0279	1.653	0.0315	4.523	0.901	0.0366	1.192	0.0386	5.646	0.642	0.0421	0.830	0.0410
	Medium				5	5			10	10	10			15	15	15			20	20
propenyl	**in vitro**	0.239	0.2176	0.296	0.2365	28.652	0.166	0.2169	0.211	0.2464	36.648	0.153	0.2157	0.213	0.2160	27.400	0.131	0.2061	0.175	0.1943
	sand	1.926	0.0270	2.151	0.0325	7.293	1.226	0.0294	1.716	0.0303	3.357	1.031	0.0320	1.354	0.0340	5.107	0.758	0.0356	0.996	0.0341
	loam	2.011	0.0259	2.465	0.0284	3.622	1.294	0.0278	1.615	0.0322	5.110	1.196	0.0276	1.581	0.0291	4.275	0.836	0.0323	1.024	0.0332
	peat	10.715	0.0049	12.912	0.0054	0.749	4.573	0.0079	5.760	0.0090	1.386	4.309	0.0077	5.583	0.0082	1.292	2.546	0.0106	3.149	0.0108
	Medium				5	5			10	10	10			15	15	15			20	20
Mustard meal	**in vitro**	24.600	0.0021	43.500	0.0016	0.090	10.200	0.0035	21.300	0.0024	0.149	7.900	0.0042	13.000	0.0035	0.320	6.800	0.0040	11.500	0.0030
	sand	50.900	0.0010	71.700	0.0010	0.079	35.000	0.0010	52.800	0.0010	0.092	31.100	0.0011	44.000	0.0010	0.128	31.300	0.0009	38.500	0.0009
	loam	65.100	0.0008	93.800	0.0007	0.057	36.800	0.0010	55.600	0.0009	0.087	28.000	0.0012	41.000	0.0011	0.125	23.700	0.0011	36.500	0.0009
	peat	441.900	0.0001	718.300	0.0001	0.006	143.000	0.0003	224.800	0.0002	0.020	118.400	0.0003	165.900	0.0003	0.350	93.300	0.0003	125.600	0.0003
	Medium				5	5			10	10	10			15	15	15			20	20
benzyl	**in vitro**	0.114	0.4561	0.182	0.3846	24.406	0.072	0.5000	0.097	0.5361	64.535	0.079	0.4177	0.103	0.4466	66.734	0.073	0.3699	0.095	0.3579
	sand	7.984	0.0065	11.635	0.0060	0.450	3.874	0.0093	4.830	0.0108	1.720	3.153	0.0105	3.935	0.0117	2.104	3.125	0.0086	4.153	0.0082
	loam	5.165	0.0101	6.641	0.0105	1.115	3.107	0.0116	4.701	0.0111	1.032	2.794	0.0118	3.517	0.0131	2.274	2.081	0.0130	2.594	0.0131
	peat	68.850	0.0008	91.549	0.0008	0.072	45.004	0.0008	54.871	0.0009	0.167	31.543	0.0010	39.446	0.0012	0.208	21.812	0.0012	27.171	0.0013
	Medium				5	5			10	10	10			15	15	15			20	20
phenylethyl	**in vitro**	0.146	0.3562	0.236	0.2966	18.230	0.117	0.3077	0.226	0.2301	15.145	0.069	0.4783	0.098	0.4694	57.780	0.077	0.3506	0.096	0.3542
	sand	26.453	0.0020	51.883	0.0013	0.065	9.027	0.0040	21.572	0.0024	0.131	4.712	0.0070	6.148	0.0075	1.145	2.925	0.0092	3.731	0.0091
	loam	26.956	0.0019	56.326	0.0012	0.056	6.612	0.0054	23.825	0.0022	0.096	5.462	0.0060	7.532	0.0061	0.794	2.839	0.0095	3.774	0.0090
	peat	945.393	0.0001	3284.843	0.0000	0.001	82.965	0.0004	207.511	0.0003	0.013	70.142	0.0005	102.477	0.0004	0.051	34.250	0.0008	44.585	0.0008
	Medium				5	5			10	10	10			15	15	15			20	20
BQMulchC root	**in vitro**	191.144	0.0003	480.362	0.0001	0.006	49.532	0.0007	102.149	0.0005	0.031	32.817	0.0010	69.985	0.0007	0.044	21.220	0.0013	35.657	0.0010
	sand	652.465	0.0001	912.562	0.0001	0.006	188.487	0.0002	251.452	0.0002	0.026	149.669	0.0002	202.865	0.0002	0.031	118.919	0.0002	145.462	0.0002
	loam	645.614	0.0001	1229.388	0.0001	0.003	142.297	0.0003	197.864	0.0003	0.030	98.137	0.0003	149.989	0.0003	0.032	78.199	0.0003	109.509	0.0003
	peat	na		na		na	na		na		na	na		na		na	na		na	

As would be expected, the pattern of response for MITC and metham sodium was closely similar. Generally, above 10 ºC the toxicity of all test materials except phenylethyl ITC tended to remain fairly constant. Most showed a relatively sharper falling-off in toxicity tending below 10 ºC, a characteristic that was much more pronounced with phenylethyl ITC. This is to be expected for this least volatile of the ITCs tested.

The vapour toxicity of all pure ITCs tested was similar *in vitro*, with the LD_{95} being in the range of around 0.1 – 1.0 mg/l/24h (Fig. 2, Fig 6). Given the vastly different volatility of the compounds (Table 1) this is at first surprising. However, it appears that contact toxicity (which may, arguably, be viewed as the 'inherent' toxicity) that has a trend in reverse of volatile toxicity, offsets the volatility effect. This raises very important questions of the mode of action of ITCs (i.e. contact or respiratory) - and how to determine it, especially in soil.

For the bioassays carried out in the three soils, in all cases the results showed a substantial (c. 10 to1000-fold or more) 'dampening' of the toxicity (Fig. 2, Figs 7-16). The materials all behaved similarly in the sand and the loam (3.26 and 6.77% organic matter respectively). The effects of the soil varied between the aliphatic ITCs, where toxicity was dampened around 10-fold, and the aromatic ITCs where it was reduced over 100-fold. The suppressive effects of the soil type on ITC toxicity were strongest for phenylethyl ITC.

It was in the peat soil (31.55% organic matter - which is an extreme example), that the toxicity was most severely dampened. It is known that methyl ITC is rapidly and strongly sorbed by soil, particularly the high organic matter peat soil (published for the same soils as used in these bioassays: Matthiessen *et al.* 1996 *J. Economic Entomology* 89: 1372-1378). It appears from the present bioassay results that the variation in the soil organic matter content between the sand and the loam, even though doubled, was below some absolute threshold where sorption effects become very strong.

This was somewhat surprising, but good, news. The sand was the coarse soil common to the Swan coastal plain, WA, and typical of the intensive horticultural operations there, while the loam was from a typical horticultural situation near Pemberton, WA where the soil would typically be considered substantially 'richer' than the sand.

In both cases of the *Brassica* tissues there was less separation of the toxicity levels between the different media than occurred with the pure ITCs. However, attempts to achieve high levels of mortality using the BQMulch(C) tissue in the peat soil proved not to be possible at the amounts of tissue that could be accommodated in the experimental apparatus.

The toxicity of the mustard tissue was markedly reduced in the peat soil at the lowest temperature (Fig. 13), while temperature below 10 ºC caused a marked decline in the toxicity of the fodder rape root tissue in all media (Fig. 16).

Figure 3 shows the slope of the dose-response lines for each bioassay. There was considerable variation in the biological activity between the compounds and tissues, as evidenced by how rapidly the response changed with increasing dose. Again, the need to accommodate the wide range of values through use of a log scale makes detailed comparisons difficult. Following figures (Figs. 17-20) show more detailed comparisons using different y-axis scales to suit the data.

Reference to Fig. 3 and Figs. 17-20 show various aspects: the slope of the dose-response lines was consistently greater for MITC and metham sodium and it tended to rise more sharply with increase in temperature for those than the other compounds, it was very steep *in vitro* for all pure ITCs but was substantially damped *in vivo*, particularly in the peat soil, while it was very flat in all test situations for the plant tissues. In summary, the presence of soil exerts the major influence in flattening the slope of the response of the bioassay insects to ITCs.

In Fig. 4 the ratio of the LD_{95} values for each compound or tissue *in vivo* is calculated relative to the value for the same compound or tissue determined *in vitro*, to gauge how the soil affects the 'pure' vapour toxicity of the compounds and tissues. Figs. 21-23 show the data on a linear y-axis scale. Generally, the toxicity of the compounds and tissues *in vivo* was a small fraction (up to c. 20%) of the *in vitro* values and was little influenced by temperature in the case of the pure ITCs.

Intriguingly, the ratio was much greater for the two plant tissues, particularly in the sand and loam. This indicated that the soils were not damping the effect of the tissues as much as the pure compounds. Why this should be the case is obscure; however, it needs to be borne in mind that the amounts of tissue required to obtain effects were always quite substantial.

In sand there was also a clear temperature effect, but with opposite trend than may have been anticipated from first principles – as temperature decreased, the extra material required to give the same effect was disproportionately less than it was at higher temperature (Fig. 21). The ratio was especially low for all compounds in the peat soil (Fig. 23).

In Fig. 5 the ratio of the LD_{95} values for each compound or tissue *in vitro* and *in vivo* is calculated relative to the value for the same compound or tissue determined for MITC *in vitro*, to gauge how the soil affects the vapour toxicity of the compounds and tissues relative to the 'pure' vapour toxicity of the active product of the compound used as a commercial fumigant. Figs. 24-27 show the same results on a linear y-axis scale.

When compared *in vitro*, the other pure compounds ranged between about 25 and 65% as toxic as MITC. The two aromatic ITCs, despite their low volatility, were at the upper end of that range (Fig. 24). The presence of soil markedly damped the ratio, bringing it down to 10% and less for the sand and peat and well under 5% for the loam soil (Figs.25-27).

The ratio for the tissues is indicative of trends only as the units of measure were not the same. The tissues behaved similarly in the sand and loam while the peat soil produced a much lower ratio that could only be measured for the mustard tissue and not the fodder rape root (Fig. 5).

In summary, MITC is the most potent of the compounds in the testing system we used, which measures the toxicity of the volatiles. This is not surprising, given that MITC is the most volatile of the compounds. Despite this, the toxicity of the poorly volatile aromatic ITCs, when exposed to the test organisms only in the vapour phase was high. This must reflect their very high intrinsic toxicity as determined by Borek *et. al*. The presence of soil strongly suppressed the efficacy of all the compounds and tissues, especially when the organic matter content was high. This was not surprising, given that our previous work had shown the high capacity for MITC to be sorbed by soil.

How to relate these results to the achievement of effects in the field is a complex and difficult issue. Borek *et. al* showed that propenyl ITC was c. 2x as toxic as MITC in contact tests, but

our assay results show propenyl ITC to be c. 0.25 as toxic as MITC at 10 ºC. We measured levels of propenyl ITC in soil following mulching and heavy watering of mustard tissue in the 2001 plots at Busselton (see Section 2) that were about 50% of the concentration of MITC achieved after application of metham sodium at the recommended rate of 500 L/ha.

Superficially, the 2-fold difference in contact toxicity could imply that half as much propenyl ITC as MITC should be as effective as the MITC. However, with the vapour toxicity of propenyl ITC being only one quarter of that of MITC, the realised toxicity of the propenyl ITC from the mustard in soil in the field may be as low as one eighth of MITC, given that its soil concentration was half that of the MITC derived from metham sodium. A further issue is that the solubility of propenyl ITC is only about one quarter of that of MITC, with the aromatic ITCs very much less.

Similarly, Borek *et. al* showed benzyl and phenylethyl ITCs to be c. 60 and c. 70 times more toxic by contact, but our vapour bioassays showed benzyl ITC to be c. 0.5 and phenylethyl ITC c. 0.2 as toxic as MITC at 10 ºC.

Just how the interplay of contact and respiratory toxicity, volatility, solubility and soil type play out in the field is still obscure and presents enormous challenges to investigate. What can be said is that even MITC in the field as derived from metham sodium is relatively inefficient. Large quantities have to be applied to allow for the very active sorption effects that occur on most soils. Also, adequate soil moisture is important for achieving good effects but it is unclear what is the relative importance of moisture for the hydrolysis reaction to produce the MITC and the presence of moisture to carry the MITC in solution through the soil.

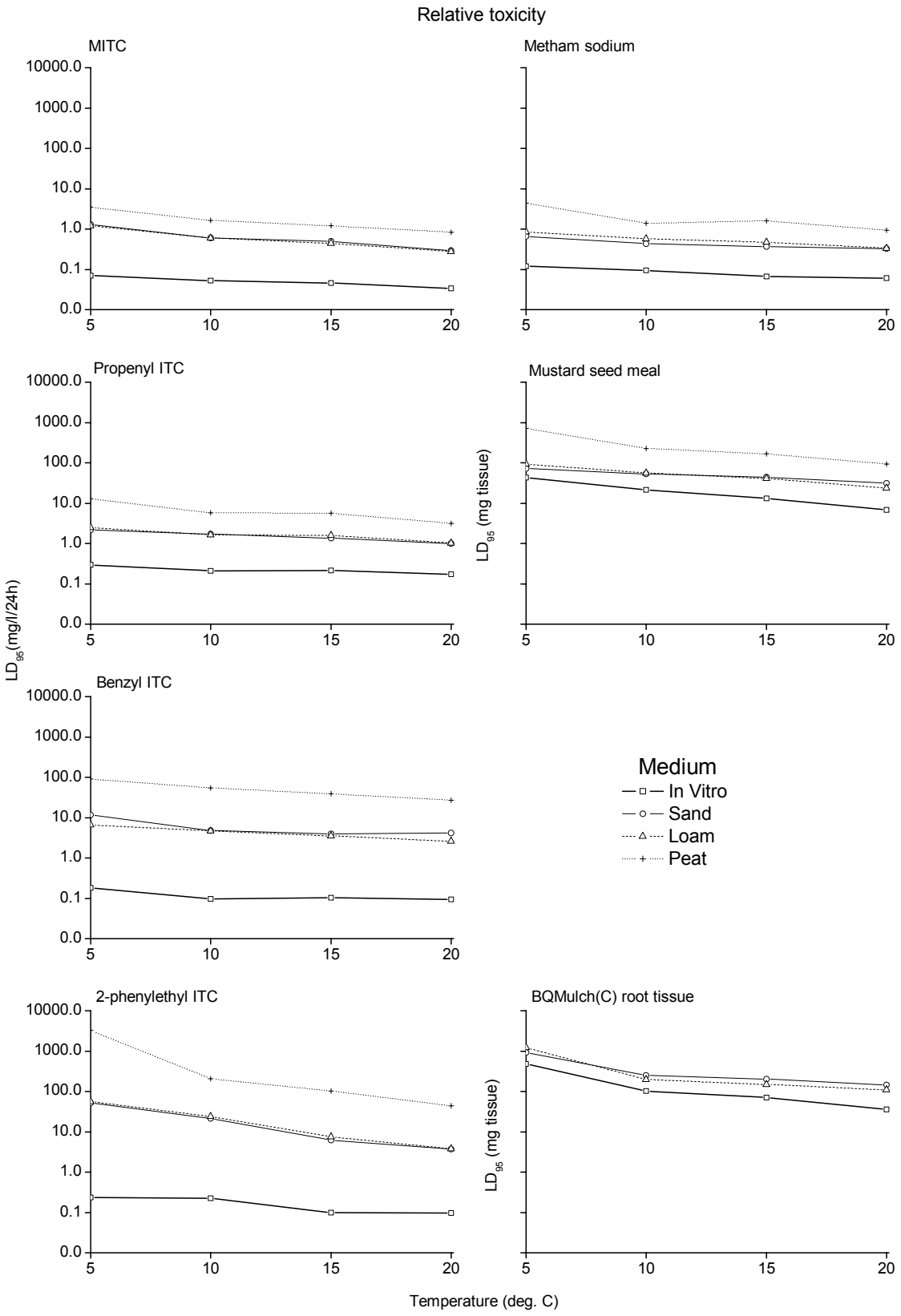

Figure 2. The relative toxicity of two aliphatic (methyl, propenyl) and two aromatic (benzyl, phenylethyl) ITCs, metham sodium and two plant tissues dominant in propenyl (mustard) or phenylethyl (fodder rape) ITC, *in vitro* and *in vivo* in three soils, at four temperatures.

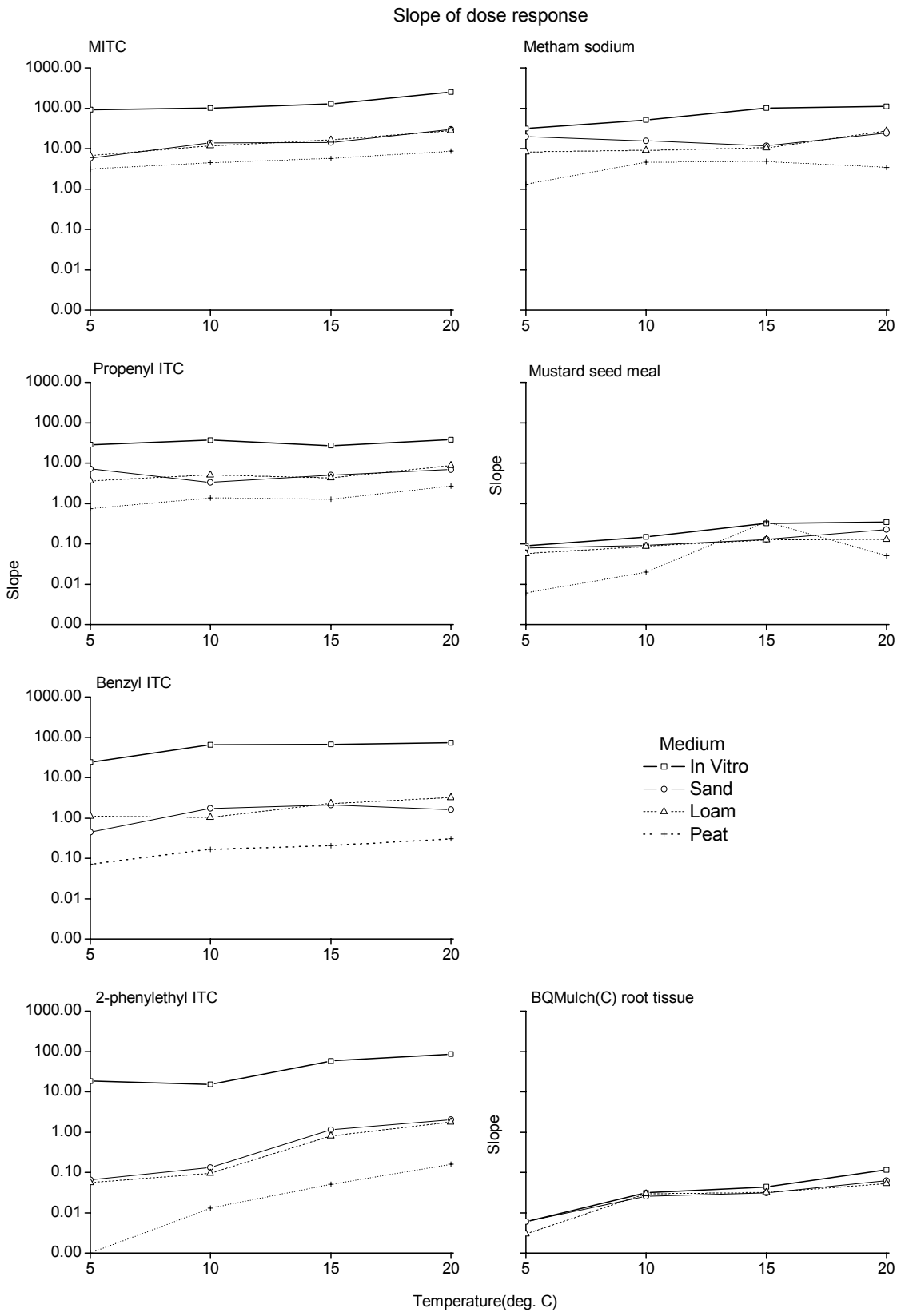

Figure 3. The slope of the probit analysis dose response lines of two aliphatic (methyl, propenyl) and two aromatic (benzyl, phenylethyl) ITCs, metham sodium and two plant tissues dominant in propenyl (mustard) or phenylethyl (fodder rape) ITC, for *in vitro* and *in vivo* bioassays in three soils, at four temperatures.

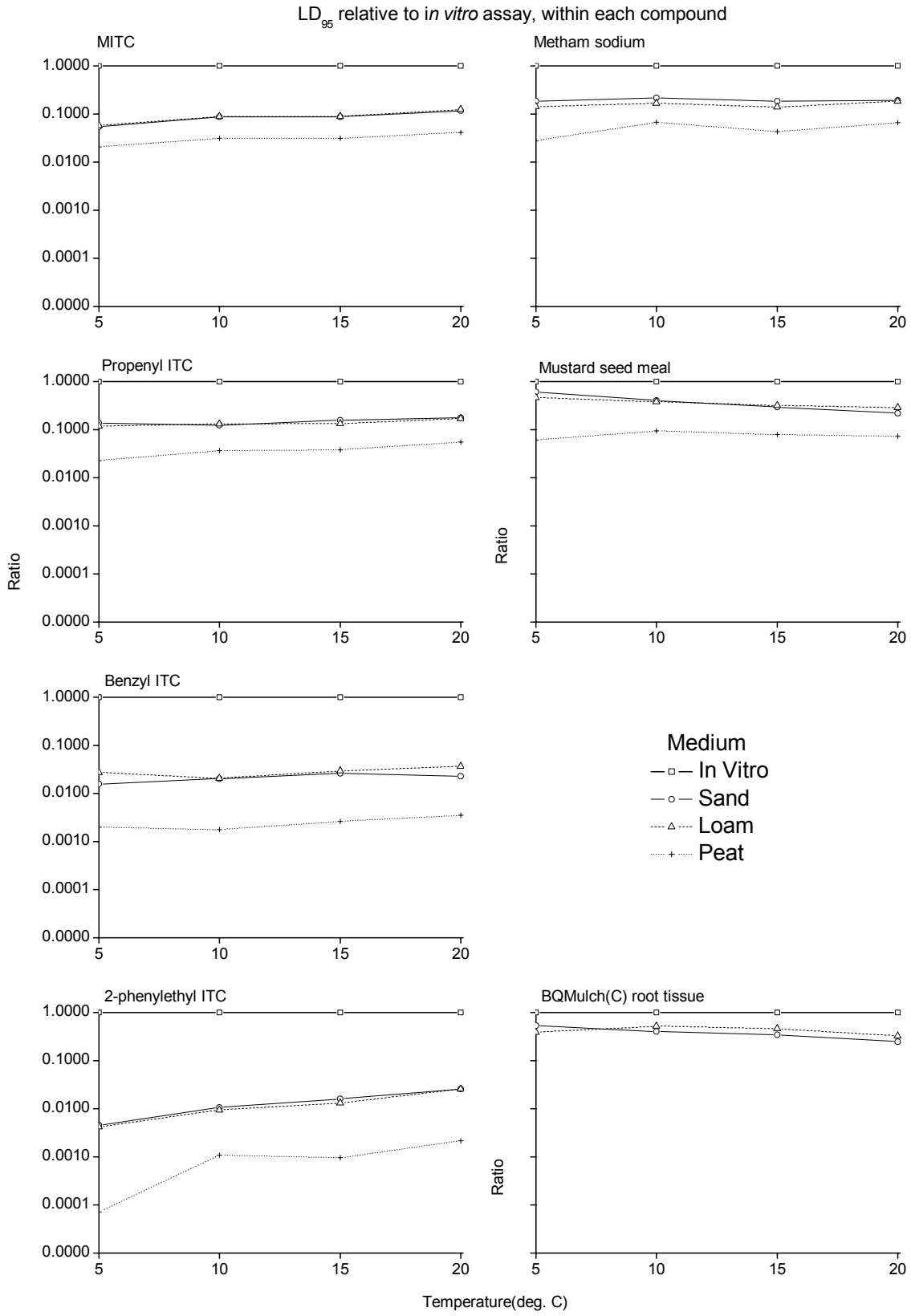

Figure 4. The ratio of the LD_{95} values of two aliphatic (methyl, propenyl) and two aromatic (benzyl, phenylethyl) ITCs, metham sodium and two plant tissues dominant in propenyl (mustard) or phenylethyl (fodder rape) ITC, *in vivo* in three soils, at four temperatures, relative to the corresponding result for *in vitro* assays within the same compound.

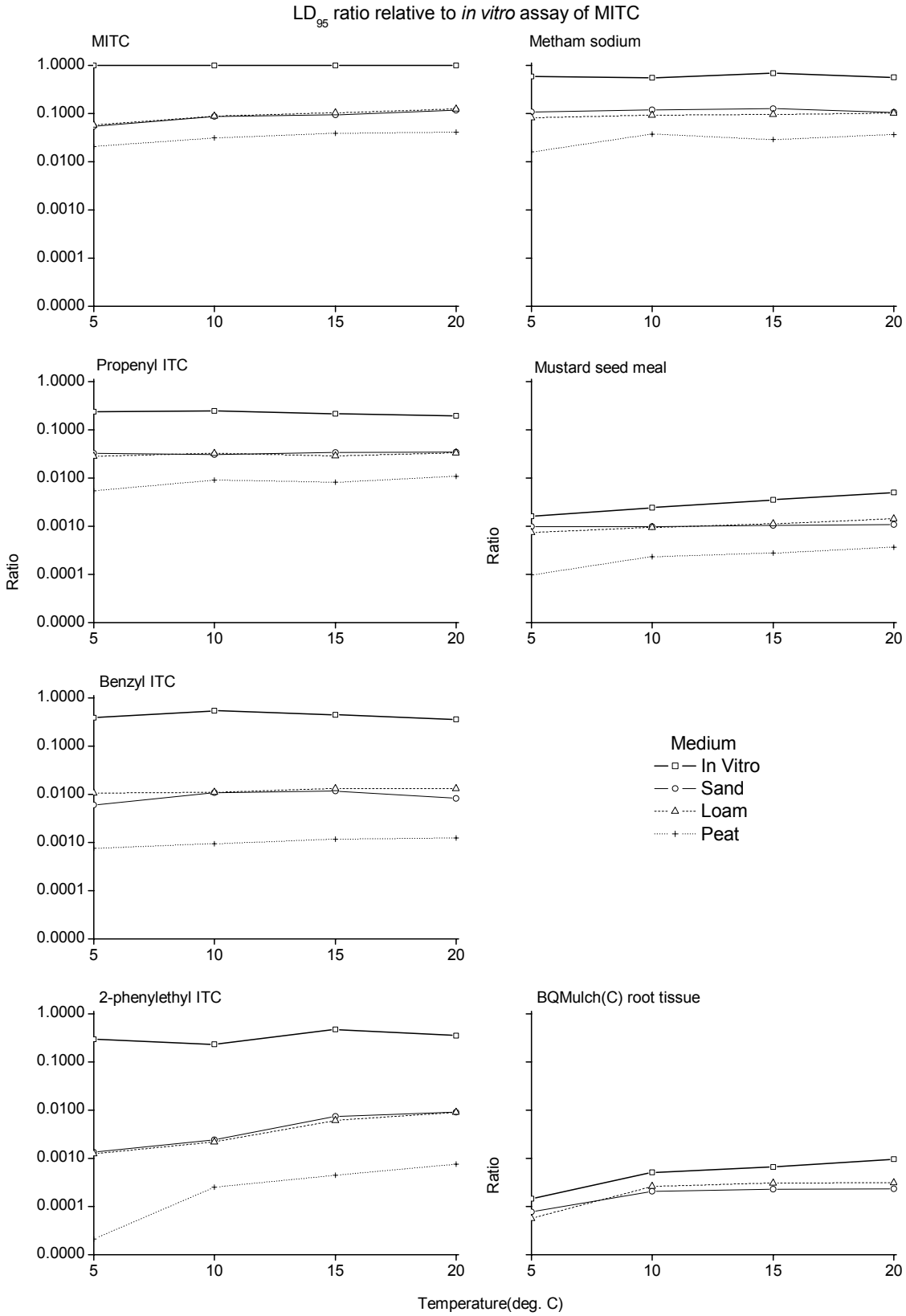

Figure 5. The ratio of the LD_{95} values of two aliphatic (methyl, propenyl) and two aromatic (benzyl, phenylethyl) ITCs, metham sodium and two plant tissues dominant in propenyl (mustard) or phenylethyl (fodder rape) ITC, *in vitro* and *in vivo* in three soils, at four temperatures, relative to the corresponding result for *in vitro* assays of MITC.

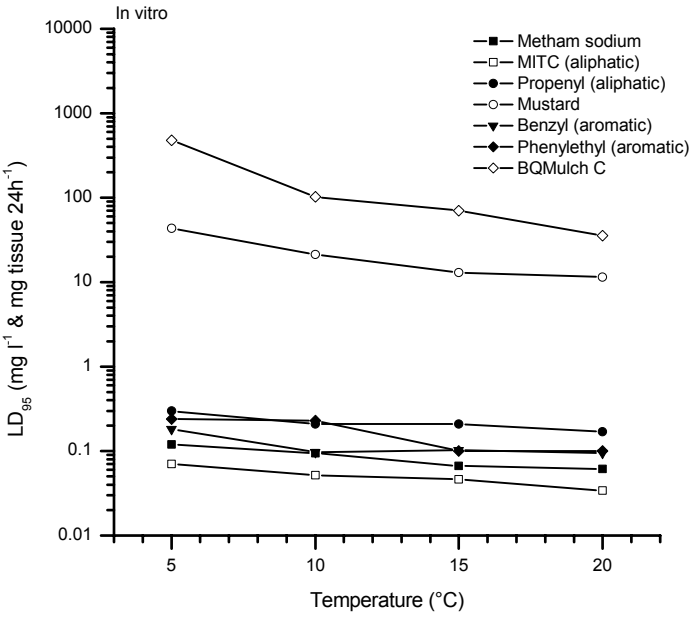

Figure 6. Toxicity of ITCs and *Brassica* tissue *in vitro*.

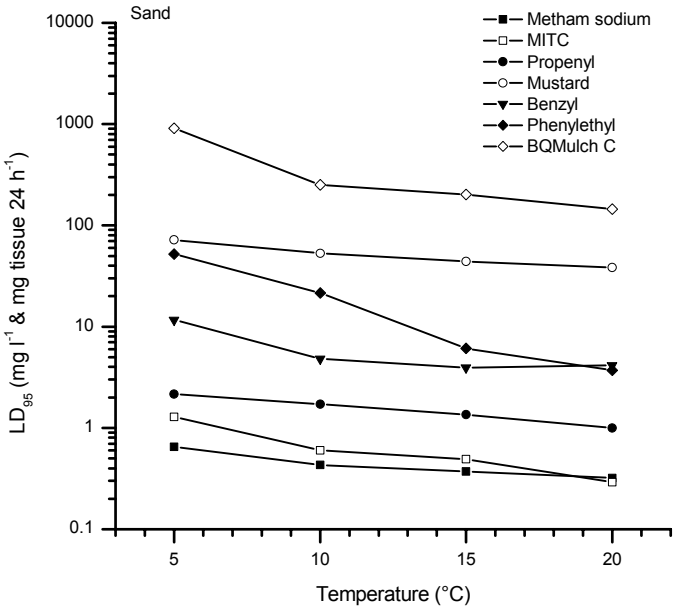

Figure 7. Toxicity of ITCs and *Brassica* tissue in sand soil.

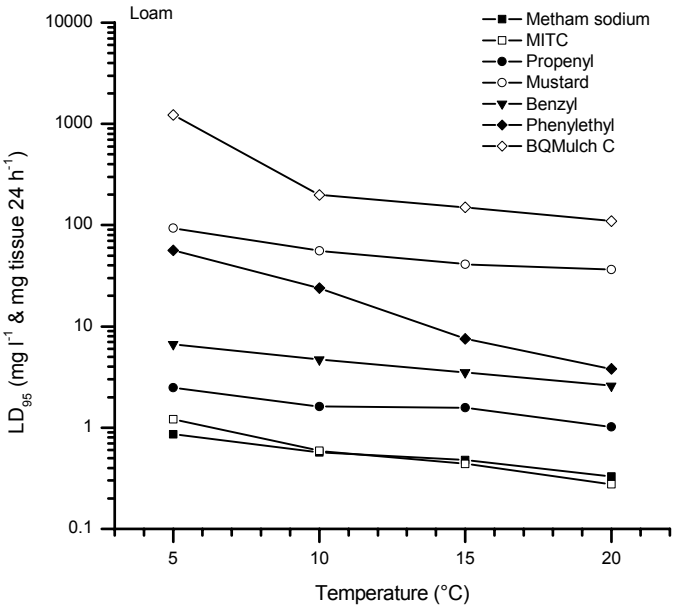

Figure 8. Toxicity of ITCs and *Brassica* tissue in loam soil.

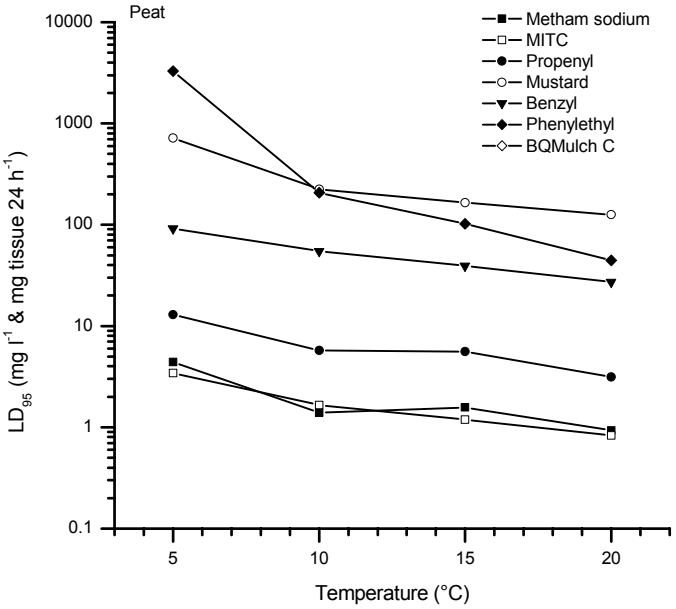

Figure 9. Toxicity of ITCs and *Brassica* tissue in peat soil.

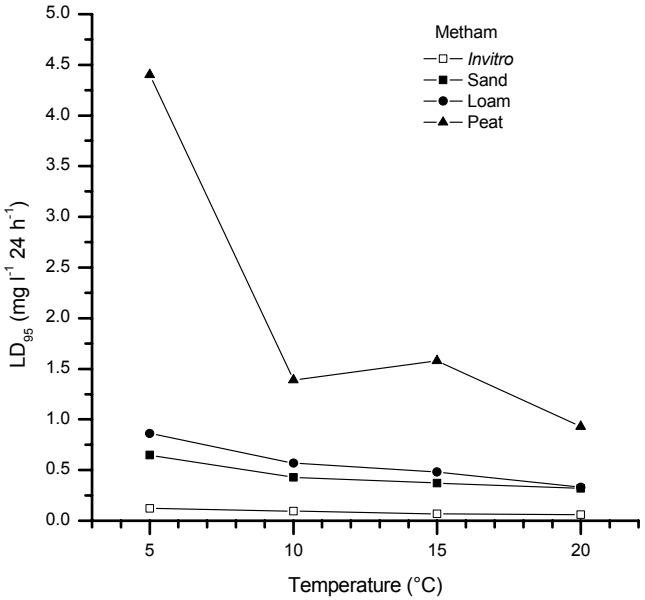

Figure 10...Toxicity of metham sodium in different media.

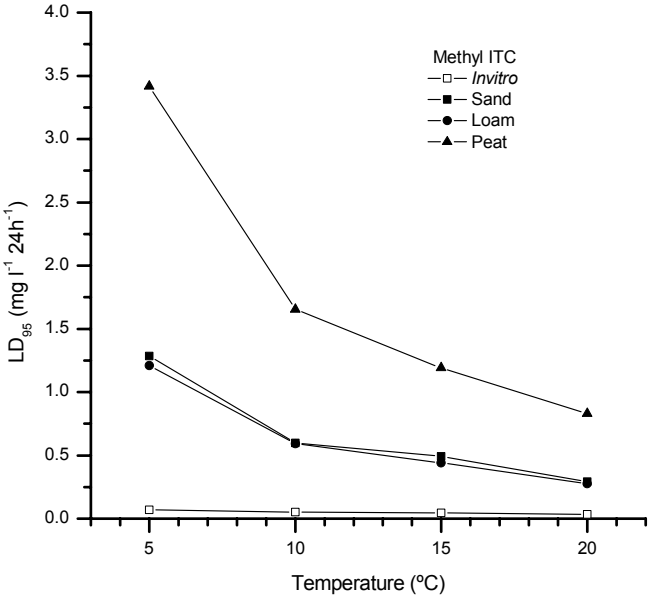

Figure 11. Toxicity of MITC in different media.

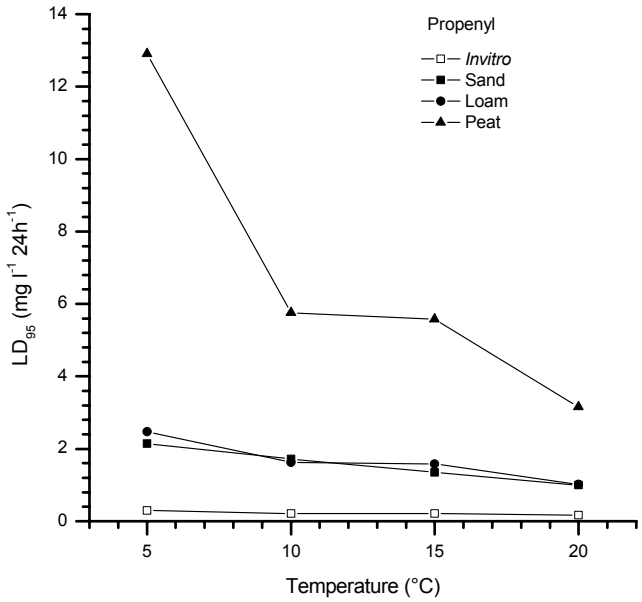

Figure 12. Toxicity of propenyl ITC in different media.

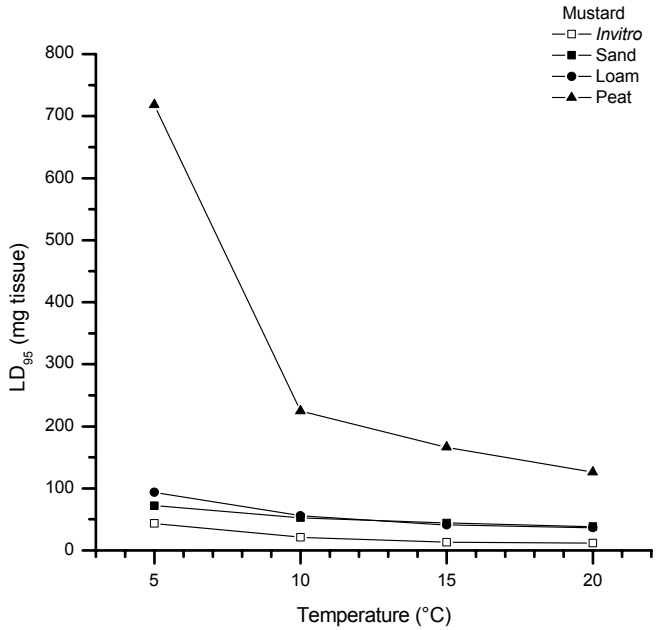

Figure 13. Toxicity of mustard tissue in different media.

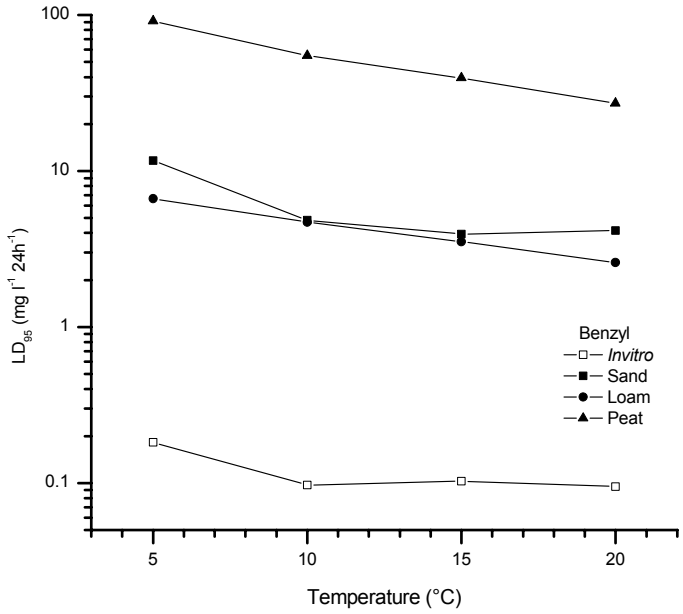

Figure 14. Toxicity of benzyl ITC in different media.

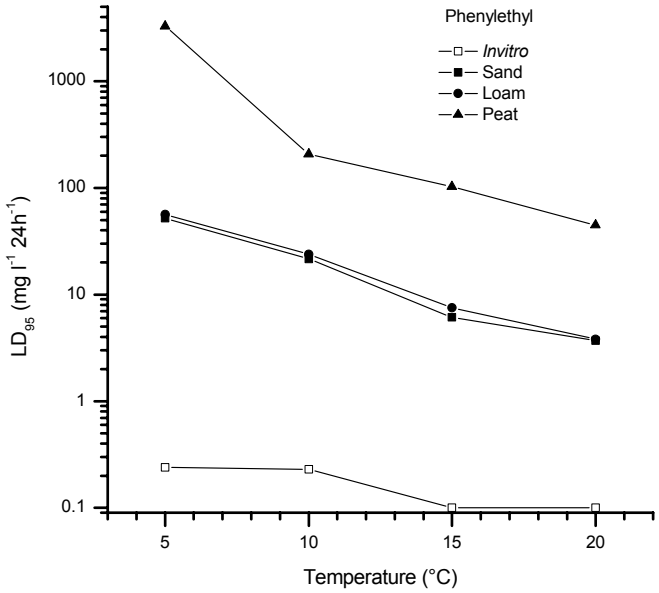

Figure 15. Toxicity of phenylethyl ITC in different media.

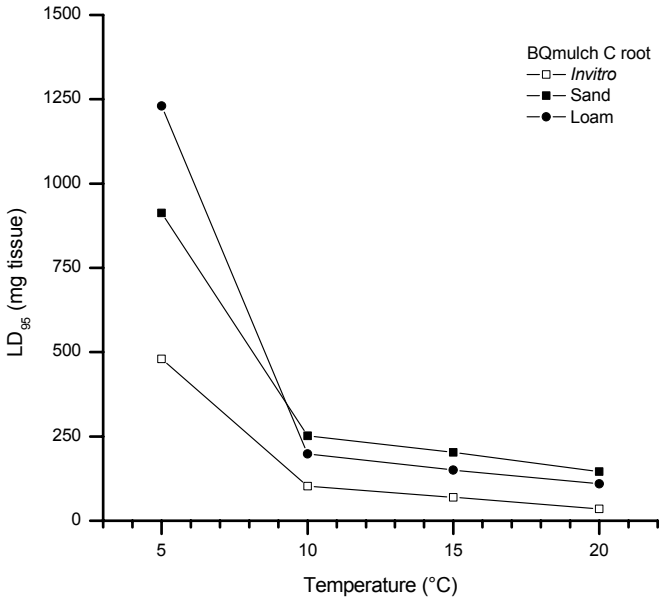

Figure 16. Toxicity of fodder rape root tissue in different media.

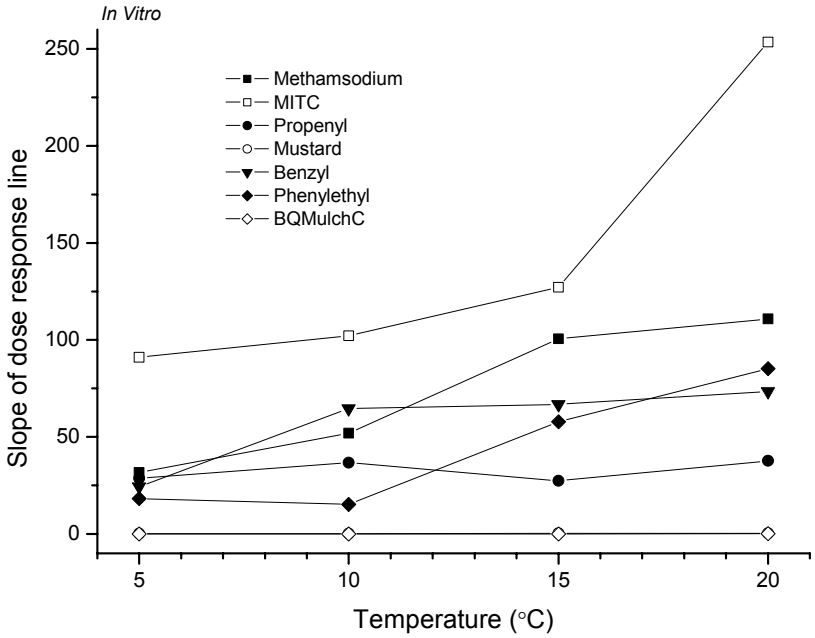

Figure 17. Slope of the probit analysis dose-response line for various ITCs and tissue *in vitro*.

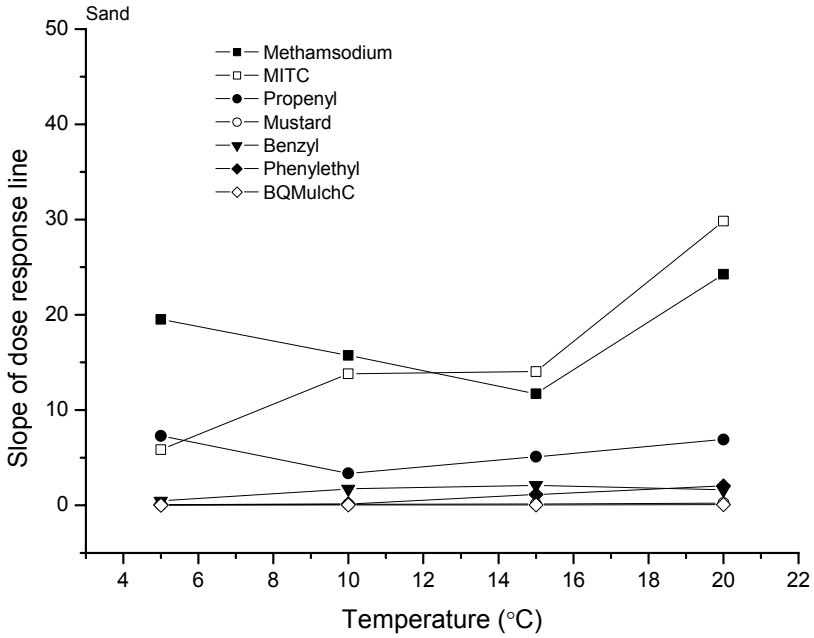

Figure 18. Slope of the probit analysis dose-response line for various ITCs and tissue in sand soil.

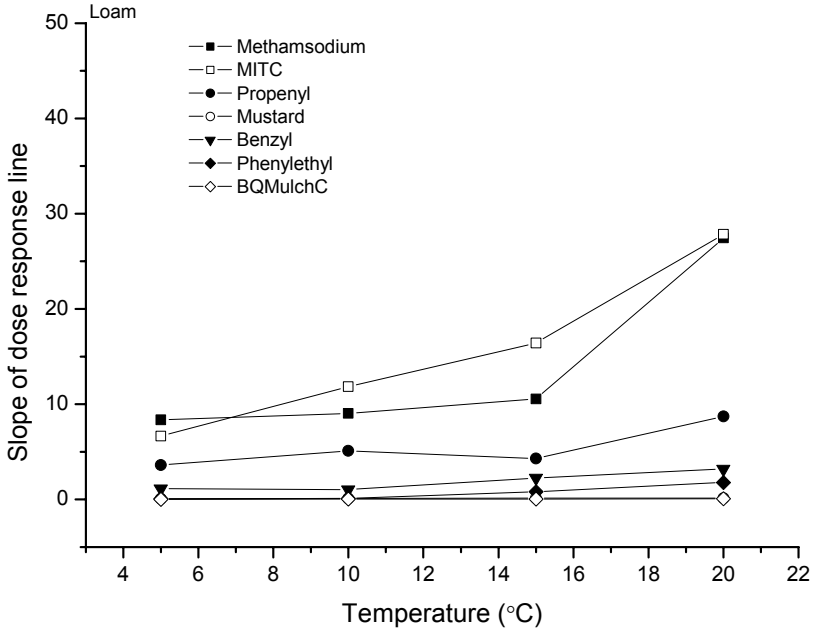

Figure 19. Slope of the probit analysis dose-response line for various ITCs and tissue in loam soil.

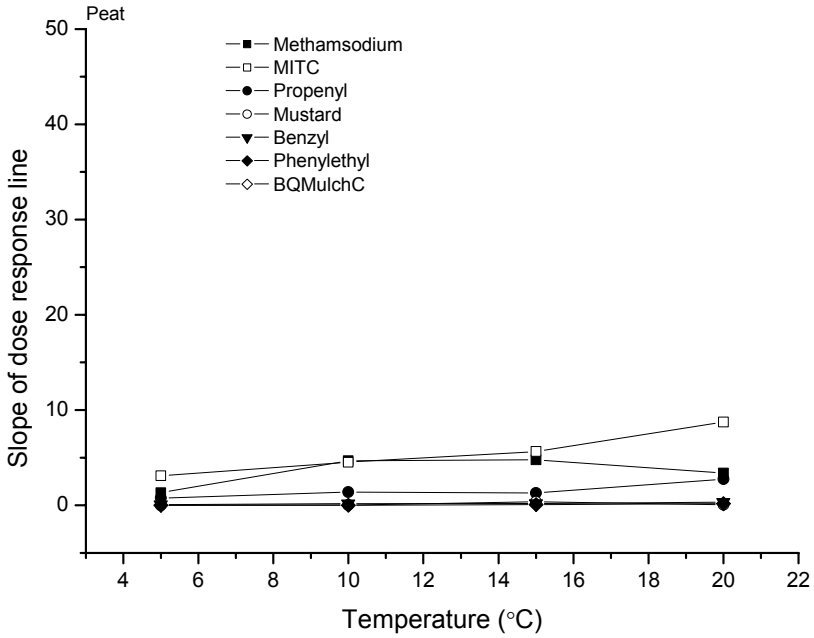

Figure 20. Slope of the probit analysis dose-response line for various ITCs and tissue in peat soil.

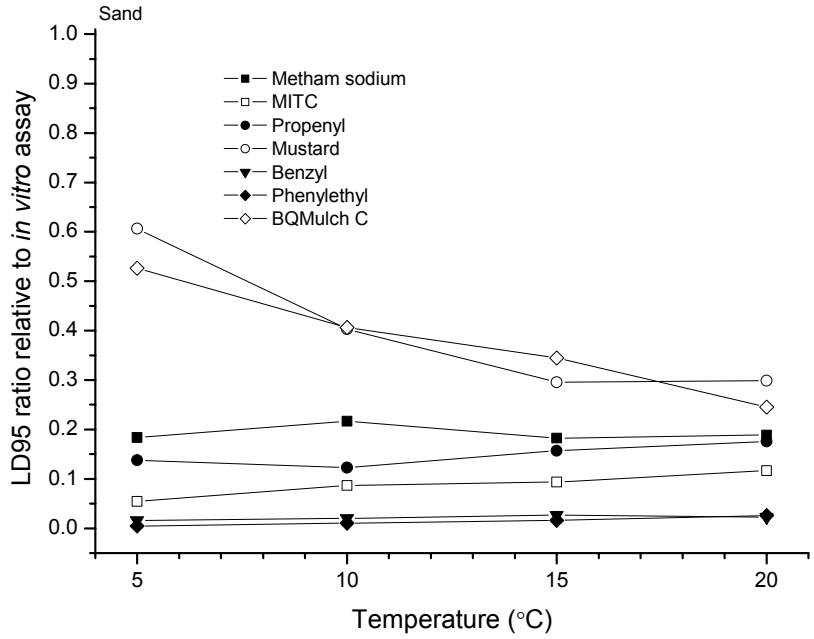

Figure 21. Ratio of LD_{95} of each compound *in vivo* to same compound *in vitro*, in sand soil.

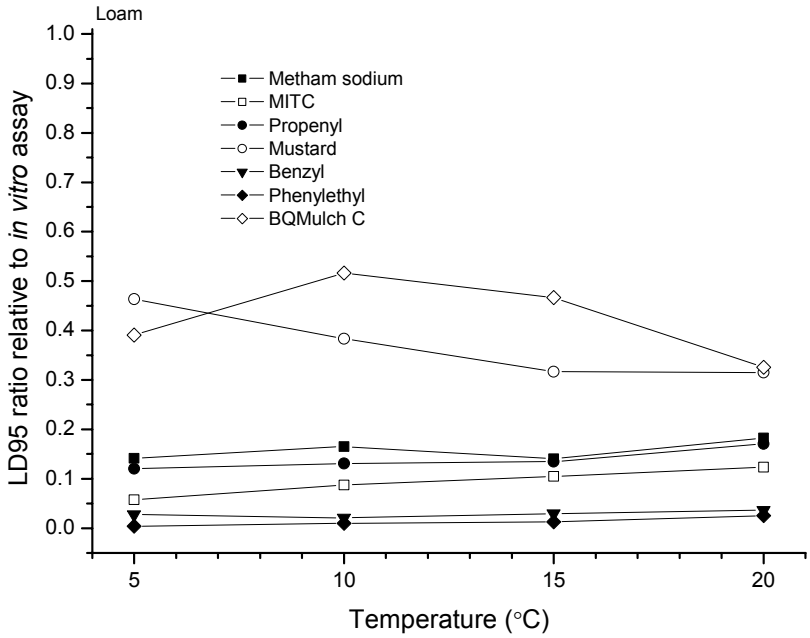

Figure 22. Ratio of LD_{95} of each compound *in vivo* to same compound *in vitro*, in loam soil.

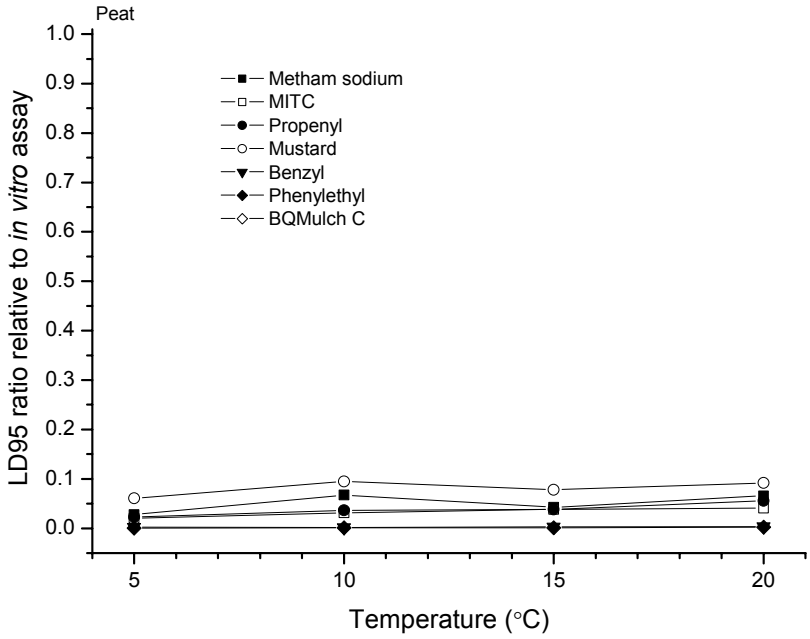

Figure 23. Ratio of LD_{95} of each compound *in vivo* to same compound *in vitro*, in peat soil.

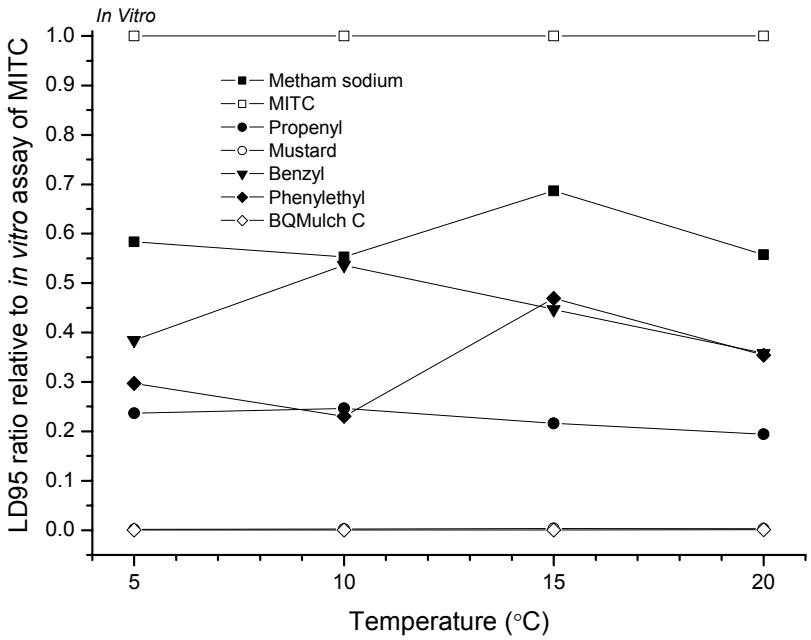

Figure 24. Ratio of LD_{95} of each compound *in vivo* to MITC *in vitro*, *in vitro*.

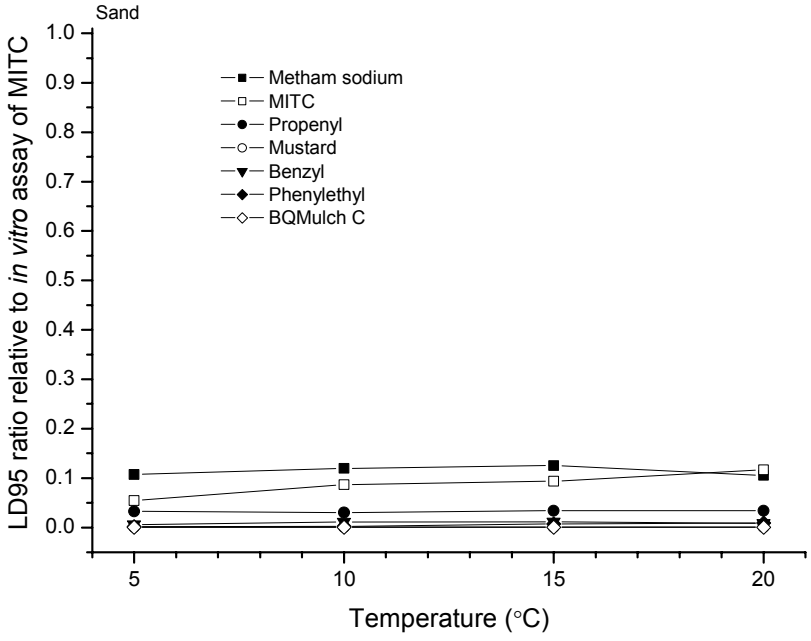

Figure 25. Ratio of LD_{95} of each compound *in vivo* to MITC *in vitro*, in sand soil.

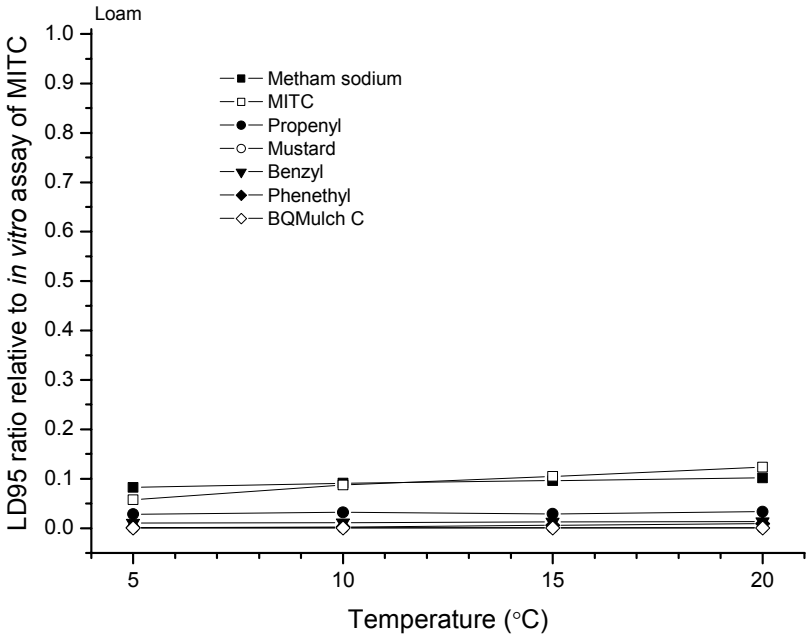

Figure 26. Ratio of LD_{95} of each compound *in vivo* to MITC *in vitro*, in loam soil.

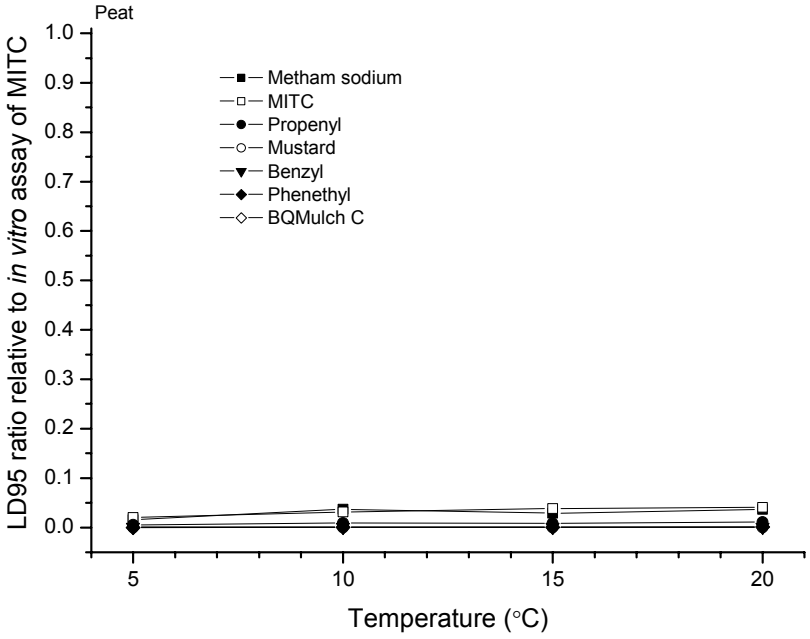

Figure 27. Ratio of LD_{95} of each compound *in vivo* to MITC *in vitro*, in peat soil.

Field assessments

Figure 28 shows the mean concentration of total ITCs in the soil of the three replicate plots of each treatment two hours after treatment of the plants. These samples were taken before any watering, so the low concentrations measured were not unexpected.

Because of the detailed nature of sampling the soil for ITCs, all other soil sampling and treatments were carried out in the adjacent plots dedicated to soil chemistry (see experimental plan above). The initial treatment of the plants (i.e. mulching, rotary hoeing) was identical in both areas, but watering was different.

The field assessment plots were sprinkler irrigated in the normal daily irrigation routine. In the soil chemistry plots irrigation was simulated in the cylinders (see section 2). As described in section 2, even after heavy watering the concentration of ITCs in the soil was low compared to what was achieved in the 2001 plots. This was attributed to insufficient pulverising of the plants because of the use of a bladed, rather than a club, mulcher.

The realisation that the mulching machinery had not provided the same level of plant pulverisation in 2002 as was achieved in 2001 came too late to modify the treatments or revise the equipment. Consequently, the results obtained are likely to not reflect what could have been achieved, certainly in terms of ITCs in the soil, with improved breakdown of the plant material that was the core objective of the study. They need to be considered in this light, as our original experimental plans were not fully met, unfortunately leaving a major question mark over the results.

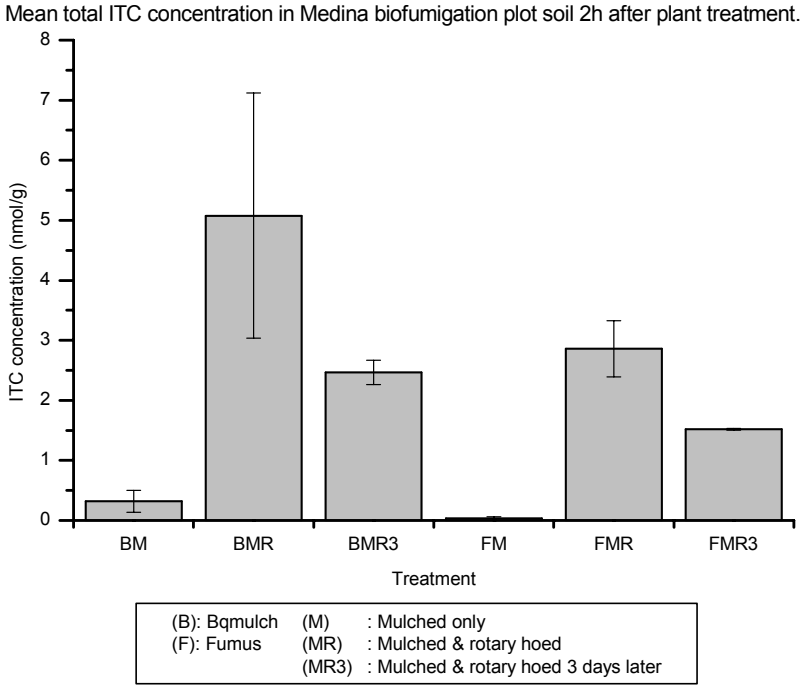

Figure 28. The mean total ITC concentration in soil at Medina two hours after treating the biofumigant plants in the biofumigation evaluation plots with various incorporation methods.

Table 6 gives the results of the seedling infection assessments, for all *Pythium* species and specifically for *Pythium sulcatum*. Analysis of variance showed that there was a significant treatment effect, with all *Brassica* treatments and the untreated showing less *Pythium* infection than the treatment where carrots preceded the test crop (Table 7).

Table 6. The levels of infection by all *Pythium* species and *Pythium sulcatum* in the roots of seedling carrots.

		linfection by all *Pythium* species (no. root sections of 5)																							
		Plot																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	Block:	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
	Tr't:	6	1	4	8	7	2	3	5	3	6	1	8	4	5	7	2	2	6	1	7	3	8	5	4
Seedling no.	1	0	0	1	1	0	0	3	1	1	0	1	0	1	1	2	2	4	0	1	2	0	2	0	1
	2	4	1	2	0	4	0	1	5	0	1	0	0	0	3	0	2	2	0	0	2	3	0	0	0
	3	1	3	0	0	3	1	1	2	2	1	2	0	0	3	1	1	0	0	0	1	0	0	0	1
	4	1	1	1	0	2	0	1	3	0	0	1	0	1	2	1	0	0	0	2	1	0	0	1	0
	5	1	0	0	0	4	0	2	3	0	0	1	0	1	2	0	1	0	0	1	2	2	1	1	0
	6	1	0	0	0	4	0	1	0	3	0	2	0	1	3	0	1	2	1	0	3	1	0	0	0
	7	0	1	0	0	2	1	1	0	0	0	1	1	0	0	0	3	3	0	0	2	0	1	0	1
	8	2	0	2	2	1	0	2	3	3	0	0	0	2	0	1	1	1	0	0	1	0	1	1	1
	9	3	1	0	1	3	0	0	1	4	2	1	0	0	3	0	2	2	0	0	3	0	1	1	0
	10	4	1	0	1	2	0	4	2	1	0	1	0	0	1	0	2	4	0	2	2	1	0	0	1
	11	1	0	1	1	1	1	1	1	1	0	2	1	0	0	1	1	1	0	2	3	1	0	0	0
	12	1	1	1	0	1	0	1	3	1	0	1	0	0	3	0	2	3	0	1	3	2	0	0	0
	13	1	0	1	1	5	1	0	1	0	1	0	2	3	0	0	1	0	1	0	3	0	1	2	1
	14	1	0	0	1	1	0	2	1	3	0	3	0	2	2	0	2	3	0	0	4	1	0	1	0
	15	0	1	0	0	3	2	3	2	1	0	1	0	1	4	0	3	0	0	1	0	0	1	0	1
	16	1	0	0	0	3	0	5	2	0	0	4	0	4	2	0	2	1	1	1	3	0	0	2	0
	17	2	0	1	2	1	1	2	2	0	1	1	0	0	0	0	3	3	1	1	3	0	1	2	0
	18	1	0	1	0	3	0	3	4	0	0	0	0	3	1	2	1	3	0	0	3	1	1	3	0
	19	1	0	0	1	2	0	2	1	1	0	0	1	2	5	1	1	3	0	1	1	0	0	1	0
	20	2	0	2	0	1	1	1	1	1	1	0	0	0	4	0	0	3	1	1	2	0	0	1	2

		Pythium sulcatum infection (no. root sections of 5)																							
		Plot																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	Block:	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
	Tr't:	6	1	4	8	7	2	3	5	3	6	1	8	4	5	7	2	2	6	1	7	3	8	5	4
Seedling no.	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	4	0	1	0	0	0	0	0
	2	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	1	2	0	0	0
	3	0	2	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
	4	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2	0	0	1	0
	6	0	0	0	0	1	0	1	0	2	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0
	7	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	3	3	0	0	0	0	0	0	0
	8	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	1
	9	0	0	0	1	0	0	0	1	2	0	0	0	0	0	0	1	2	0	0	1	0	0	1	0
	10	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	2	4	0	1	0	0	0	0	1
	11	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	1	1	0	2	0	1	0	0	0
	12	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0
	13	0	0	0	0	0	1	0	1	0	0	0	2	0	0	0	1	0	1	0	0	0	0	1	0
	14	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	2	3	0	0	1	1	0	0	0
	15	0	1	0	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
	17	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	3	3	0	1	0	0	0	2	0
	18	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	0
	19	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	3	0	1	0	0	0	1	0
	20	0	0	1	0	0	1	1	0	1	1	0	0	0	0	0	0	3	0	1	0	0	0	0	1
	Total & % (severity)	0	6	7	4	1	8	4	8	6	2	5	4	0	2	4	19	37	3	12	6	4	1	6	3
	Incidence (no. plants infected)	0	5	7	4	1	7	4	7	4	2	5	3	0	2	4	13	15	3	10	5	3	1	5	3
	% incidence	0	25	35	20	5	35	20	35	20	10	25	15	0	10	20	65	75	15	50	25	15	5	25	15

Mean incidence (%)		Treatments:
Treatment 1	33.3	1 Untreated
Treatment 2	58.3	2 Carrots
Treatment 3	18.3	3 Mustard, mulch, RH t=0
Treatment 4	16.7	4 Mustard, mulch, RH t=3d
Treatment 5	23.3	5 Mustard, mulch, no RH
Treatment 6	8.3	6 Rape, mulch, RH t=0
Treatment 7	16.7	7 Rape, mulch, RH t=3d
Treatment 8	13.3	8 Rape, mulch, no RH

Table 7. Summary of the ANOVA on seedling infection (incidence and severity)

Seedling infection - Medina																
					Mean no. of 20 infected (incidence)					Mean no. root segments infected (of 100) (severity)						
						LSD groups (P=0.05)										
Treatments:																
1	Untreated				6.7		*			7.7		*				
2	Carrots				11.7	*				21.3	*					
3	Mustard, mulch, RH t=0				3.7		*	*		4.7		*				
4	Mustard, mulch, RH t=3d				3.3		*	*		3.3		*				
5	Mustard, mulch, no RH				4.7		*	*		5.3		*				
6	Rape, mulch, RH t=0				1.7			*		1.7		*				
7	Rape, mulch, RH t=3d				3.3		*	*		3.7		*				
8	Rape, mulch, no RH				2.7		*	*		3.0		*				

As described above, the carrot crop sown to evaluate the treatments suffered growing problems that precluded a realistic assessment of yield. All harvest results are based on the proportion of the number of carrots sampled exhibiting the measured characteristics.

There was an array of categories related to both the expression of *Pythium* infection as cavity spot disease on the carrots and its effect on placement of a carrot into market categories, to size ranges related to export or domestic marketability or rejection, deformed or otherwise reject, with all the possible combinations of these measures.

Table 8 gives the percentage of carrots in each plot in each category. There was considerable variation between plots, which was hampered for analysis by only three replicates. Analysis of variance showed almost no treatment effects. The only category with any significant treatment effect was the forked carrots with no cavity spot. As the carrots were forked and therefore unmarketable, this was of little clear meaning (Table 9).

While further statistical analysis may reveal some elements of the data, it seems only likely to be hints of things that may have happened rather than practical results. Realistically, too many other issues hampered the field study to ultimately allow it to be a reliable indicator of what the treatments may have achieved.

Table 8. The percentage of carrots in the range of *Pythium* infection and marketability categories at harvest.

Medina Carrot Assessment June 2003																	
Biofumigation Plots - Total Percentage No.																	
		Cavity Spot No.					Export	Short	Total			Forked/					
Trt	Plot	0	1	2	3	>3	marketable	market	market	Undersize	Oversize	Stumped	Misshapen	Split	Other		
1	2	26.2	24.6	12.6	11.5	25.1	19.9	12.6	32.5	14.1	0.0	50.8	1.0	1.6	0.0	Bare ground	
1	11	54.1	17.9	8.7	9.2	10.1	27.1	10.1	37.2	6.0	0.0	55.5	1.4	0.0	0.0		
1	19	40.1	24.4	15.2	7.6	12.7	34.5	18.3	52.8	6.1	0.0	35.5	4.6	1.0	0.0		
	Avg	**40.1**	**22.3**	**12.2**	**9.4**	**16.0**	**27.2**	**13.6**	**40.8**	**8.7**	**0.0**	**47.3**	**2.3**	**0.9**	**0.0**		
2	6	17.2	21.3	16.7	10.9	33.9	30.8	17.6	48.4	6.8	0.0	31.2	12.7	0.9	0.0	Carrots	
2	16	35.8	26.0	14.0	8.4	15.8	43.7	20.0	63.7	7.0	0.0	26.5	2.8	0.0	0.0		
2	17	21.1	18.9	20.0	14.3	25.7	14.9	4.6	19.4	1.7	0.0	78.3	0.6	0.0	0.0		
	Avg	**24.7**	**22.1**	**16.9**	**11.2**	**25.2**	**29.8**	**14.1**	**43.9**	**5.2**	**0.0**	**45.3**	**5.3**	**0.3**	**0.0**		
3	7	53.9	21.5	10.0	6.4	8.2	34.2	21.0	55.3	8.2	0.0	33.3	2.7	0.5	0.0	Mustard mulch t=0	
3	9	54.8	25.6	9.5	7.5	2.5	12.6	5.5	18.1	6.0	0.0	71.9	3.5	0.5	0.0		
3	21	25.7	25.0	12.5	13.9	22.9	23.6	2.8	26.4	3.5	0.0	68.8	1.4	0.0	0.0		
	Avg	**44.8**	**24.0**	**10.7**	**9.3**	**11.2**	**23.5**	**9.8**	**33.2**	**5.9**	**0.0**	**58.0**	**2.5**	**0.3**	**0.0**		
4	3	24.9	25.4	10.3	8.6	30.8	21.1	13.5	34.6	7.0	0.0	57.3	1.1	0.0	0.0	Mustard mulch t=3d	
4	13	25.9	17.8	15.7	17.3	23.2	12.4	1.6	14.1	2.7	0.0	77.8	5.4	0.0	0.0		
4	24	17.7	16.0	14.7	15.2	36.4	41.6	19.0	60.6	7.8	0.0	27.3	3.5	0.9	0.0		
	Avg	**22.9**	**19.8**	**13.6**	**13.7**	**30.1**	**25.0**	**11.4**	**36.4**	**5.8**	**0.0**	**54.1**	**3.3**	**0.3**	**0.0**		
5	8	14.6	12.7	9.4	7.1	56.1	35.8	9.0	44.8	6.6	0.0	39.6	7.5	1.4	0.0	Mustard mulch, no RH	
5	14	36.3	35.3	13.5	6.0	8.8	49.8	14.9	64.7	7.0	0.0	27.0	1.4	0.0	0.0		
5	23	30.8	29.9	12.1	11.2	16.1	48.2	19.6	67.9	6.7	0.0	20.1	4.0	1.3	0.0		
	Avg	**27.2**	**26.0**	**11.7**	**8.1**	**27.0**	**44.6**	**14.5**	**59.1**	**6.8**	**0.0**	**28.9**	**4.3**	**0.9**	**0.0**		
6	1	33.3	19.9	15.9	9.5	21.4	6.0	1.5	7.5	5.5	0.0	86.1	1.0	0.0	0.0	BQ mulch t=0	
6	10	41.8	26.4	12.1	8.4	11.3	36.4	19.7	56.1	11.3	0.0	26.4	6.3	0.0	0.0		
6	18	51.5	19.2	12.7	6.1	10.5	57.2	17.9	75.1	2.6	0.0	17.5	4.4	0.4	0.0		
	Avg	**42.2**	**21.8**	**13.6**	**8.0**	**14.4**	**33.2**	**13.0**	**46.2**	**6.5**	**0.0**	**43.3**	**3.9**	**0.1**	**0.0**		
7	5	42.1	28.2	9.1	9.1	11.5	10.5	2.9	13.4	4.8	0.0	80.4	1.4	0.0	0.0	BQ mulch t=3d	
7	15	46.0	21.8	13.3	6.2	12.8	32.2	19.9	52.1	9.5	0.0	36.5	1.4	0.5	0.0		
7	20	23.1	21.3	10.9	10.9	33.9	48.0	15.4	63.3	6.8	0.0	27.1	2.7	0.0	0.0		
	Avg	**37.1**	**23.8**	**11.1**	**8.7**	**19.4**	**30.2**	**12.7**	**43.0**	**7.0**	**0.0**	**48.0**	**1.9**	**0.2**	**0.0**		
8	4	14.7	18.8	18.2	14.7	33.5	32.4	10.6	42.9	5.9	0.0	42.4	8.8	0.0	0.0	Bq mulch, no RH	
8	12	17.1	16.1	12.7	15.6	38.5	48.8	14.6	63.4	7.8	0.0	27.3	1.5	0.0	0.0		
8	22	51.2	20.8	12.4	9.6	6.0	44.8	24.8	69.6	8.4	0.0	17.2	4.8	0.0	0.0		
	Avg	**27.7**	**18.6**	**14.4**	**13.3**	**26.0**	**42.0**	**16.7**	**58.7**	**7.4**	**0.0**	**29.0**	**5.0**	**0.0**	**0.0**		

Table 9. The only significant treatment effects observed at harvest.

Harvest								
No significant treatment effects in all major parameters.								
CS0Forked was the only category with 2 LSD groups (P=0.05):								
Treatments:								
1	**Untreated**				21.3	*	*	
2	**Carrots**				10.4	*	*	
3	**Mustard, mulch, RH t=0**				25.7	*		
4	**Mustard, mulch, RH t=3d**				14.3	*	*	
5	**Mustard, mulch, no RH**				9.8	*	*	
6	**Rape, mulch, RH t=0**				16.2	*	*	
7	**Rape, mulch, RH t=3d**				21.9	*	*	
8	**Rape, mulch, no RH**				6.7		*	

Overview of stage of growth and size of plants at time of incorporation treatments at Medina 7 October 2002.

After mulching and rotary hoe incorporation of BQMulch, in foreground plots.

Various mulching, incorporation and left on the surface treatments.

Irrigating, with plots for 3-day incorporation of mulched plant material left on surface covered with plastic only during watering until then.

BIOFUMIGATION WORKSHOP

Busselton, Western Australia
11-13 September 2002

Convenor:

John Matthiessen
CSIRO Entomology, Perth

Introduction

Background

Biofumigation, in the context used by the CSIRO researchers working on the concept in Australia, is the use of *Brassica* plants as biologically-active rotation and green manure crops for the suppression of soil-borne pests and diseases through a particular focus on the capacity of such plants to produce an array of isothiocyanates (ITCs).

There are diverse data and anecdotal reports to show that brassicas can have beneficial effects as a break in cropping cycles. It is recognised that these effects within brassicas may not come solely from the liberation of ITCs. Toxic or otherwise beneficial effects analogous to these 'biofumigation' attributes can arise through the liberation of other compounds - in both brassicas and other species - and through other attributes of these plants such as the physical impact of their agronomic characteristics on the soil.

It is also recognised that the word 'biofumigation' a little too easily carries with it connotations of oversold 'silver bullet' cures for the very difficult issues that soil-borne pest and disease management present, especially in horticulture. There is a natural tendency to lapse into shorthand, but the word 'biofumigation' relates to the broader beneficial effects that can be obtained from the biological activity of 'biofumigant green manure crops'.

The focus of CSIRO research has unashamedly been on the ITC-generating aspects of brassicas as biofumigants in a specific endeavour to systematically work through the mechanisms of the observed beneficial effects and their apparent close association with ITC production, notwithstanding other effects.

The objective has been to harness and improve the beneficial aspects through the toxicity of ITCs to offer farmers a more comprehensive suite of options or alternatives for the vexed issue of soil-borne pest and disease management, than either sole reliance on synthetic fumigants or doing nothing.

Rationale for workshop and timing

In the last year or so, results from laboratory and field work in Canberra (John Kirkegaard and Matt Morra), and field work in Western Australia (John Matthiessen, Ben Warton, Mark Shackleton) have shown vast improvements in the release of ITCs by *Brassica* plant tissue, resulting from the way it is treated.

In summary, it has been found that disruption of tissue at the cellular level (first noted by freezing and thawing tissue, and subsequently replicated in the field by pulverising tissue with a 'mulcher') leads to a substantial increase in ITC release. The addition of water further improved that effect, apparently either through facilitating greater hydrolysis and/or carrying greater amounts of ITC into the soil.

In both the laboratory and the field the release of ITCs was improved around 100-fold by these 'new' ways of treating the tissue. These results were very exciting, and put a new complexion on the research.

The findings also marked a concerted push by the CSIRO group in Western Australia to develop methods for measuring concentrations of ITCs produced in soil from brassicas treated in different ways (and of methyl ITC from application of metham sodium).

A further implication was that it raised the question of what was the importance of plant treatment methods relative to the main early focus of endeavouring to build ITC-releasing capacity in brassicas by selecting for high concentrations (and type) of the precursor glucosinolates.

As a consequence of these apparently significant advances and the potentially large shifts in approach the new information could engender, it was felt timely to convene a small workshop of people of diverse background knowledgeable about aspects of soil-borne pests and diseases, the agronomy and breeding of brassicas, chemistry and horticultural production systems to discuss issues and critique approaches.

The aim was to add to everyone's knowledge, discuss recent findings, help keep focus, ensure high levels of cross-fertilisation of knowledge, implications and ideas across disciplines, help in the process of disseminating practical information to horticultural producers by ensuring workshop participants were 'hands-on' people, and develop potential collaboration.

In keeping with the objective of an informal, practical orientation, the workshop was convened for spring and at a country location to allow visits to sites where trials were under way and the brassicas were nearing the stage of being treated to obtain biofumigation effects.

Accordingly, the workshop was held at Busselton, Western Australia where the CSIRO Entomology group has been doing field work with the assistance and collaboration of potato farmers Keith and Paula Taylor. A field visit was also made to the Department of Agriculture, WA's Medina research station where CSIRO has trials in the *Pythium* disease 'nursery' developed by Elaine Davison and colleagues.

Participants

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17. Keith Taylor, farmer, Busselton
18. Paula Taylor, farmer, Busselton
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Program

Wednesday 11 September

Morning: Travel Perth-Busselton
Afternoon: Workshop session

Thursday 12 September

Morning: Seminar by Dr Sandro Palmieri, specialist glucosinolate biochemist from the Research Institute for Industrial Crops, Bologna, Italy and McMaster Fellow with John Matthiessen. Seminar entitled: "Brassicaceae: a good source of industrial oil, high value proteins and bioactive derivatives."
Keith & Paula Taylor's potato farm
Afternoon: Workshop session

Friday 13 September

Morning; Travel Busselton-Medina research station
Biofumigation trials with carrots and *Pythium* Medina Research Station
Afternoon: Laboratory visit, CSIRO

Workshop summary

The workshop discussion ranged widely over the multitude of issues that invariably come to the fore when discussing methods for managing soil-borne pests and diseases and the implications of various approaches.

In brief, topics covered included:

History
Reasons for workshop & aims
Expertise/interests of participants
Factors driving soil fumigation
Recent developments in biofumigation
Current focus of research
Industry expectations
Managing 'hype' and over-expectations
Chemistry – in plants and in soil
Other beneficial effects of 'green manure biofumigant crops'
Practical growing issues
Incorporation methods
Comparison with metham sodium
Fitting to crop rotations and systems
Longevity of effects/plantback times
Breeding
Pathogens – recalcitrant resting stages
Co-hosting issues
'Soil health'/ecological functioning
Seed companies' perspective
Economics/cost effectiveness
Setting realistic expectations
General upside/downside issues
Collaborative interactions
Application in tropics/developing countries

Inevitably, for such a complex and multi-facetted issue, some of the issues were complex and the hurdles high but it was resolved to utilise the diverse expertise present to contribute to putting out a simple as possible information sheet for industry.

The plan is that this information brochure will be drafted during the next few months and circulated amongst workshop participants for comments in order to make it as comprehensive and practical as possible, before being made available for widespread distribution to industry.

Acknowledgements

Funding support for the workshop was provided by Horticulture Australia Limited and Wrightson Research.

MCMASTER FELLOWSHIP

John Matthiessen was awarded a CSIRO McMaster Fellowship in 2002 to fund the visit to Australia of Dr Sandro Palmieri, Director, Biochemistry Section, Istituto Sperimentale per le Colture Industriali, Italian Ministry of Agriculture, Via di Corticella, 133, I-40129, Bologna, Italy. Dr Palmieri's research group has over many years painstakingly isolated 22 of the most widely occurring GSLs in brassicas. It is the only group in the world to possess such a collection, and the group's members are recognised world leaders in GSL chemistry. The following is Dr Palmieri's report on his fellowship visit.

Report of Dr. Sandro Palmieri of the Research Institute for Industrial Crops, Bologna, Italy as McMaster Fellow, September 2002.

Duration, location and timing: 9-20 September, 2002; one week at CSIRO Entomology Floreat, WA and one week at CSIRO Plant Industry, Black Mountain, Canberra.

Host Division and host Scientists: Entomology – John Matthiessen (Principal Research Scientist & Project Leader, Sustainable Soil Pest Management); John Kirkegaard (Principal Research Scientist & Project Leader, Crop Management, CSIRO Plant Industry).

Visit aim: Participation at the Biofumigation Workshop and discussion of the main aspects regarding the chemistry of the myrosinase-glucosinolate system as a defence structure in Brassicaceae, as well as its exploitation in green manure and in special formulations after glucosinolate isolation.

Presentations: A seminar entitled: "Brassicaceae: a good source of industrial oil, high value proteins and bioactive derivatives." was presented at the Biofumigation Workshop on 12 September, and at CSIRO Plant Industry on 17 September.

Report

For some years, attention on bioactive natural molecules has strongly increased because public opinion considers them as a mild, safe and reliable option to control different plant pathogens, thus limiting the use of synthetic pesticides in agriculture. If these bioactive molecules are used as they are in plant tissues, without applying any chemical procedure of extraction and isolation, they meet even more interest and support. Among several bioactive molecules, glucosinolates (GLs) and their enzymatic degradation products (GLDPs) via myrosinase (MYR) stand out as a valid alternative due to the variety of compounds that can be produced of high cytotoxic activity.

The MYR-GLs system contained mainly in Brassicaceae appears to be of great value not only in crop protection but also in fine chemistry and food technology. At present several studies are in progress to find the best techniques for its practical use in suitable forms in the above sectors. Prominent among these is research being carried out by CSIRO Entomology and Plant Industry, and my own Research Institute for Industrial Crops at Bologna. Visits by Australian researchers to Bologna in recent years have established a good collaboration. This McMaster Fellowship has further advanced and cemented that relationship and provided opportunities for further collaboration.

One of the most interesting uses of the MYR-GLs enzymatic system is for pest control in horticulture, given the high economic value of horticultural products. In organic agriculture, which today is finding greater favour with farmers and consumers, the exploitation in different forms of the MYR-GLs system seems to be one of the few acceptable ways for controlling pests in soil. In this context, it is easy to predict an increasing interest to develop and apply different techniques based on the activity of the MYR-GLs system in agriculture.

In the last years several research teams have been involved in the exploitation of the MYR-GLs system in different ways and forms. One of the most active research groups is working at CSIRO Entomology and CSIRO Plant Industry, especially in the practice of biofumigation by green manure of brassicaceae, for controlling soil-borne pests and diseases, while my institute has focused on the detailed biochemistry of the MYR-Gls system. The research of the two teams is very complementary, and provided the impetus for this McMaster fellowship application.

Field trials carried out at CSIRO and at the Research Institute for Industrial Crops (Bologna, Italy) (ISCI) indicate that biofumigation practice, using some Brassicaceae such as *Brassica juncea*, *Brassica napus, Eruca sativa, etc.*, is useful to improve the yield and quality of the following crops. In Australia this has been successfully obtained on wheat using canola (*Brassica napus* ssp. oleifera) and Indian mustard (*Brassica juncea)* as previous crops. This positive effect has been obtained, at least in part, by breaking the life cycle of the soil borne pathogens as consequence of the cytotoxicity of the GLDPs, essentially isothiocyanates (ITCs) released from the roots in the soil.

In the same period, the results of the green manure trials carried out in Italy also confirm the advantages on the yield and quality of following crops. In Italy these trials were made with the aim of protecting some horticultural crops such as strawberry and some vegetables (lettuce, carrots etc.) by green manure using Indian mustard (*Brassica juncea*), *Rapistrum rugosum*, and rocket (*Eruca sativa*).

In spite of the uncontestable practical benefit obtained by the use of the biofumigation carried out with different techniques and plants, some important theoretical aspects have still to be defined to completely exploit the advantage of brassicas use in crop rotation or in green manure. For instance, it is not clear if the ITCs found in the soil during the cultivation of brassicas are leached as they are from the roots or they are formed in the soil after the GLs degradation by the action of soil microorganisms.

Another important aspect is the fate of these cytotoxic compounds in the soil after their formation and protection of the plants against soil diseases. Finally, the study of the enzymatic degradation chemistry of some GLs such as the phenyl ethyl and indolyl appears to be a further important aspect that has to be defined in detail to completely understand their potential in crop protection when plants or their tissues containing these GLs are used in biofumigation.

Although these studies are not so easy to carry out, particularly considering the complexity of relationship between roots (tissues) and soil, at present several experiments are in progress at CSIRO and at ISCI with the objective to make clear some of these points. At ISCI a simple approach has been chosen, based on the study of the enzymatic reaction in vitro, using pure MYR and GLs and by determining the produced GLDPs by chromatographic methods, whereas at CSIRO more complex experiments are in progress, aimed at the study of the entire

plant-soil system, which involves the investigation of complex chemical reactions and related microbiological outcomes. Again, these studies are complementary and provide for elucidation of complex issues.

It is clear that a sound understanding of the fundamental basis of biofumigation is matter of primary importance to maximize the benefit due to the application of this technique and, above all, to define reliable agrotechnical pathways for its correct management, according to the different agroclimatic conditions in which the technique has to be applied. Thus taking into account the specializations of the two groups, it will be important to set up suitable common and complementary experiments not only to immediately have practical advantages, but also to unequivocally ascertain which are the strengths and the weak points of this technique, possibly in relationship with agroclimatic conditions and pathogen infestation levels.

Another important aspect for environmental and economic reasons is the use of the MYR-GLs system to control phytophagous insects. At present however the natural defense mechanism based on this enzymatic system in Brassicaceae against non-specialist insects is still not completely explained. In fact, it is known that when the insect attacks the plant it generally makes a wound with its ovipositor, stilet or masticatory apparatus, putting GLs and MYR in contact and thereby triggering an overproduction of cytotoxic compounds only in the point of attack. Although this reaction has been demonstrated for several noxious insects attacks, in some cases it was observed (using pure GLs in vitro, of which the ISCI group has a unique collection purified from many Brassicaceae), that also some intact GLs show a toxic activity.

It would be interesting to determine if this finding is due to the presence of a MYR-like enzyme inside the insect or must be ascribed to an intrinsic toxicity of the intact GL towards a specific insect. Also this point could be studied and clarified with a collaborative project between CSIRO Entomology and ISCI, given that CSIRO has set up a reliable insect bioassay suitable to test pure GLs, while ISCI has available more that 22 different GLs in pure form. ISCI also has experience in enzyme isolation and purification, to have available two pure MYRs as reference.

In conclusion, the visit in Australia has been of great and twofold interest. From one side the participation at Biofumigation Workshop held in Busselton has made it possible to focus on the most important theoretical, practical and potential aspects of this technique for controlling soil-borne fungi, nematodes and insects in different climatic and cultivation conditions. Visits to field trials (potato and carrots) were important to directly assess the beneficial effect of biofumigation. From the other side, the meetings and discussion at CSIRO with the inviting scientists of CSIRO Entomology and Plant Industry, as well as with many other researches and project leaders of these two CSIRO Divisions made it possible to become more aware of the wide significance and the high level of research carried out at the above Institutions.

Acknowledgments: I thank the McMaster Fellowship Committee of CSIRO for the opportunity to undertake this visit to Australia to offer the experiences of my team at ISCI, Bologna, and to build further relationships with the Australian research teams in their home bases.

Dr Sandro Palmeri
3 October 2002

PUBLICATIONS

During the project considerable effort was put into publishing the results in a wide variety of outlets to ensure broad dissemination of information and uptake of results, both nationally and internationally.

The '*Biofumigation Update*' newsletter was continued as a means of disseminating information in a timely manner. Six issues of the '*Biofumigation Update*' were produced at six-monthly intervals during the project.

The mailing list reached approximately 650, comprising a diverse mixture of farmers, consultants and researchers, nationally and internationally. It was well-received by stakeholders, with positive feedback common. It was also published on the World Wide Web at:
http://www.ento.csiro.au/research/pestmgmt/biofumigation/newsletter_list.html.

Images of the newsletters follow.

Horticulture
Biofumigation Update
CSIRO

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The Glucosinolate-Myrosinase System — A Natural & Practical Tool for Biofumigation

An integrated team at the Research Institute for Industrial Crops (ISCI)—Italian Ministry of Agriculture, at Bologna, has done much research ranging from basic chemistry to applications of the glucosinolate-myrosinase system. The team has put together the following account of their varied activities.

Introduction and rationale

Over the last decade, attention to bioactive natural molecules has strongly increased because public opinion considers them as a mild, safe and reliable option to prevent or to fight not only several diseases in humans but even different plant pathogens, thus reducing use of pesticides.

Among bioactive molecules, glucosinolates (GLs) and their enzymatic degradation products (GLDPs—which includes isothiocyanates) via myrosinase (MYR) stand out as promising, through the variety of compounds that can be produced and for their high bioactivity and selectivity.

In vitro studies, using purified GLs and MYR, confirm that GLDPs show high biocidal activity. For this reason, some GLs or GLDPs could be used in controlling post-harvest fruit pathogens, in killing soil nematodes and some soil-borne pathogenic fungi.

We have demonstrated that the biocidal activity of GLDPs is strongly affected by the chemical structure of their side chain. In particular, thiofunctionalised GLs (glucoiberin, glucocheirolin, glucoerucin, glucoraphenin), some alkenyl GLs (sinigrin, glucocapparin) and benzyl GLs (glucotropaeolin, gluconasturtin) are precursors of GLDPs with high biological activity.

The reasons for this good performance, which is better than other GLDPs, such as hydroxy-GLDPs (goitrin and *epi*-goitrin), are still not clear. Studies are in progress in our laboratory to explain the action mechanism of these molecules *in vivo*.

One of the most important common properties of these more toxic molecules is their hydrophobicity. This could help their penetration through the hydrophobic lipid bilayer cell membranes, and may explain the good potential in crop protection of these bioactive natural compounds.

At present, the simplest way to exploit the GL-MYR system is the use of plants containing this enzymatic system as biocidal green manures. This 'biological' crop protection method, in particular to fight soil pathogens (biofumigation), appears to be practical for good agronomic, economic and environmental reasons linked with the use of 'green molecules' such as the GLDPs.

Our group is also engaged in finding convenient ways for the exploitation of these molecules in other fields such as in fine chemistry and food technology.

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Chemistry and analytical aspects

Plants are complex organisms where many biochemical pathways operate simultaneously. Generally it is hard to understand the role of each distinct reaction in the whole system. Sometimes it is useful to study a single biochemical reaction, using a suitable laboratory model, and then strive to transfer that knowledge to the real situation.

To exploit the potential of plants with the GL-MYR system in crop protection, we have isolated by chromatographic techniques the MYR from *Sinapis alba* ripe seeds and several GLs from various *Brassicaceae* ripe seeds where each GL is the unique or main GL.

Using these compounds in pure form, the GLs-MYR-catalyzed hydrolysis, which produces several bioactive isothiocyanates, nitriles and thiones, has been studied in detail. The effect of the most important parameters such as temperature, pH, ionic strength on the yield and quality of GLDPs has been determined for each substrate type.

To have available sufficient amounts of the most important GLDPs, MYR was immobilised on nylon and a small bioreactor was built to continuously hydrolyse pure GLs. The availability of these compounds, the understanding of the reaction kinetics for each GL in different conditions, as well as information about the chemical and physical properties of GLDPs made it possible to evaluate the biocidal activity of GLDPs on soil-borne pathogens *in vitro*.

Studies of the chemical properties of GLs and the determination of their concentration in plant material to be used as green manure are of fundamental importance to choose the best plants and the right management procedures to make biofumigation effective.

We normally use the official method of the European Community, which was set up for rapeseed analysis. To obtain reliable results, and when different cruciferous material is analysed, the method is modified, depending on materials and GL profile.

Finally, studies for establishing the dynamics of the GLDPs and their biophysical properties such as penetration through the soil, toxicity and losses to the atmosphere or from secondary chemical reactions during the green manure treatments are very important to understand the real potential of the GL-MYR system in biofumigation. To this end, unique and reliable analytical methods, involving special devices, are in progress in our laboratories.

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Agro-technology

Starting from the known biocidal activity of the hydrolysis products of GLs, we have selected some new ecotypes for their biomass and for the type and amount of their GL content, with the aim of amending soil with good amounts of active GLDPs by their cultivation and green manuring.

We have identified some new catch crops (*Eruca sativa* cv. Nemat) of the sugar beet cyst nematode (*Heterodera schachtii*) and some new ecotypes containing thio and alkenyl GLs with a good toxic activity toward some soil-borne fungi.

Among these selections, *Brassica juncea* sel. ISCI20, characterised by a very high

(Continued on page 2)

The grains and horticultural industries are supporting research on the biofumigation concept through GRDC & HRDC.

HRDC GRDC

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Horticulture Pests and Diseases

(Continued from page 1)
biomass production (more than 100 t ha^{-1} of fresh matter) and a GL content around 20 μmol g^{-1} dry matter of sinigrin (allyl-GL), seems to be particularly promising for a full field application as biocidal green manure.

Its cultivation did not require any particular agronomic techniques, full mechanisation of field work was possible and no pest control or irrigation was needed. Only ploughing required particular attention to minimise the losses of active compounds released during cutting, chopping and incorporating of the plants, because of their high volatility.

In full pre-plant field trials in strawberry *B. juncea* green manure produced a yield not significantly different from that obtained on fumigated soil with methyl bromide, and that was higher than a traditional green manure not containing GLs (pigeon bean, barley), and an untreated soil (Table 1).

Table 1. Effect on strawberry yield of biocidal green manure. Numbers followed by the same letter are not significantly different.

	Strawberry yield (g/plant)	
	1998	2000
Methyl bromide	464a	501a
B. juncea ISCI20	381ab	445ab
Barley (cv. Baraka)	318b	352c
Untreated control	317b	379bc

Studies to optimise this technique in Italian agroclimatic conditions and to increase knowledge on the effect of these plants on other soil pathogens are going on. From a commercial point of view, *Brassica juncea* ISCI20 was commercialised in 1999 by an Italian Seed Company (Cerealtoscana – Leghorn – Italy) and it will be applied in 2000 autumn sowing on more than 50 hectares.

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Soil-borne pathogen control

In vitro studies on the fungitoxicity of GLDPs carried out over the last five years showed a different sensitivity of phytopathogenic fungi to these compounds. In particular, among soil-borne fungi, *Phytophthora* spp., *Pythium* spp. and sterile fungi (*Rhizoctonia* sp. and *Sclerotium rolfsii*) are more sensitive than imperfect fungi (*Fusarium* spp., *Verticillium dalhiae*, *Trichoderma* spp.).

Studies on the toxicity of GLDPs, in sterile soil, toward *Pythium irregulare* and *Rhizoctonia solani* confirmed that thiofunctionalised and alkenyl-GLDPs are more active than hydroxy-GLDPs and that the interaction between GLDPs and the inert and/or organic soil components does not inhibit their activity in controlling fungal growth.

Pythium and *Rhizoctonia* and *S. rolfsii* are the main targets of our studies on biofumigation with *Brassica* selections. Green manure in naturally infected soil with different ISCI selections of GL-containing plants showed a suppressive activity toward *Pythium* correlated to the type and amount of GL in their fresh tissues.

Practically, the effect of biocidal selections for green manuring showed not only a reduction of *Pythium* growth in soil after fresh tissue incorporation, but also increases in microbial activity in amended soil when compared with conventional cover crop green manuring. In fact, using the same biomass rate, soil incorporation with *Brassica* ISCI selections greatly increased mycoflora and total bacteria when compared to soil incorporation of some conventional cover crops.

The suppressive effect of the biocidal selections toward *S. rolfsii* was similar to that observed on *Pythium*.

Green manuring with brassicas selected for particular GL content provides a new disease control approach. It combines the traditional positive effect of green manure (increase of nutrient availability, etc) with the suppressive effect of GLDPs released by plant residues towards soil-borne pathogens, as well as the stimulation of beneficial microflora in soil.

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Potato Grower's Practical Experience of Growing Brassicas for Biofumigation

Potato farmers Keith and Paula Taylor of Vasse, near Busselton in coastal south-western Australia are interested in growing brassicas for suppression of soil-borne pests and diseases, and as a soil amendment in their potato rotation.

Not being equipped with a seeder for small seeds, Keith tried various options this season to assess the best way of sowing brassicas in his situation.

He sowed several hectares of BQMulch® fodder rape and Fumus® mustard, on both a sandy loam soil and a coarse sand. The loam had a long fertiliser history from horticulture, whereas the sand was old pasture.

The sandy loam area had been under sweetcorn in the 1999/2000 summer. Residues were rotary hoed to break them up in March, and the ground was irrigated. The winter rainy season began in mid autumn.

Best results were obtained in the sandy loam by mouldboard ploughing in early June to invert the soil profile. This produced a very clean seed-bed, and left the surface somewhat rough (more 'crumbs' than 'lumps').

About 1-2 weeks later, with rain forecast, the seed was mixed with double superphosphate in a rotary fertiliser spreader and applied to the surface. The flow rate was cut right back, and two passes were made in order to achieve as even a distribution as possible. The seed was applied at around 10 Kg/ha and the fertiliser at 70 Kg/ha.

The seed could be seen on the surface of the ground. Following a substantial rainfall event, the surface smoothed down. Many seeds were lightly buried in this process, although many also germinated on the surface.

Continued rain and cool weather ensured that the surface never dried, and the soil remained uncompacted for a substantial period. This soil has a sufficient clay fraction (8-12%) that it can pug quite firmly if compressed when wet and be difficult for small-seeded plants to establish and grow.

This combination of factors resulted in excellent establishment and uniform growth. Also, the area is not fully exposed to strong winds.

The wheel tracks from the sowing were followed during the growing season for four applications of approximately 50 Kg/ha of urea each.

A dense stand of both brassica types resulted, giving a total biomass of Fumus® of 15.7 t dry matter/ha, and of BQMulch® of 11.2 t dry matter/ha by early November, and still growing. We have used these plots for studies of isothiocyanate profile and to track growth, over time, and for some first direct measurements of isothiocyanates in soil to develop sampling techniques.

Keith's conclusion is that on this sandy loam soil, with a good seed-bed as described and good moisture, there is no need to harrow seeds in. His view also is that the seeding rate could be reduced in good situations such as this.

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Desktop publishing by John Matthiessen, CSIRO Entomology, Perth

Horticulture
Biofumigation Update
CSIRO

No. 13 | Compiled by John Matthiessen & John Kirkegaard | May 2001

A Complex Mode of Action for Biofumigation?

Previous research into the effects of biofumigation has centred on the direct effects on pathogens. However, biofumigation is a 'gentle fumigant'. Many organisms are not affected - evident from the fact that biofumigation appears to only work effectively on some pathogens.

In addition to the effects on pathogens, there are the beneficial components of the soil microflora to consider. An intriguing possibility is that in some instances biofumigation may also lead to changes in the overall microbial community. These could lead to improved plant growth, which might explain observations of growth responses in wheat crops following canola which cannot be attributed to reduced disease or nutrition.

With this in mind **Brendan Smith** and **John Kirkegaard**, working on a GRDC grant at CSIRO Plant Industry, Canberra, included some non-pathogenic organisms when they surveyed a range of pathogens for their tolerance to the main isothiocyanate produced by the roots of canola, 2-phenylethyl isothiocyanate (2-PE ITC).

The aim was twofold: to identify which pathogenic species should be targets of control by biofumigation and to assess the differential effects of the chemical on a wider range of organisms, including beneficials. Approximately 130 fungal and bacterial isolates were screened.

The results were very encouraging, as many of the most sensitive organisms have been controlled in field studies, including the fungi *Gaeumannomyces graminis* var. *tritici* (take-all of wheat), *Aphanomyces* (root rot of beans), *Pythium sulcatum* (cavity spot of carrots), *Theilaviopsis basicola* (root rot of cotton, beans and sesame) and the bacteria *Ralstonia solanacearum* (bacterial wilt).

What was startling about the results is that some fungi and bacteria thrived at extremely high levels of 2-PE ITC. The most tolerant of the fungi were *Trichoderma* species (Fig. 1), which grew luxuriantly at levels well beyond what killed other organisms, and which are likely to occur in soil.

This group of fungi has been widely studied for their biocontrol potential, and it is the proliferation of these fungi in acid soils of Western Australia which is thought to disfavour take-all.

The different groups of fungi differed markedly in susceptibility to 2-PE ITC (Fig.1). At the lower end of the tolerance *Gaeumannomyces* was on average approximately 50 times more sensitive than the most tolerant group, *Trichoderma*.

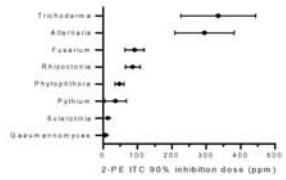

Fig. 1. Fungal responses to 2-PE ITC.

However, this does not tell the whole story. While the average tolerance of the *Pythium* species tested is in the medium range, individual species respond quite differently.

Pythium sulcatum and *P. violae* are highly sensitive at levels that seem to correlate with disease control in the field (ie. less that 10 ppm, Fig. 2). However *Pythium ultimatum* is only significantly affected at levels well beyond those achievable in soil.

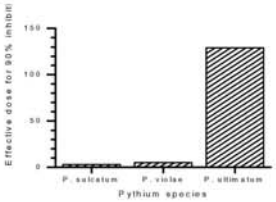

Fig. 2. Relative sensitivity of three *Pythium* species to 2-PE ITC.

Thus biofumigation is not a panacea and will need to be targetted at specific disease organisms. The bright side is the correlation between the tested sensitivity of a pathogen in a simple lab. assay and its reported control in the field by biofumigation. This offers the opportunity of forward screening for diseases which could be potentially controlled by biofumigant practice.

The fact that many organisms, both 'bad' and 'good', were tolerant of the chemical may change the way we think about, and implement, biofumigation. It raises the possibility that changes to the microbial community favouring beneficial organisms might also contribute to disease suppression and improved plant growth.

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ITCs Measured in Soil

There have been few attempts to measure field soil concentration of isothiocyanates (ITCs), either after incorporation of plants or during growth.

In the last year **Ben Warton** and **John Matthiessen**, working on an HRDC (now Horticulture Australia Ltd) grant at CSIRO Entomology, Perth, and **Matt Morra** (University of Idaho, Moscow, ID) working on a GRDC Visiting Fellowship with **John Kirkegaard** at CSIRO Plant Industry, Canberra have been investigating ITCs produced in soil from brassicas, using different methods.

Ben and John set out to develop a technique to measure individual ITCs in the soil around two fodder rapes (*B. napus*) and mustard (*B. juncea*), and to do a preliminary analysis of how well their profile matched between the plants and the soil. Soil gently shaken from the roots of intact plants was extracted with ethyl acetate to capture the ITCs, which were subsequently measured by GC-FPD.

Matt & John measured aggregated total ITCs in soil following rotary hoe incorporation of rape and mustard by derivatising all the ITCs to a common product measurable by HPLC.

The four main ITCs in the plants (2-propenyl, 3-butenyl, 4-pentenyl and 2-phenylethyl) were detected in soil while the plants were growing, plant:soil ratios were roughly equivalent, and the two methods detected similar total concentrations at two contrasting sites.

In the coming season we will examine in more detail the release of ITCs into soil and their profile there relative to the source plants, with the aim of developing methods to maximise ITC release.

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The horticultural and grains industries are supporting research on the biofumigation concept through HRDC & GRDC.

HRDC GRDC

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Horticulture Pests and Diseases

Can Poor Resistance Undermine the Biofumigation Effect?

Recent studies have confirmed the toxicity of 2-phenylethyl isothiocyanate (2-PE ITC) to a range of soil-borne pathogens. The release of this chemical from *Brassica* root tissue after mechanical damage or natural breakdown is thought to be at least partially responsible for the biofumigation effect associated with brassicas

Leaf tissue of brassicas has also been observed to provide a biofumigation effect, although it is currently unclear if this is due to the release of ITCs or some alternative system (or a combination). The growth and incorporation of a *Brassica* crop as a biofumigant should therefore provide a reasonably reliable effect, reducing our reliance on unsustainable and costly chemical pesticides.

However, studies have revealed that the biofumigation effect is currently not highly reliable. The levels of chemicals produced by the tissues can vary greatly due to uncontrollable environmental factors, and their release is probably not efficient, and organisms differ in susceptibility (see P1).

A further complication is the level of resistance of the *Brassica* to pathogens. A biofumigant crop may allow multiplication of some pathogens, which could undermine any biofumigation effect that the tissue may eventually provide.

This certainly has been the case when seeking to control the root lesion nematode (*Pratylenchus neglectus*) using canola (*B. napus*) within the cereal rotation. *P. neglectus* thrives on all canola varieties tested, often generating numbers comparable with those following a susceptible wheat crop.

As this cropping system, unlike horticulture, cannot afford to green manure the oilseed, we rely solely on the root tissues for a biofumigation effect. Only rarely does this crop provide a good biofumigant effect against *P. neglectus*. Indeed, in most years nematode numbers have increased in the presence of our biofumigant crop.

However, there is hope. It seems that plants producing higher root levels of 2-PE glucosinolate (2-PE GSL) show greater resistance to invasion and multiplication by this nematode (Fig. 3).

While only a few individual plants (<15%) within the population are particularly susceptible, this is enough to result in significant multiplication of the nematode in the field. However, plants producing sufficient 2-PE GSL in their roots lose this susceptibility and so will be more reliable in controlling nematode numbers during the season.

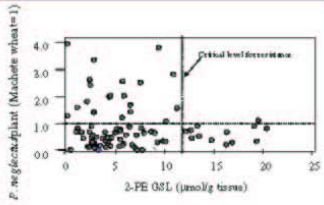

Fig. 3. Relationship between root 2-PE GSL and resistance of canola (cv. Dunkeld) to root lesion nematode.

Populations bred to produce different levels of 2-PE glucosinolate do confirm this finding (Fig. 4). It is important to note that the root tissue produced by such populations also produces a stronger biofumigation effect (Fig. 5) - providing two-pronged pathogen control.

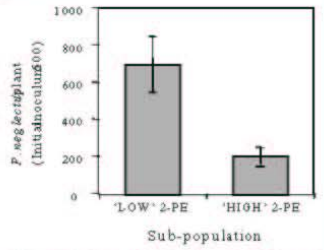

Fig. 4. Impact of increased root 2-PE GSL level on resistance to root lesion nematode.

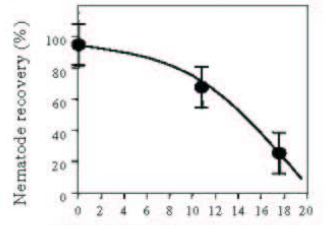

Fig. 5. Effect of a 1% soil amendment of *B. napus* root tissue containing different 2-PE GSL levels on mortality of root lesion nematode.

The lessons learnt in this study may well be applied to improving the reliability of biofumigant crops within horticultural rotations.

First and foremost, they illustrate the importance of ensuring that the crop used is actually resistant to the pathogen in question.

Further, the variation in root 2-PE GSL levels found in individual canola plants is likely to also be present in many of the fodder brassicas available as biofumigants. It may therefore be possible to increase levels in a similar fashion, leading to fodder crops that provide a more reliable biofumigant effect through a combination of improved resistance and more reliable biofumigation.

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US Grower's Successful Use of Mustard Green Manure in a Two-year Potato Rotation

Dale Gies of Moses Lake, Washington, USA, has been successfully growing a short season, fresh market potato in a two-year rotation with spring wheat for the last six years. Between the wheat and potato crops, he uses a white mustard (*Sinapis alba*) green manure crop. Dale has the mustard seed flown onto his wheat fields at 11.2 kg/ha just before wheat harvest. Then after harvest, he packs the field without incorporating the wheat stubble and irrigates the mustard crop.

Dale uses a selective herbicide to kill the volunteer wheat. The mustard crop (cv. Martigena) grows quickly in September and is chopped and disked, with the wheat stubble, into the soil in late October or early November. The following spring, Dale applies metam sodium, and then plants Russet Norkotah potatoes. This is a short season fresh market potato very susceptible to infection by *Verticillium dahliae*.

On-farm research shows that this rotation has increased soil organic matter levels. Water infiltration rates have also been measured to be 2-4 times that of neighboring fields with the same soil type. In a 2000 study, the yields of potatoes after the yellow mustard with no fumigant application were not significantly different than those after mustard with fumigant. These averaged 71 tons/ha of US #1 potatoes (>113.4 g) with total yields being just over 84 tons/ha. This study will be repeated in 2001.

Dale does not have root-knot nematodes in his fields, but the levels of other parasitic nematodes have decreased under this rotation. In addition, there appears to be no buildup of *Verticillium dahliae* after three potato crops in six years.

This research is being expanded to include growers of long-season potatoes that will go to processors, and growers who have fields with parasitic nematode problems. Other information available at http://grant-adams.wsu.edu.

- ***Andy McGuire***, Washington State University (amcguire@wsu.edu).

Desktop publishing by John Matthiessen, CSIRO Entomology, Perth

HORTICULTURE
Biofumigation Update
CSIRO

No. 14 | Compiled by John Matthiessen & John Kirkegaard | November 2001

Does Incorporation Strategy Hold the Key to Biofumigation Success?

New research results suggest that the method used to incorporate biofumigant crops may be the most important factor influencing the success of biofumigation for pest control.

The research was conducted by **Matt Morra** (University of Idaho) and **John Kirkegaard** (CSIRO Plant Industry) during a recent one-year GRDC Visiting Fellowship to CSIRO by Matt to investigate factors influencing the release of isothiocyanates (ITCs) from *Brassica* biofumigant crops.

The framework used for the research is shown in Fig. 1.

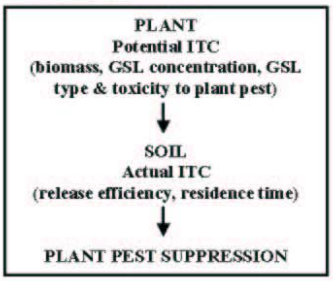

Fig. 1. Theoretical framework in optimising GSL-containing plants as sources of ITCs for suppression of soil-borne pests and diseases.

Research on biofumigation to date has been focussed on an understanding of the types and concentration of glucosinolates (GSLs) present in *Brassica* biofumigant crops and the toxicity to pest organisms of the main ITCs liberated.

This work indicated the 'biofumigation potential' of various biofumigant crops from the total ITC-liberating GSLs in the tissues.

However, pest suppression is reliant on the release of these ITCs into the soil following incorporation. This aspect has received less attention.

Some of Matt's earlier studies suggested release efficiency may be less than 5%. This indicates that there may be potential for considerable improvement in the release of ITCs into the soil.

Two currently available high GSL biofumigant crops (BQ Mulch and FUMUS) and two low GSL breeding lines were grown in the field and incorporated using two passes of a rotary hoe at flowering.

The ITCs in the soil were measured periodically for 72 h following incorporation.

The method developed by Matt involved soil sampling, methanol extraction and then derivatising the ITCs in the soil extracts using 1,2-benzenedithiol to form a more stable compound readily quantified using HPLC.

The results (Fig. 2) show that the highest amounts of ITCs were detected 2 h after incorporation, with a decline during the following two days. The increase at the end of the sampling period was associated with rainfall (11 mm).

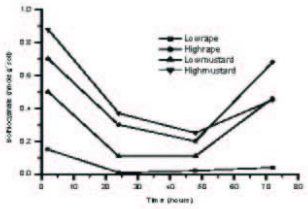

Fig. 2. ITC concentration in soil after field incorporation of rape and mustard shoot tissues with low and high GSL content.

The high GSL varieties of both species gave higher levels of ITCs in soil. However, the maximum ITC concentration measured in soil did not exceed 1.0 nmol g^{-1}, which was barely 1% of the ITC potentially available in shoots at the time of incorporation.

A laboratory study was conducted to investigate the impacts of tissue disruption and soil water content on ITC release from mustard leaf tissues.

Small leaf discs (3.7 cm) from a high GSL mustard variety were added to soil in glass jars either fresh (immediately after cutting) or after freezing. The freezing was designed to cause maximum cellular disruption and ITC release. The soil was kept either moist or waterlogged.

Results (Fig. 3) show that fresh leaf discs produced ITC concentrations less than 1 nmole g^{-1} throughout the experiment.

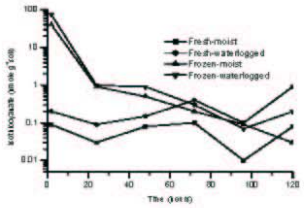

Fig. 3. ITC concentration in soil of two different moisture contents after laboratory incorporation of fresh or frozen mustard tissues. Note log scale on y-axis.

In contrast, freezing the tissues prior to incubation produced a large flush in ITC concentration up to 75 nmole g^{-1}, increasing release efficiency from <1% to 26%.

Wetter soil also maintained higher ITC concentrations for both fresh and frozen tissues.

The results show that soil-borne pest suppression is likely to be improved by choosing a high GSL variety and providing adequate moisture to increase ITC release and retention.

But the results further indicate that the greatest improvements in the use of *Brassica* biofumigants to control soil-borne plant pests will be achieved by developing methods to increase cell disruption and thereby increase ITC release.

Both physical and chemical methods are now being explored.

- ***John Kirkegaard***, CSIRO Plant Industry, Canberra (02 6246 5080; John.Kirkegaard@csiro.au).

The horticultural and grains industries are supporting research on the biofumigation concept through Horticulture Australia & GRDC.

Horticulture Australia GRDC

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Horticulture Pests and Diseases

In Vitro Suppression of Potato Pathogens by Volatiles Released from *Brassica* Residues

Recently, **Robin Harding**, SARDI, Adelaide conducted a laboratory trial comparing the antifungal properties of *Brassica juncea* (Indian mustard), *B. napus* (rape or canola), *Raphanus sativus* (radish), oats, *B. juncea* seed meal and pellet formulations on the fungal growth of *Verticillium dahliae* (Verticillium Wilt), *Colletotrichum coccodes* (Black Dot), *Rhizoctonia solani AG3*, and *AG8* (Black Scurf), *Phytopthora erythroseptica* and *P. cryptogea* (Pink Rot).

In summary, findings were as follows:

- growth of all fungal isolates were reduced when in the presence of all amendments; however, rates of amendment varied between species.
- fungal suppression generally increased with increasing levels of amendment.
- leaf and root tissue of all brassicas suppressed all fungi greater than stem tissue.
- *Phytopthora spp.* were the most susceptible whilst *V. dahliae* was the least susceptible.
- *B. juncea* and *B. napus* leaf tissue were more inhibiting than *R. sativus*.
- *B. juncea* meal and pellet formulations had the most prominent effect.
- 100% suppression of all pathogens occurred within *B. juncea* meal treatments, whereas pelletised meal formulation only suppressed *P. erythroseptica* and *P. cryptogea* by 100%.
- glucosinolates that were isothiocyanate (ITC)-liberating were highest in *B. juncea* meal (48 µmol g^{-1}) and lowest in *R. sativus* stem tissue (2.1 µmol g^{-1}).
- of all plant species and parts, GSL levels were highest in leaf tissue from *B. juncea* (38 µmol/g).

Table 1 gives results of the percentage inhibition of mycelial growth in the fungi after exposure to the maximum quantity of tissues tested (0.1 mg).

These results show that volatiles emanating from leaf and root tissue of *B. juncea* and *B. napus* and meal from *B. juncea* are inhibitory to a number of significant fungal pathogens of potatoes.

They also show that substantial variation in GSL production exists between *Brassica* species and plant parts, and the variation in the sensitivity to these volatiles between the fungal species.

The higher levels of suppression by the *B. juncea* meal extract compared to the pelletised formulations are consistent with higher concentrations of ITC contained within the meal.

This high inhibitory effect of the meal suggests that future research should be directed at developing this material for use as an economical soil amendment.

- ***Robin Harding***, South Australian Research and Development Institute, Adelaide (08 8389 8804; harding.robin@saugov.sa.gov.au.

Table 1. Inhibition (%) of mycelial growth after exposure to volatiles released from 0.1 mg *Brassica* residues, and concentration of ITC-liberating GSLs.

Fungus	*B. juncea* meal	*B. juncea* pellets	*B. juncea* leaf (root)	*B. napus* leaf (root)	*R. sativus* leaf (root)	Oats leaf
Glucosinolate (µmol/g)	**48**	**10**	**38 (16)**	**25 (36)**	**6 (12)**	-
Rhizoctonia AG3	100	34	50 (40)	44 (39)	4 (2)	16
Rhizoctonia AG8	100	36	23 (28)	31 (27)	9 (8)	12
V. dahliae (A & B)	100	27	34 (37)	33 (38)	12 (14)	13
C. coccodes (A & B)	100	17	52 (40)	51 (43)	23 (18)	14
P. erythroseptica	100	100	50 (13)	-	0 (11)	1
P. cryptogea	100	100	55 (11)	-	0 (9)	0

Mustard Hits Bacterial Wilt

FUMUS® F-E75 and F-L71 are *Brassica juncea* (Indian mustard) cultivars developed for use as biofumigants in broadacre and horticultural farming systems in a joint venture between AgSeed Research and Agriculture Victoria.

In an attempt to determine the efficacy of these cultivars against pathogens such as bacterial wilt and *Fusarium* that are a problem for some potato growers, several growers spread along the coast of Victoria have recently trialled either cultivar.

A 4.5 ha potato paddock just east of Melbourne infected with bacterial wilt was sown with varying treatments of FUMUS® F-E75. Some very interesting results stemmed from this exercise.

The paddock in question would generally not have been sown to potatoes in the summer of 1999/2000. Rather it would have been left open to the hot conditions in an attempt to decrease the severity of bacterial wilt.

A crop of FUMUS® F-E75 was sown into approximately two thirds of the total area in June 1999 and ploughed in at 20-30% flowering in early September 1999.

The paddock was left for 2-3 weeks before half of the previously sown area (one third of the total area) was sown to FUMUS® F-E75 a second time in late September 1999. This crop was destroyed in mid December at 20-30% flowering.

The total 4.5 ha was sown to potatoes in the first week of January. The results of the 0, 1 and 2 mustard crop phases were quite marked.

From the area where no crop had been sown (area 1) the grower harvested just 300-500 ***kg***/ha.

The area where one FUMUS® F-E75 crop had been sown (area 2) yielded 10-12 tonnes/ha. The final area in which two sequential crops of FUMUS® F-E75 were sown (area 3) yielded 20-22 tonnes/ha.

This anecdotal trial suggests that population of bacterial wilt in soil can be decreased by the use of FUMUS® F-E75 crops as green manures.

- ***Kate Light***, AgSeed Research, Horsham (03 5382 1269; katie@agseed.com.au).

Desktop publishing by John Matthiessen, CSIRO Entomology, Perth

HORTICULTURE
Biofumigation Update
CSIRO

No. 15 | Compiled by John Matthiessen & John Kirkegaard | April 2002

Plant Maceration and Moisture Hold the Key to Biofumigation Success

In the last *Biofumigation Update*, **John Kirkegaard**, CSIRO Plant Industry, Canberra and **Matt Morra**, University of Idaho, Moscow, Idaho USA reported on laboratory and small-scale field experiments carried out during Matt's GRDC Visiting Fellowship in Canberra.

Their results indicated the importance of cell-level disruption of *Brassica* tissue, and adequate moisture to increase ITC release and retention in the soil.

Disruption of the plant tissue at the individual cell level, by freezing and thawing, dramatically increased the amount of ITC formed – by an average of about 400-fold. The results showed that the efficiency of conversion of GSLs to ITCs could be increased from under 1% to over 25%.

Having the plant tissue in saturated soil in turn approximately doubled the concentration of ITC in the soil. Also, rainfall after rotary hoeing brassicas into soil caused a re-activated 'flush' of ITCs into the soil several days after incorporation.

Surprisingly, higher GSL brassicas did not result in ITCs in the soil that reflected the relative concentration of GSLs in the plants. For example, a mustard cultivar with 5x higher GSL than another only gave 2x the ITC in the soil.

Similarly, a fodder rape cultivar with 20x higher GSL than another only gave a 3x greater ITC concentration in the soil. There is, as yet, no explanation for these puzzling findings.

Overall, the results suggested that the major factor limiting biofumigation potential in the field is ITC release from plant tissue, and that greatest improvement should be achieved by developing methods that increase cell disruption and maximise hydrolysis of GSLs to ITCs.

The combined effect of cellular disruption and excess moisture causing a major increase in GSL to ITC conversion efficiency suggested that methods used to break down plant tissue and the pattern of ITC release and retention in soil need to be better understood to remove an apparent bottleneck in the system.

Recent work has given cause to believe that substantial improvements in the effectiveness of biofumigation can be achieved by management strategies that growers can readily adopt.

Field studies

With the background information provided by the laboratory and field experiments in Canberra, **John Matthiessen** with research chemist **Ben Warton**, technician **Mark Shackleton**, of CSIRO Entomology, Perth, and with the involvement of Busselton, Western Australia, potato and vegetable farmers **Keith** and **Paula Taylor**, carried out an extensive range of field experiments to test various practical incorporation techniques on the amounts of ITCs detectable in soil.

With different analytical facilities at their disposal, the Canberra team measured the combined total of all ITCs in soil, while the WA team was able to measure individual ITCs.

The soil in which the study was carried out was a sandy loam. The brassicas, a fodder rape and a mustard, were sown in mid-June, and grown without irrigation.

Plant treatment and incorporation operations were carried out in the first week of October when the mustard was in the early stages of flowering (the fodder rape does not usually flower in the mild winter conditions of the Busselton region). Soil moisture then was around field capacity.

Two different methods for breaking up the plant tissue were employed. The plants were either chopped and incorporated with a rotary hoe, or the above-ground parts were pulverised with a mulcher.

The mulching was aimed at breaking apart the plant tissue more thoroughly than rotary hoeing, in an endeavour to obtain the cell-level disruption achieved by freezing and thawing in the laboratory experiments. The process appeared successful, turning the plant tissue into a pulp with juice running freely into the soil.

The mulched plant tissue was either left on the surface of the ground, or was immediately incorporated into the soil using the rotary hoe (images at: http://www.ento.csiro.au/research/pestmgmt/soil_pests/biofumigation_info.html).

Fig. 1 shows the total ITCs in the soil under each of the three treatments for the fodder rape and mustard at 0 and 2 hours, and 1, 2, 7 and 14 days, after treatment.

Every treatment resulted in an early 'spike' of ITCs in the soil. However, mulching and immediate rotary hoeing produced a 5-10x greater ITC concentration than rotary hoeing alone or mulch on the surface – a substantial improvement, especially noticeable for the mustard.

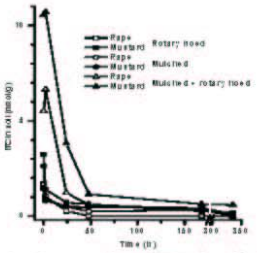

Fig. 1. Concentration of ITCs in soil after three different incorporation methods, for fodder rape and mustard.

Both rotary hoeing and mulching alone caused only a very brief and low-level 'flush' of ITC, with concentration dropping quickly during the first two hours.

In contrast, the mulched and rotary hoed material slightly increased the ITC concentration in the soil during the first two hours before declining relatively slowly over 1-2 days, with the mustard showing the best persistence.

Effect of water

The effect of adding water to the various plant treatment and incorporation methods was tested at various time intervals. No rain fell during the period.

PVC cylinders 30 cm in diameter were hammered into the ground in the various treatment areas and the equivalent of 14 or 42

- Continued over page

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Horticulture Pests and Diseases

mm of water was added.

The amounts of simulated irrigation were chosen to test whether the fairly typical 10-15 mm watering a farmer may be accustomed to giving a crop would have effects on ITC release, or whether substantially heavier application of water may be needed to maximise effects. It is likely that the loamy soil where these tests were done will require more water to produce effects than in a sandy soil.

In general, the lower irrigation had considerably less effect in the study soil, so the results for the 42 mm simulated irrigation are presented to illustrate the principles.

Fig. 2 shows the effect of adding water to the soil two and seven days after rotary hoe incorporation of the brassicas and sampling the soil for ITCs either two or 24 hours later. Water added after two days produced a large flush of ITCs in the mustard area two hours later, while in the fodder rape the effect was greatest when the water was added after seven days.

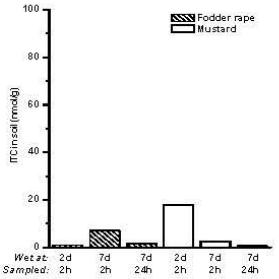

Fig. 2. Concentration of ITCs in soil following rotary hoe incorporation, with the soil wet either 2 or 7 days later and sampled 2 or 24 hours after that.

However, the really big surprise is shown in Fig. 3, where the mulched plant material was left on the surface of the ground and watered immediately after the mulching operation, and the soil sampled two or 24 hours later.

For both brassicas, but the mustard in particular, this treatment resulted in a massive flush of ITCs into the soil. Encouragingly, the concentration of ITCs under the mustard of almost 100 nmol/gram soil was even greater than the concentration in the laboratory experiments with frozen and thawed tissue (see *Biofumigation Update 14*).

For comparison, we estimate that a typical application of metham sodium will produce a methyl ITC concentration of around 400 nmol/gram soil if distributed uniformly in the top 30 cm. However, we know that many of the ITCs that occur in the brassicas are a great deal more toxic than MITC, so the comparative difference could be less than it appears.

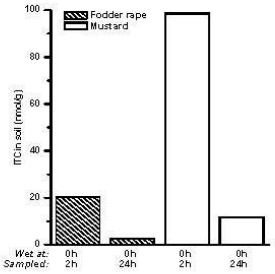

Fig. 3. Concentration of ITCs in soil following mulching and leaving mulch on the surface, with the mulch wet immediately and sampled 2 or 24 later.

The concentration of ITCs in the soil under the mustard was about 5x higher than under the rape. We estimated the total potential ITCs in the plants (shoots plus roots) to be 45 mmol m^{-2} metre for the mustard and 15 mmol m^{-2} for the fodder rape, and the GSL to ITC conversion efficiency for both plant types to be in the range 30-40% - a very encouraging figure.

A closer correlation of the 5-fold difference of the ITCs in the soil under the mustard and the fodder rape was with the level in shoots alone. This totalled 42.8 m^{-2} for the mustard and 8.7 mmol m^{-2} for the fodder rape – also a 5-fold difference.

After 24 hours the total soil ITC concentration under both types of plant had fallen to about 10% of the level after two hours.

When the areas that had been mulched and immediately rotary hoed had water added at different times, ITC production was reactivated. In Figure 4 it can be seen that watering separate parts of these areas one and two days after incorporation resulted in quite a good ITC flush after two hours.

Again, as was indicated in Figure 2 which showed effects after rotary hoe incorporation of whole plants, the effects of water seemed to lag more for the fodder rape than they did for the mustard.

This may be related to the different GSL profiles of the two types of plants. Precursor GSLs of much more volatile ITCs predominate in the mustard.

What it means

While there are still a lot of details to examine further, it is now clear that biofumigation effects have a much greater chance of being maximised if the plants can be pulverised, rather than chopped. The mulcher seemed to do the job as well as freezing and thawing plant tissue, which is very encouraging as freezing is obviously impractical in the field!

It is also clear that a thorough watering will help maximise the production of ITCs, again confirming laboratory results. It is likely that the water will also help the dispersal of the ITCs through the soil.

The results suggest that growers who could water heavily immediately after mulching a biofumigant could benefit from leaving the pulverised plant material on the surface of the ground. Further work is needed to see how long the interval between mulching and watering could be before losing effects through drying.

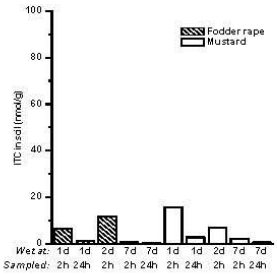

Fig. 4. Concentration of ITCs in soil following mulching followed immediately by rotary hoeing, with the soil wet after 1, 2 or 7 days and sampled either 2 or 24 hours after that.

Growers unable to water quickly may be better off incorporating the mulched plant matter and watering later. The 're-activation' effect seems to last for some time, apparently more so for the fodder rape than the mustard.

It seems likely that the amount of water required for maximum effect will vary with the soil type. We examined only two widely different simulated irrigation levels in a single soil type in the field. In the loam soil, the beneficial effects were not strongly evident with 14 mm, yet 42 mm may have been more than the minimum required to obtain the effects.

More research on these phenomena is still required to help optimise the effects in different situations. The results give us great encouragement that substantial improvements in the efficiency of ITC production and dispersal in soil can be readily achieved by growers in a practical way, and that various options can be employed to suit individual circumstances.

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Desktop publishing by John Matthiessen, CSIRO Entomology, Perth

HORTICULTURE
Biofumigation Update
CSIRO

No. 16 | Compiled by John Matthiessen & John Kirkegaard | November 2002

Potato Growers' Positive Experiences With Biofumigant Green Manure

Potato growers **Geoff, Joan** and **David Hobson** from Thorpdale in Gippsland, Victoria have recently related their experiences in using mustard as a biofumigant green manure in their intensive rotation. The practical information should be of wide interest.

They plant potatoes from August to mid-November each year, harvesting in February-May. Annual average rainfall is above 950 mm, winter-dominant.

As soon as the field is cleared and waste tubers eaten by sheep, the land is prepared for sowing mustard in May-June. The soil, which is friable red volcanic clay with good water infiltration characteristics, is cultivated using a chisel plough.

The fine seed bed needed for the small mustard seeds is obtained with a power harrow working to about 7-10 cm. The seeder is piggy-backed onto the power harrow, dropping the seed behind, and the combination has a trailing roller.

The roller is made from solid rubber forklift tyres, in place of the conventional metal cage roller, to firm the seeded bed. However, Geoff feels that pneumatic tyres would be better.

The mustard is grown for about 100 days, until early flowering. It is then mulched using a 4.5 m wide 4-row profiled pulveriser normally used to destroy potato stems in preparation for harvest.

Because this implement is set up with short and long blades to follow the contours of the hilled potato beds, some of the blades are changed to obtain uniform lengths.

The pulveriser then works uniformly across the flat surface in which the mustard is grown. This maximises the utility of the implement and avoids the need for a separate mulcher.

Fig. 1. Pulverising mustard green manure.

Immediately after pulverising, the mustard is incorporated into the soil using a mouldboard plough. It is then left for about one month before being disc harrowed at least 2-3 times at approximately two-week intervals.

A deeper cultivation is then carried out with a chisel plough. In preparation for planting potatoes, a four-row bed former is then used to form the hills, and the annual cycle resumes.

Usually the mustard is not sown with fertiliser as there is sufficient left after the potato crop, depending on the field's history. About 50 Kg/ha of urea is spread when the mustard is around 30 cm high.

Geoff feels that the *Brassica* rotation needs to go through about three cycles to achieve optimum results.

They have been using mustard in winter only in place of rye-corn in this rotation for the last five years, on land that has been in potato production for 15 years without a total break, and are very satisfied with potato yields and quality.

Previous rotations have included winter wheat and barley for 3-4 years, but they are happier with the mustard.

The only drawback Geoff notes is that the pulverised mustard carries with it a lot of moisture into the soil when ploughed in.

They are trying to reduce this by pulverising and ploughing a little earlier before the mustard develops too much biomass, and to give more time between ploughing and planting potatoes for weathering down.

Figure 1 shows the mustard being mulched. The .jpg file of the colour photographs is moderately large (1.2 Mb), but those who would like it e-mailed, please e-mail John.Matthiessen@csiro.au. Alternatively, view them in *Biofumigation Update 16* at:
http://www.ento.csiro.au/research/pestmgmt/biofumigation/newsletter_list.html.

- ***Geoff Hobson***, Thorpdale (03 5634 6397; hobson@sympac.com.au).

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Horticulture Pests and Diseases

Successful Use of Biofumigant Green Manure Crops for Soil-Borne Disease Control

Recent field trial studies on green manure crops by **Hoong Pung, Susan Cross** and **Dennis Patten** of Serve-Ag Research in Tasmania have shown that biofumigant green manure crops could increase marketable yields of subsequent lettuce crops by reducing tipburn, bacterial rot, and *Sclerotinia* disease. The benefits appear to depend on local soil conditions and the type of crop used.

Green manures were sown in July 2001 and rotary hoed to 25cm when the mustard, rape and broad beans were flowering in October-November. Lettuces were planted in December (Trial 2) and January 2002 (Trial 1).

Trial 1 was located at Cambridge, in an area that has a history of lettuce production and severe *Sclerotinia* disease. Trial 2 was located in Forth in an intermittently cropped paddock with a low level of *Sclerotinia*.

These differences were reflected in the initial sclerotia count in soil samples from the two sites at the beginning of the trial, before green manure crops were sown. The Trial 1 site had relatively high levels of *S. minor* sclerotia, while very few or no sclerotia were found in the Trial 2 area (Table 1 & 2).

In Trial 1, the biofumigants BQMulch and Fumus significantly reduced the percentage of plants with *Sclerotinia* wilt (Table 1). BQMulch appeared more effective than Fumus in reducing wilted plants. Oats and broad beans had little or no effect.

In Trial 2, at close to commercial harvest, the lettuce plants had a relatively high incidence of tipburn and/or bacterial rot, which was reduced by the Fumus, resulting in a increase in the percentage of marketable lettuces (Table 2).

BQMulch, broccoli and broad beans had little or no effect on tipburn and/or bacterial rot, and hence did not significantly increase the marketable yield compared to the untreated control.

Bacterial rot often occurred on the inner leaves of plants also showing tipburn. Tipburn on inner lettuce leaves is usually attributed to calcium deficiency, which also makes them susceptible to bacterial rot.

The biofumigant crops used in the trials were selected through conventional breeding for their high levels of isothiocyanates (ITCs). Apart from any biofumigation effects, the crop residues also increase organic matter and nutrients, and improve soil structure. Fumus is a variety of mustard, while BQMulch is two rapes.

In both trials, Fumus flowered approximately two weeks earlier than BQMulch. Analysis of the brassicas, sampled prior to incorporation into soil, showed that Fumus produced higher levels of ITCs in their shoots, whereas most of the ITCs for BQMulch were in the roots (Table 3).

The better *Sclerotinia* disease control with the BQMulch plants may be due to the higher ITC levels in their roots. The biofumigant plants from Trial 1 produced much lower levels of ITCs than plants from Trial 2.

These differences may be due to the different soil types. The crops were sown in poor clay loam soil at Cambridge, compared to rich, friable ferrosol soil at Forth.

In the final assessment for sclerotia levels in soil from Trial 1, even though BQMulch and Fumus plots tended to have lower levels, the differences with other treatments were relatively small (Table 1).

Table 1. Effects of green manures on *Sclerotinia minor* sclerotia and disease in a subsequent lettuce crop. Trial 1.

Treatment	Mean no. sclerotes/200g soil: Initial count (5/7/01)	Final count (1/3/02)	% plants with *Sclerotinia* wilt
Untreated control	26	10	31c
Oats	16	nt	23bc
Broad bean	15	11	24bc
Fumus	16	9	17ab
BQMulch	25	7	3a

Values with same letter are not significantly different at 5%. nt-not tested.

Table 2. Effects of green manures on subsequent lettuce marketability. Trial 2.

Treatment	Initial count (19/7/01) Mean no. sclerotes/200g soil	% marketable lettuces	% unmarketable: % tip burn & soft rot	% unmarketable: % other*	% unmarketable: % *Sclerotinia*
Untreated control	0	72a	24	4	0.4
Broccoli	0.3	78a	19	3	0
Broad bean	0	79a	19	1	0.9
Fumus	0.3	88a	9	3	0.9
BQMmulch	0	74a	22	4	0

*Values with same letter are not significantly different at 5%. *Plants rejected due to physiological disorder.*

Table 3. Total ITC concentration in plants (µmol/g)

Trial	Variety	Tissue	ITC conc'n
1	BQMulch	Shoot	2.7
		Root	16.6
	Fumus	Shoot	6.1
		Root	5.9
2	BQMulch	Shoot	2.7
		Root	42.2
	Fumus	Shoot	32.2
		Root	23.4

This indicated that biofumigation by BQMulch and Fumus appeared to have little effect on the sclerotia viability in the soils. It is possible that the biofumigant crop residues act by inhibiting the mycelial growth of the pathogen instead.

At the end of the trials, when the lettuce crops were ready for commercial harvest, relatively high levels of BQMulch and Fumus crop residue were still evident in the trial areas. In contrast, there was little or no broad bean, broccoli or oat crop residue left in the soil.

The noticeable improvement of the poor clay loam soil structure, with reduced soil surface crusting and cracking and better water infiltration, especially in the BQMulch plots in Trial 1, may be due to the slow decay of *Brassica* residues. The long-term effect of the remaining *Brassica* residue on the mycelial growth of *Sclerotinia* is unknown.

In both trials, the broad beans generated relatively high quantities of crop residues, similar to those of the *Brassica* plants. The differences observed in the soilborne diseases appeared to be related to the increased organic matter alone.

These trials were conducted as part of a three-year project funded by the vegetable growers' levy and Horticulture Australia Ltd. Plant analysis for isothiocyanates was conducted by **Mark Shackleton** at CSIRO Entomology, Perth.

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HORTICULTURE
Biofumigation Update
CSIRO

No. 17 | Compiled by John Matthiessen & John Kirkegaard | May 2003

Soil Impacts on Canola Resistance and Biofumigation to Root Lesion Nematode

Recent work by **Mark Potter** at the South Australian Research & Development Institute shows that variation in soil qualities, such as pH, total dissolved salts (TDS), texture and trace element levels, impact not only on the ability of canola to resist disease, but also on the 'biofumigation potential' of the tissues as they degrade in the soil.

Ten different soils were collected from the field to represent different areas of the key canola cropping districts of South Australia.

Soils were gamma irradiation sterilised, and assessed for parameters including pH, TDS, texture, organic matter, nitrate, phosphate, sulphate, chloride and fluoride.

Doubled haploid (DH) canola was employed to allow discrimination between environmental and genetic effects (no genetic variation between plants when using DH lines).

Plants grown in each of the soils showed significant variation in resistance status to the root lesion nematode *Pratylenchus neglectus*.

Soils containing higher organic matter and lower levels of sand led to plants containing fewer nematodes at the end of a six week period. High pH (>6.5, in water) and TDS also led to reduced nematode numbers.

The different soils resulted in significant variation in root glucosinolate levels, although no soil parameter (including nitrate and sulphate) was associated with the variation.

DH plants with elevated levels of glucosinolates contained fewer nematodes than their otherwise identical counterparts.

A strong relationship was also observed between root glucosinolate level (total and 2-phenylethyl) and the toxicity of powdered freeze-dried tissue amendments to *P. neglectus* as they degraded in a standard soil (Fig. 1). (Note that by graphing % nematode recovery, the graph shows that the toxicity effect on the nematodes is greater as % recovery declines).

An identical sample of tissue was amended into the range of soils, revealing significant variation in toxicity due to soil environment.

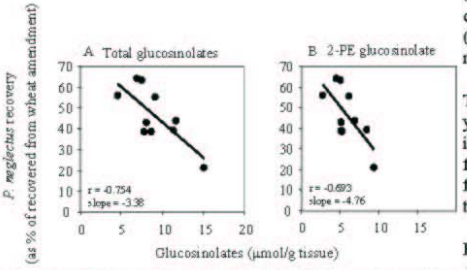

Fig. 1. Relationship between root glucosinolates and toxicity of amendments to *Pratylenchus neglectus*.

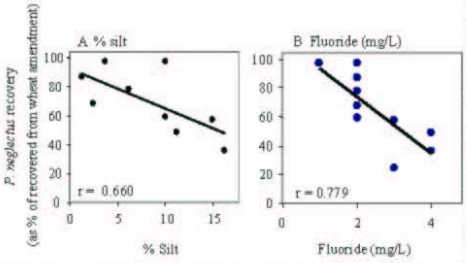

Fig. 2. Relationship between toxicity of identical tissue amendments and soil parameters. (P=0.05, 9 d.f., r=0.632).

Tissue amended into soils containing high levels of silt was more toxic than when it was amended into lower silt soils (Fig. 2A).

However, very interestingly, a strong relationship was observed between tissue toxicity and soil fluoride levels—the more fluoride in the soil, the more toxic was the *Brassica* tissue to the nematodes (Fig. 2B).

Our current hypothesis is that the fluoride impacts on the glucosinolate system.

Fluoride has been shown to be involved in the formation of sugar/glycosidase intermediates required to allow the cleavage of a glucose unit from fruit sugars such as xylan (Sidhu *et al.* (1999). *Biochemistry* **38**: 5346).

Parallels between xylanase and myrosinase exist (Becker *et al.* (2000). *Journal of Biochemistry* **345**: 315), suggesting that fluoride might be involved in the efficient breakdown of glucosinolate, so increasing the yield of isothiocyanates (ITCs) in soils containing glucosinolates.

This may in part explain the low yield of ITCs from tissues degrading in many soils, and may soothe the frustration of researchers who have failed in seeing a reliable biofumigation effect in field studies.

Further research is required to better assess the role of fluoride in the glucosinolate system.

These results also raise the prospect that other soil factors such as minerals not examined in these studies could influence biofumigation effects.

At the very least it shows the unexpected and not readily apparent complexities that can occur in soils when trying to manage soil-borne pests and diseases in alternative ways to heavy doses of pesticides, and the challenges that identifying and managing these subtleties present to growers and researchers.

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The horticultural and grains industries are supporting research on the biofumigation concept through Horticulture Australia & GRDC.

Horticulture Australia GRDC

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Horticulture Pests and Diseases

Can Brassicas be Used to Manage Root-knot Nematode in Tropical Vegetable Production?

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint to the production of vegetables in tropical regions.

One method of management has been rotations with crops that are poor hosts to nematodes to break the nematode life cycle.

But what are the comparative benefits of using poor hosts compared to using plants that may be toxic to nematodes, or a combination of both?

Brassica species are moderate hosts to nematodes, but contain glucosinolates (GSLs) that hydrolyse to form products including toxic isothiocyanates (ITCs) that are reputed to control nematodes.

The aim of these trials carried out by **Tony Pattison**, **Tanya Martin**, **Caroline Versteeg** (QDPI, South Johnstone), **Steve Akiew**, **Joanna Arthy** (QDPI, Mareeba) and **John Kirkegaard** (CSIRO, Canberra) was to assess the feasibility of using isothiocyanate-liberating *Brassica* biofumigant rotation crops as part of an integrated root-knot nematode management strategy in tropical vegetable crops.

Brassica resistance

Commercial brassicas were tested for resistance to root-knot nematode in a pot trial.

Brassicas were found to be only partially resistant to *M. javanica*. Therefore, Brassicas have the potential to carry over nematodes to the following crop (Fig. 1).

The most resistant variety was the radish (*Raphanus sativus*) cultivar Weedcheck. However, no *Brassica* was as resistant as sorghum to nematode multiplication.

Toxicity effect

The toxicity effect of *Brassica* leaves was tested in a laboratory petri dish assay by adding 1,000 nematode eggs to 30g soil and placing either 0.75, 1.5 or 2.25g (equivalent to 2.5, 5.0 or 7.5% leaf to soil) of frozen leaf material on the soil surface. (Leaves were frozen to ensure tissue disruption and ITC release).

The *B. juncea* cultivars Fumus and Nemfix were as effective in reducing the development of eggs to juvenile root-knot nematode as methyl ITC (ie. metham sodium).

Both Fumus and Nemfix had relatively high concentrations of propenyl GSL (36.0 and 33.6 µmole g leaf^{-1}) in the leaf tissue compared to the other varieties. Propenyl ITC is more volatile than forms of ITC from the other varieties.

The higher rates of leaf material added to the soil increased the mortality of nematodes for all brassicas tested, but addition of sorghum did not.

This suggested that nematode mortality in the assay was affected by volatile compounds within the leaf and not solely by the addition of organic matter.

The incorporation of leaf material into the soil reduced nematodes recovered from the roots of tomato plants (Fig. 2).

Brassica tissue was ineffective at rates less than 0.02 g per g of soil (2% W/W), while sorghum was effective at all rates.

Together, the direct mortality and tomato infestation results suggest an overall impact of both organic matter addition and chemicals in the leaf.

As well as the production of volatile compounds, water-soluble compounds may also be produced when brassicas are added to soil, and there could also be stimulation in parasites of nematodes as the organic matter decomposes.

The effect of these different components must be separated to determine their relative importance in nematode control.

In summary:

- Brassicas are partially resistant to *M. javanica*. The radish cultivar Weedcheck had the best resistance, although not as high as forage sorghum.
- Brassicas are able to develop volatile, nematicidal compounds within their leaves that are released when their cells are disrupted.
- Incorporation of *Brassica* leaf material affects nematodes in at least two ways: by organic matter and by toxic compounds from within the leaf.

Some Brassicas exhibiting both high resistance and high toxicity are currently being tested in the field.

New *R. sativus* varieties are currently being tested to determine if they have improved resistance to root-knot nematode.

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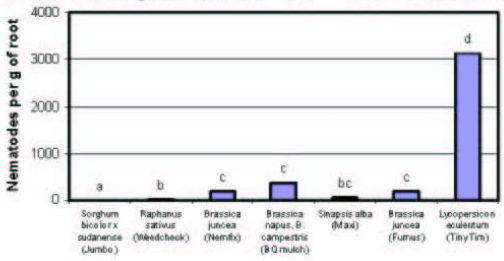

Figure 1. Resistance of commercial brassicas to root-knot nematode (*M. javanica*) relative to tomato (bars with same letters are not significantly different from one another (P<0.05)).

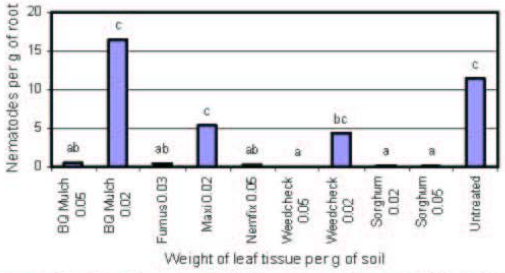

Figure 2. Root-knot nematode (*M. javanica*) recovered from the roots of tomato plants grown after incorporation of leaf material into infected soil (bars with same letters are not significantly different from one another (P<0.05)).

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CONCLUSIONS

This project made major progress in various facets of our understanding of biofumigation, particularly in revealing the key elements of how to achieve dramatically greater release of ITCs from plant material and to maximise their incorporation into the soil. In that regard, it achieved its title aims of optimising the process of biofumigation as it relates to the production, release and incorporation of the toxic elements of brassicas that were the foundation of the biofumigation concept.

The capacity to achieve leverage to carry out the specialist chemistry measurements through involvement of Ben Warton, the post-doctoral chemist employed on the concurrent project HG98034 was of great benefit. It illustrates how projects very often cannot be complete singular self-contained entities. The integration of these two closely-aligned projects was of very great value. Similarly, the interactions and collaboration maintained with John Kirkegaard's team added great value to this project, especially in giving the first indications of the importance of cell-level tissue disruption in achieving orders of magnitude increases in ITC release, and the crucial importance of water in amplifying the ITC release and transport into soil. John and Matt Morra's work in this regard was a watershed in the approach to biofumigation that we were able to greatly build on.

One of the issues that this project could not investigate was the benefit of brassicas as rotation crops that may lie outside the specifically-focussed ambit of biofumigation as conceived in relation to the release of toxic ITCs. There is evidence emerging, notably from the work of Hoong Pung at Serve-Ag in Tasmania that these plants may have other benefits in terms of soil structure, water infiltration and so on. It seems likely that such benefits may be expressed more obviously in heavy soils. However, it indicates the point that rotation-crop benefits are likely to be multi-facetted and vary with the environment in which they are used.

It was disappointing that the field trial to investigate impacts on a soil pathogen were inconclusive in that they could not test the core question of what happens when plant disruption methods that dramatically increase ITC release are used. Nevertheless, it pointed up the crucial issue that the type of plant mulching equipment used has an enormous effect on the quantity of ITCs released from the plants.

There will never bee a single, simply-applied biologically-based solution to suppression of soil-borne pests and diseases. That would be naïve and unrealistic. But I feel that the work carried out during this project, and its links to the work of others, has added greatly to the knowledge base of how better effects of biologically-based methodologies may be employed as an option or alternative for horticultural producers to consider. They will be better armed to do their own trials to test how the concepts can be made to fit their many and varied production systems as they seek to achieve sustainable production systems.

John Matthiessen
Principal Investigator
10 October 2003