PT447
Integrated management with biofumigation to control soil pests and diseases in potatoes

John Mattiessen, John Kirkegaard and Stewart Learmonth
CSIRO Entomology, CSIRO Plant Industry, Agriculture Western Australia

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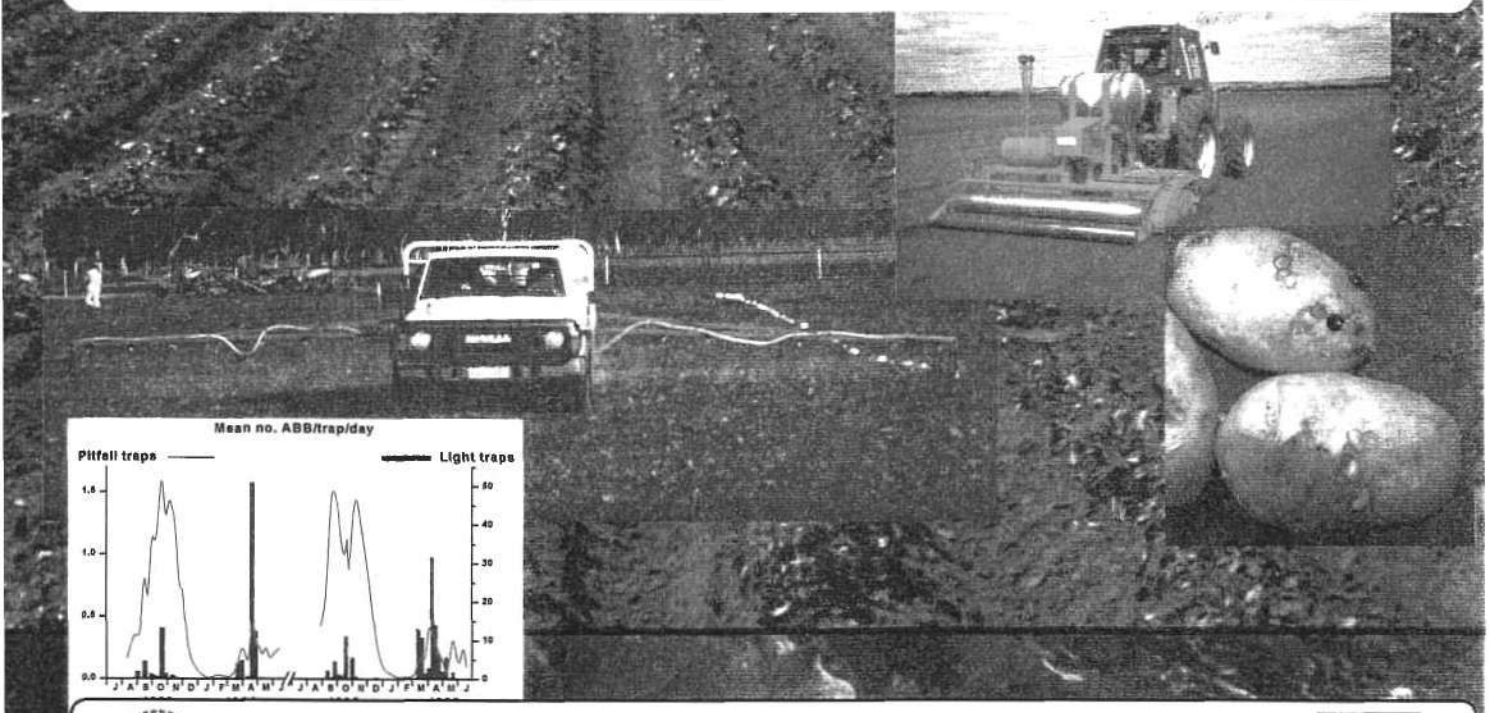

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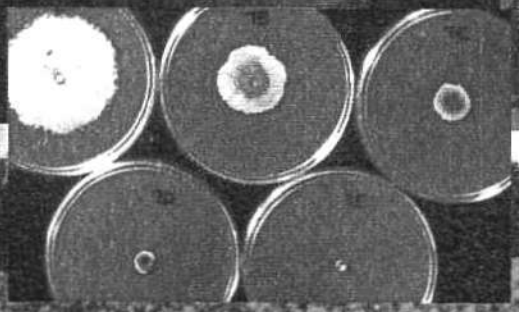

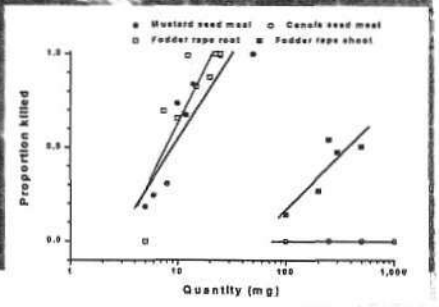

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Integrated Management With Biofumigation to Control Soil Pests and Diseases in Potatoes

HRDC Project PT447

A joint research project of:

CSIRO Entomology

CSIRO Plant Industry

Agriculture Western Australia

Investigators:

John N Matthiessen	John A Kirkegaard	Stewart E Learmonth
Principal Research Scientist	Senior Research Scientist	Entomologist
CSIRO Entomology	CSIRO Plant Industry	Agriculture Western Australia
Private Bag PO Wembley	GPO Box 1600 Canberra	Manjimup
Western Australia 6014	ACT 2601	Western Australia 6258
Ph. 08 9333 6641	Ph. 02 6246 5080	Ph. 08 9771 2444
j.matthiessen@ccmar.csiro.au	j.kirkegaard@pi.csiro.au	stewartl@manjdo.agric.wa.gov.au

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Research team

John Kirkegaard, John Matthiessen, Diana Fisher, Stewart Learmonth, Muhammad Sarwar, Mark Shackleton

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INDUSTRY SUMMARY

The control of soil-borne pest and diseases is a major production issue for potato producers s they strive to enhance quality and maximise yield while maintaining safe working practices and sustainable production systems. The key conventional approach to integrated pest management for the more easily seen or diagnosed foliar pests and diseases of scouting and treating when thresholds are exceeded is not readily implemented for soil-borne organisms because they are hidden and treatments are not easily applied during crop growth.

The traditional approach to soil-borne pest and disease management has been to adopt a prophylactic approach, usually as pre-planting incorporation of chemical pesticides into the soil using such devices as structure-damaging rotary hoes to ensure uniform distribution. A recent trend has been towards the use of the broad-spectrum soil fumigant metham sodium as an agent for the control of a wide range of pests and diseases. There are concerns about the sustainability of the use of, and over-reliance on, such a powerful biocide. It was recognised that alternative approaches, or optional strategies, for management of soil-borne pests and diseases would need to maintain a preventative approach because of the practical difficulties of implementing reactive strategies.

Methyl isothiocyanate, the active agent in metham sodium, was established as the most toxic fumigant to whitefringed weevil larvae. The larvae were also killed by volatile isothiocyanates emitted by *Brassica* tissues which also killed or suppressed the growth of fungal pathogens. The production by many *Brassica* species of different types of isothiocyanates, usually in mixture, suggested that there was potential to harness appropriate species or cultivars as biological sources of fumigant vapours ('biofumigants') that could be grown as green manures in rotation with potatoes to suppress soil-borne pests and diseases. Many different *Brassica* species and cultivars were chemically analysed for their isothiocyanates to assess biofumigation potential and the effects of environment on that potential. A large variation of types and concentrations of isothiocyanates occurred in various brassicas, with high levels in some lines suggesting that they offered high potential as biofumigants. The capacity of these lines to kill or suppress the growth of soil insects and fungal pathogens was confirmed in bioassays where the vapours from the plant tissues were exposed to the pests and diseases in sealed vessels. Tests were also conducted using several pure isothiocyanates which showed that there was variation in the susceptibility of different pathogens to different types of isothiocyanates. This suggested that *Brassica* cultivars could be selected for biofumigation capacity on the basis of their chemical profile.

Integrated pest management approaches for the controlling soil insect pests focussed on using improved knowledge of their biology to better target conventional insecticide applications. Scouting for whitefringed weevil adults in pasture during summer was a useful guide to the probability of damaging larvae being present in the following spring potato crop, and hence the need to treat with metham sodium. Metham sodium applied with a blade or tined applicator gave as effective control as rotary hoe incorporation, with the advantage of minimising operator exposure to toxic vapours.

The normally subterranean adults of African black beetle were found to have a high level of previously unsuspected surface crawling activity in spring, coinciding with mating and egg-laying. Spraying chemical insecticide onto the surface of pasture in early spring killed adults before they were able to lay eggs, substantially reducing the subsequent summer population which damages the newly-sown potato crops. The technique allowed use of reduced quantities of insecticide, did not require incorporation into the soil with a rotary hoe and allowed use of 'softer' insecticides.

This research has provided a high level of basic scientific information on the biology and ecology of soil pests, the chemical profile of brassicas, methods for testing the effects of alternative strategies on soil-borne pests and diseases and the effectiveness of those alternatives. This information and expertise will underpin continued development of alternative, soundly-based strategies for the sustainable management of soil-borne pests and diseases in horticulture.

RESEARCH SUMMARY

The management of soil-borne pests and diseases in sustainable ways is a critical production issue facing potato producers. Development and implementation of integrated pest management strategies for soil-borne pests and diseases is challenging because control techniques that require disturbance of the soil cannot easily be implemented during crop growth, and low density populations of soil insects are usually highly damaging. Incorporation of chemical pesticides into soil and a trend towards the use of metham sodium soil fumigant as a broad-spectrum chemical control agent for a wide range of noxious soil-borne organisms raise questions of long-term sustainability of such practices, and concerns about reliance on a single method of control.

This research focussed on maintaining the prophylactic approach that is necessary for managing soil-borne pests and diseases, but in ways that maximise impact on pest populations through application of better biological knowledge and the development of alternative strategies that are not reliant on expensive soil fumigants. A key component of the project was to evaluate the potential of various *Brassica* species and cultivars to act as biofumigants, through their natural ability to produce glucosinolates which are precursors of toxic isothiocyanates. Methyl isothiocyanate was established as the most toxic fumigant to whitefringed weevil, giving impetus to the notion that isothiocyanate-producing plants offer potential as green manures with biofumigant properties.

A large number of field-grown *Brassica* species were chemically analysed for the concentration of glucosinolates in their root and shoot tissues. The plants were grown under various conditions, in various geographical locations and in different seasons, with their biofumigation potential being estimated from chemical concentration, type of glucosinolate and biomass. Glucosinolate profile and growth varied widely between species and cultivars, resulting in a wide range of biofumigation potential.

Methods were developed for bioassaying the effect of *Brassica* tissue on pathogens and pests insects. Bioassays carried out against both fungal pathogens and soil insects showed that volatile compounds emitted from *Brassica* tissue can be toxic or have the capacity to suppress growth. Effects varied between species of pathogens, with *Pythium* being notably more tolerant of the volatiles than other pathogens. Newly-hatched larvae of the soil insect whitefringed weevil were a particularly good bioassay subject because of the ability to obtain eggs in large numbers, to stockpile them for long periods, and because of their longevity in the absence of food or shelter. Species or cultivars of brassicas that had not been heavily selected for seed oil or foliage palatability traits contained greater concentrations of isothiocyanate-producing glucosinolates and were more toxic to pest organisms.

Adoption of integrated pest management approaches in the use of conventional chemical pesticides aimed to enhance the efficiency, impact on pest populations and operator safety of such methods. Examination of alternative methods of applying metham sodium suggest that implements that inject the material into the soil under a blade or tines offer control of whitefringed weevil as good as rotary hoe incorporation, without the disadvantageous effects of excessive cultivation on soil structure, and with advantages of reduced operator exposure to the deeper-placed pesticide.

Alternative insecticidal approaches for management of African black beetle were developed that aimed at taking advantage of a trait whereby the normally subterranean adults exhibit high levels of surface crawling activity in the spring. By directing insecticidal sprays against adults in early spring, prior to the commencement of egg-laying, large reductions in insect abundance during the following summer potato-growing season are achieved. This integrated pest management approach to control of African black beetle allows use of lower quantities of insecticide, less toxic materials and avoids the need to incorporate insecticide into the soil. It requires growers to implement control some five months before the conventional pre-planting soil-incorporated approach.

This research provided a high level of basic scientific information on the biology and ecology of soil pests, the chemical profile of brassicas, methodology and biological effects that will underpin continued development of alternative, soundly-based strategies for the sustainable management of soil-borne pests and diseases.

INTRODUCTION

One of the most critical issues facing potato producers is the management of soil-borne pests and diseases in sustainable ways. The subterranean nature of the tubers exposes them to a wide variety of noxious organisms. Probably the most clearly visible damage is that of soil-dwelling insects. These can attack the potato plants, reducing yield through plant destruction, or they can damage tubers directly, making them unmarketable. A diverse array of microscopic soil-borne diseases and nematodes also have adverse effects on crop quality or production through effects on the plants and tubers.

The worst soil-dwelling insect pests of potatoes in Australia are African black beetle *Heteronychus arator* (Fabricius) and whitefringed weevil *Graphognathus leucoloma* (Boheman). Whitefringed weevil occurs as a pest of potatoes in Western Australia, South Australia, Victoria, New South Wales and Queensland, and is present in Tasmania. African black beetle is even more widely distributed, but appears to be a pest of potatoes mainly in Western Australia, where it is severe in some districts. Diseases of many types are a widespread problem of varying magnitude in all potato production areas.

Predicting the threat of soil pests and diseases is often difficult because they occur hidden in soil. Control is also difficult because very low populations are often highly damaging, the organisms are hidden and unevenly distributed in the soil, damage is cumulative from the time tuber development commences and a fixed population of soil insects can cause increasing damage because they attack multiple tubers. Often the risk of attack or infection is so pervasive that prophylactic application of pesticides prior to planting the crop is common. It is usually too late and impractical to effectively apply pesticides for control of soil-borne organisms during crop growth. In any event, scouting for soil organisms to decide whether to treat or not is not the practical proposition that it is for foliar pests and diseases.

The use of residual pesticides to control such things as soil insects is no longer permitted. Recently there has been a trend to the use of the soil fumigant metham sodium to control particularly threatening and difficult to control pests such as whitefringed weevil. At the same time, benefits in the control of soil-borne diseases from the use of fumigation were also claimed. The use of metham sodium has been widely and rapidly adopted by producers in the Manjimup-Pemberton area of Western Australia. It is, however, an expensive product estimated to cost in the vicinity of $700-800 per hectare. Despite perceived benefits, it is uneconomical for the potato industry in many parts of Australia.

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. Producers would undoubtedly prefer not to use highly toxic and expensive materials if they could be avoided. Also, the use of broad-spectrum biocides in soil would be widely viewed as not the most biologically desirable long-term practice. 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 produce and develop markets.

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. 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 sustainability.

This project sought to develop integrated pest management practices to apply to soil-borne pests and diseases in the potato industry. The focus was on the development of the concept of biofumigation - the use of biologically active plants to suppress undesirable organisms in the soil, and improved methods of using pesticides, based on improved knowledge of the biology and incidence of various pests and diseases that impact on potato production.

BIOFUMIGATION

Introduction

Biofumigation refers to the use of biologically-active plants as green manures, cover crops or rotation crops to suppression soil-borne pests and diseases. The concept stems principally from the knowledge that many *Brassica* species, in particular, have within their tissues glucosinolates which are the precursors of isothiocyanates. These are often volatile chemicals known to be toxins. Methyl isothiocyanate is the active compound resulting from decomposition of the commercial soil fumigant metham sodium.

The notion that these properties could be used as a pest and disease management technique came 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. Anecdotal accounts, usually from 'old-timers' in potato industry tended to corroborate the research findings in the cereals industry, with comments about 'cleaner' potatoes from crops grown following such plants as *Brassica* vegetable and fodder crops.

Investigations of the phenomenon revealed that, while it was known that many different glucosinolate precursors of a large array of isothiocyanates occurred in different brassicas, little was known about their level of occurrence in different species and cultivars, the concentrations in different plant tissues, and the relationship to biological activity. To have any opportunity to harness and enhance the observed biofumigation effects it was clear that a key area for research was to establish the glucosinolate, and hence the isothiocyanate, profile of various brassicas to gauge their biofumigation potential.

An associated aspect of these investigations was the need to establish the toxicity of pure isothiocyanates to pest and disease organisms as a benchmark against which to evaluate biofumigant effects, and to determine the effect of factors such as temperature and soil type on their toxicity to pest organisms. Such baseline information was essential to underpin future studies aimed at advancing the concept of biofumigation to achieve specific and maximal desired effects.

A large part of the research effort in this project was directed to determining this benchmark information to establish a sound foundation for future work and for 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. 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.

To further this coordination aspect, and to enhance technology transfer, a regular newsletter, the 'Biofumigation Update' was instigated very early in the project. The newsletter 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 following section provides details of studies related to biofumigation undertaken in this project.

Comparative efficacy of fumigants against hatchling whitefringed weevil larvae and their sorption by soil

John Matthiessen[A], Jim Desmarchelier[B], Le Vu[B] & Mark Shackleton[A]

[A] CSIRO Entomology Private Bag PO Wembley, Western Australia 6014
[B] CSIRO Entomology, GPO Box 1700 Canberra, ACT 2601

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Abstract

The toxicity of the fumigants methyl isothiocyanate, carbonyl sulfide, carbon bisulfide, and methyl bromide (control) was assessed against first instars of the soil-dwelling pest whitefringed weevil, *Graphognathus leucoloma* (Boheman), by injection into sealed glass jars containing the insects. Methyl isothiocyanate was the most toxic compound, with a steep dose-response relationship, followed by methyl bromide, carbonyl sulfide, and carbon bisulfide; all of which had flatter response lines. Sorption of the fumigants by three contrasting soil types was determined by injecting them into the headspace of sealed glass jars containing soil at different temperatures and moisture contents, and sequentially analysing subsequent headspace concentrations over 24 h by using gas chromatography. Methyl isothiocyanate was rapidly and strongly sorbed by all soils, with ≈1% of the starting concentration remaining after 2 h. Carbonyl sulfide was the next most sorbed fumigant (its sorption increased with time), whereas methyl bromide was sorbed least. Soil that was rich in organic matter was most sorptive. Except for high sorption of methyl bromide by dry soil, we detected little effect of temperature and moisture content on the extent of sorption. Despite the high potency against whitefringed weevil of methyl isothiocyanate, the primary bioactive degradation product of metham sodium, its rapid and high sorption precludes field use of low quantities of this costly chemical.

Introduction

The soil-dwelling larvae of whitefringed weevil, *Graphognathus leucoloma* (Boheman), are highly destructive pests of horticultural crops such as potatoes, peanuts, and sweet potato, as well as lucerne (alfalfa) forage and pasture (Young et al. 1950). This accidentally introduced South American insect is a major pest of peanuts and sweet potato in the southeastern United States (Young et al. 1950), and of potato in southwestern Australia (Matthiessen and Learmonth 1994). In southwestern Australia potato crop protection relied on prophylactic preplant soil incorporation of residual cyclodiene insecticides until they were withdrawn from agricultural use. The next most suitable insecticide was chlorpyrifos, but it was less effective at reducing tuber damage (Crisp et al. 1992), because it has lower impact deep in the soil where most of the larvae occur (Matthiessen and Learmonth 1995).

Recently, potato producers in regions of Australia infested with whitefringed weevil began using the soil fumigant metham sodium because of their general dissatisfaction with the efficacy of chlorpyrifos. Despite an approximately 5-fold greater cost, its widespread use indicates that producers find it sufficiently more reliable in reducing the risk of whitefringed weevil damage to justify the additional expense.

Metham sodium is widely used to control noxious soil-borne organisms in horticulture. It is notable in potato production against fungal pathogens and nematodes in the United States (Powelson and Rowe 1994), and against potato cyst nematodes (*Globodera* spp.) in the Netherlands (Leistra and Smelt 1974). Although the properties of metham sodium have a broad spectrum, and soil insect control is often mentioned as a benefit, few quantitative studies of the efficacy of metham sodium or its primary breakdown product, methyl isothiocyanate, against soil-infesting arthropods have been published. Laboratory and field tests showed metham sodium to be toxic against larval sugarbeet wireworm, *Limonius californicus* (Mannerheim), but economically unacceptable for protecting potatoes from tuber damage (Toba 1984). Variability of efficacy is, however, a recognized characteristic of the use of metham sodium (Munnecke 1967).

Although metham sodium appears to be the most effective agent available for control of whitefringed weevil, instances of failure are reported by potato producers. This problem raises questions about the mode of action of the agent against whitefringed weevil and the effects of other factors, such as characteristics of the soil and aspects of insect biology. Because whitefringed weevil mainly damages spring-planted potatoes (Matthiessen and Learmonth 1993), most metham sodium is applied in late winter or early spring before planting. At that time, approximately 95% of the population is in the first instar (Matthiessen 1991; Matthiessen and Learmonth 1992), which is a nonfeeding stage capable of prolonged survival (Gough and Brown 1991). In the study described here, we quantified the susceptibility of hatchling whitefringed weevil larvae to methyl isothiocyanate and other fumigants, and determined how the fumigants interact with contrasting types of soil under varied moisture and temperature conditions.

Materials and Methods

Insects

Whitefringed weevil adults were collected in the field and maintained in the laboratory on lucerne foliage. Eggs laid under pieces of wood scattered over the base of plastic cages (Gross and Bartlett 1972) adhered to the wood and were collected twice weekly. After maturation at 26°C and 95% RH for 14 d, the eggs were transferred to 15°C and 95% RH, which allows prolonged storage (Gross et al. 1972). When hatchling larvae were required, sufficient pieces of wood were removed and the eggs were sprayed with water to induce hatching (Gross et al. 1972). They were maintained at 26°C and 100% RH overnight, by which time most eggs had hatched. Larvae were collected and used for bioassays within 36 h of eclosion.

Bioassays

The fumigants used in the bioassays were methyl isothiocyanate (CH_3NCS), carbonyl sulfide (COS), carbon bisulfide (CS_2), and methyl bromide (MeBr). Carbon bisulfide has long been used as a commercial fumigant; carbonyl sulfide has recently been found to have useful fumigant properties against insects (Desmarchelier 1994). Both compounds are also among the possible degradation products of metham sodium (Turner and Corden 1963, Kaufman 1967, Rosario et al. 1994); consequently they are of intrinsic interest for potency against whitefringed weevil. Methyl bromide, as a standard soil fumigant, was included for comparison.

Tests were conducted in 120-ml glass bottles equipped with an airtight cap that allowed gas injection through a septum. A piece of black filter paper (1 by 1 cm) moistened with water was placed in each bottle to maintain humidity and to assist in assessing insect mortality. Fifty larvae were added to each bottle, which was then sealed. Fumigants were injected into separate bottles with a pressure-lok glass microlitre syringe used for gas chromatography after removal of a volume of air equal to that of the fumigant. Controls were sets of 50 larvae in sealed bottles. We tested two replicates for each test.

Fumigant concentrations were calculated from the known concentrations of methyl bromide and carbonyl sulfide in cylinders of the gases, from the volume of carbon bisulfide liquid added with a syringe, and from the saturated vapour concentration of methyl isothiocyanate in a sealed 120-ml bottle containing 2 g of solid material (Worthing 1980). Preliminary tests were done to narrow fumigant concentrations to appropriate ranges to assess their efficacy against whitefringed weevil larvae.

After 24-h exposure at 20°C, the caps were removed and the bottles were placed in a fume hood for 2-3 h to allow the gases to disperse. Caps were then replaced loosely and the bottles kept overnight at 20°C and 50% RH. Mortality was assessed the following morning by examining the larvae under a dissecting microscope. Control mortality was done using Abbott's formula (Abbott 1925), and evaluating effects using probit analysis (Finney 1971). Although we recognize the theoretical importance of end-point mortality (Winks 1986), testing soil insects in vitro presented major logistical constraints in attempting to nullify control mortality. Because the purpose of our study was to determine relative fumigant efficacy against hatchling whitefringed weevil larvae broadly, we viewed it as reasonable to assess acute mortality. During the course of the tests, fumigant concentrations were

measured with gas-liquid chromatography. Experimental units that had initial concentrations outside the expected range were discarded.

Sorption by Soil

Contrasting soils were collected from three locations where potatoes are grown in southwestern Australia: sand (Myalup), loam (Pemberton), and peat (Albany). Basic characterisation tests were done. Water-holding field capacity was estimated by saturating soil in plant pots (305 mm diameter, 270 mm deep), which were covered with a plastic bag and drained for 24 h, after which the moisture content of a sample of soil from the top of the pot was determined gravimetrically by oven drying to constant mass. A sample of each soil was ashed to determine its organic matter content, pH was measured using a standard calcium chloride solution, and a density estimate was made by measuring the weight of soil resembling the consistency of ploughed soil (at ≈50% field capacity) in a measuring cylinder (1 litre).

Samples of each soil at 100% field capacity were heat-sealed in plastic bags in 500 g lots, and the remainder of the bulk sample of each soil was air dried. After moisture content was determined, sufficient water was added to bring subsamples to 25, 50, and 75% field capacity; these were broken into 500 g lots stored in heat-sealed plastic bags until required. For testing of sorption, 50 g of a soil was placed into a 120-ml glass bottle as used for the larval bioassays. Vapour concentrations of individual gases, or microlitre quantities of carbon bisulfide, sufficient to give a nominal concentration of 40 mg/litre in an empty bottle, were injected into the air space above the soil (headspace), after removal of a volume of air equal to that of the fumigant. Each sample was replicated twice. Standards with no soil were injected to give a 10 mg/litre fumigant concentration, to be nearer the range expected after interaction with the soils.

The presence of soil in the bottle raised the initial concentration of fumigant in the headspace over the nominal 40 mg/litre, depending on the space occupied by each soil type and assuming no immediate sorption by the soil. The net volume of each soil (ie, excluding air spaces) was determined by placing 50 g in a measuring cylinder, adding a measured excess of water, stirring and allowing the soil to settle, then reading the volume of the soil.

The fumigant concentration in the headspace was determined by extracting 30 μl with a pressure-lok syringe and injecting it into the column of a gas chromatograph appropriately configured for the fumigant being measured. Methyl isothiocyanate was analysed on a Varian 3300 (Varian Associates, Sunnyvale, CA) gas chromatograph, equipped with a thermionic specific detector, after isothermal separation on a 15m megabore column, Dbwax. Carbonyl sulfide and carbon bisulfide were analysed on a Tracor 220M (Tracor Inc., Austin, TX) gas chromatograph, equipped with a flame photometric detector, sulfur mode, after isothermal separation on a 1.5 m glass column filled with Chromosorb W, high performance, coated with 20% SE30. Methyl bromide was detected on a Shimadzu GC6AM (Shimadzu Seiakusho Ltd., Kyoto, Japan) gas chromatograph, equipped with a flame ionization detector, after isothermal separation on a 2 m glass column filled with Chromosorb W, high performance, coated with 6% SP2401, 4% SE30.

The initial sample (referred to as T=0) was taken 1-5 min after injection of the fumigant into the bottle. The bottles were placed in controlled temperature cabinets at the required temperature between measurements. Samples were taken at T=0, 1-2 (depending on processing delays), 4, 6, and 24 h. All soils, with moisture contents of 25, 50, 75 and 100% field capacity, were measured at 4 and 20°C; those at 75% field capacity were also measured at 12 and 30°C. A few subsamples of soil were oven dried to zero moisture and tested, as was water alone. All readings were later calculated against the standards to give the concentration of fumigant remaining in the atmosphere above the soils. The mean headspace concentration of fumigant across each time interval between samples was calculated from the average of the two concentrations multiplied by the number of hours in that interval. Summation of these figures gave the estimated concentration × time product (mg h l^{-1}), or the average level of fumigant to which any organisms in such soil would have been exposed in the 24 h.

Results

Bioassays

The results of the in vitro bioassays of the fumigants against the whitefringed weevil larvae are summarized in Table 1 as concentration × time multiples that resulted in 50 and 95% mortality. Methyl isothiocyanate was by far the most toxic substance, with a very steep response line. Methyl bromide, carbonyl sulfide and carbon bisulfide, all of which had flatter response lines, were the next most toxic.

Sorption by Soil

The characteristics of the soils tested for their response to the fumigants showed that peat was markedly different from sand and loam in its water-holding capacity, organic matter content, and density (Table 2). Mean net volume occupied by 50 g of soil was 23.5, 23.8, and 31.2 ml for sand, loam, and peat, respectively. Hence, actual headspace concentrations after injection of the fumigants to produce the nominal 40 mg litre^{-1} in the empty bottle, when recalculated, averaged 49.7, 49.9, and 54.1 mg litre^{-1}, respectively, for the sand, loam and peat.

The concentration of fumigant measured in the headspace at each sample was expressed as a percentage of the recalculated starting concentration of each fumigant in the headspace. Results for samples of soil at 75% field capacity moisture and held at 20°C are illustrative of the general trends observed in concentration changes over time (Fig. 1). For all soils, methyl isothiocyanate was removed from the headspace extremely rapidly with only ≈1% of the starting concentration remaining 2 h after injection. Generally, the other fumigants were removed more steadily; methyl bromide was consistently removed least. After methyl isothiocyanate, carbonyl sulfide was the most affected; its removal accelerating with time on the loam, and more on peat. Peat was markedly more active than both sand and loam in reducing the headspace fumigant concentration, with methyl isothiocyanate and carbonyl sulfide disappearing by 4-6 h after injection (Fig. 1).

Figure 2 shows change in the concentration × time product with temperature for each fumigant and soil at a moisture content 75% of field capacity. Except for carbonyl sulfide, concentration of the fumigants was generally not affected by temperature. A decrease in the concentration of methyl isothiocyanate over peat at 20°C appears to be slightly aberrant, suggesting that the degradation seen in Fig. 1 was slightly exaggerated. For each fumigant, the concentration remaining in the headspace was consistently least with peat, and generally similar for sand and loam. Carbonyl sulfide showed the greatest downward trend with increasing temperature, and the greatest degree of difference between peat and the other soils.

The $L^{\odot} \times T)_{50}$ and $L^{\odot} \times T)_{95}$ values determined in vitro at 20°C for each fumigant against whitefringed weevil larvae (Table 1) were incorporated into Fig. 2 for comparison with concentrations remaining after exposure to the different soils. Despite the high degree of removal of methyl isothiocyanate from the headspace, its extremely high potency against whitefringed weevil larvae ensured that in all soil types, and over a wide range of temperature, the concentration remaining was consistently well above the toxic levels determined at 20°C. The greatest contrast between soil types in the concentration of fumigant remaining in the headspace occurred with carbonyl sulfide and peat where it was reduced below levels toxic for whitefringed weevil larvae. Although carbon bisulfide was poorly removed by the soils (Fig. 1), its low toxicity ensured that the concentration in the headspace remained sublethal, whereas methyl bromide remained lethal by virtue of its higher toxicity (Fig. 2).

Table 1. In vitro toxicity of fumigants to first instar whitefringed weevil

Fumigant	n	Slope ±SE	L(CxT) mg h litre^{-1} (95% CL)		Heterogeneity χ^2	df
			$L(CxT)_{50}$	$L(CxT)_{95}$		
Methyl isothiocyanate	8	5.072 ± 0.787	1.31 (1.22-1.40)	1.64 (1.53-1.85)	19.22	6
Carbonyl sulfide	22	0.068 ± 0.004	100.2 (98.5-101.9)	124.4 (121.2-126.4)	29.07	20
Carbon bisulfide	6	0.011 ± 0.001	487.9 (453.9-521.4)	642.7 (594.5-732.7)	20.67	4
Methyl bromide	7	0.164 ± 0.015	27.98 (26.40-29.32)	37.99 (36.28-40.33)	10.42	5

Table 2. Characteristics of the soils tested for their ability to sorb fumigants

Soil	Field capacity (% moisture)	pH ($CaCl_2$)	Organic matter (%)	Density (g ml^{-1})
Sand (Myalup)	21.6	7.21	3.26	1.30
Loam (Pemberton)	19.3	4.89	6.77	1.35
Peat (Albany)	45.2	5.69	31.55	0.90

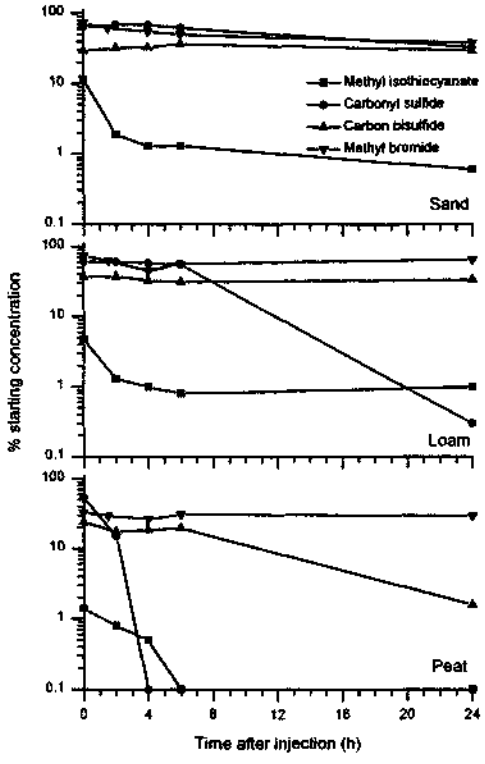

Figure 1. Percentage of starting concentration of fumigants remaining in the headspace of sealed bottles over time for soils at a moisture content 75% of field capacity and held at 20°C.

The main effect of moisture variation in the soil was reduction of residual fumigant in the headspace of samples of oven-dried soil (ie. methyl bromide in all soils and carbon bisulfide particularly with peat) (Fig. 3). Carbonyl sulfide was the only fumigant to show a major decline in headspace residues with increasing moisture, but only in the peat soil. The trend for carbonyl sulfide data suggests that the apparent lack of sorption at 25% field capacity may have been aberrant. Methyl isothiocyanate retained a consistently potent residual concentration, relative to toxicity values determined for whitefringed weevil larvae at 20°C and high RH in all soils. The decline

in residual fumigant at zero moisture was greatest in peat, causing sufficient removal of methyl bromide to take the residual concentration below the levels determined to be toxic to whitefringed weevil larvae. Water alone produced results similar to soil at field capacity (Fig. 3).

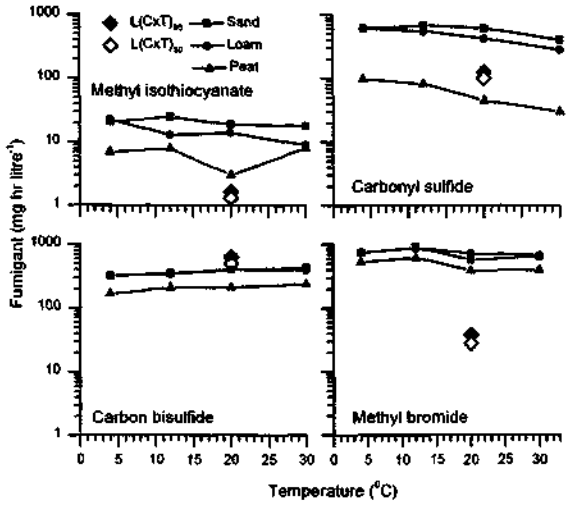

Figure 2. Concentration × time product of each fumigant remaining in the headspace of the bottles, related to moisture content of the soil (percentage of field capacity) and water alone, and compared with $L(C \times T)_{50}$ and $L(C \times T)_{95}$ values for toxicity against first instar whitefringed weevil determined in vitro at 20°C.

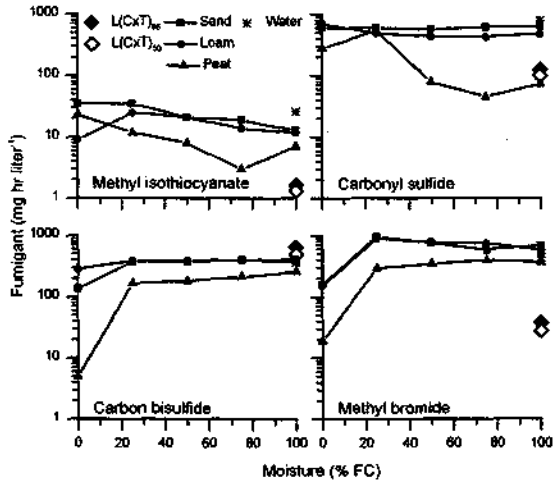

Figure 3. Concentration × time product of each fumigant remaining in the headspace of the bottles, related to moisture content of the soil (percentage of field capacity) and water alone, and compared with $L(C \times T)_{50}$ and $L(C \times T)_{95}$ values for toxicity against first instar whitefringed beetle determined in vitro at high humidity.

Discussion

The extremely high potency of methyl isothiocyanate against first instar whitefringed weevil found in these in vitro tests verify the empirical findings of the general potency of metham sodium in reducing damage caused by whitefringed weevil in potatoes. For whitefringed weevil control in potato production, metham sodium is typically applied at 500 litres/ha and incorporated into the soil to a depth averaging 25 cm. Metham sodium is 42.3% (wt:vol) sodium N-methyldithiocarbamate, which could yield a theoretical concentration of methyl isothiocyanate in the field of 47.9 mg/litre of soil when applied as described. This rate is close to the starting concentration used in our soil tests, in which the residual concentrations after sorption by the soils consistently remained above levels that were highly toxic to first instar whitefringed weevil larvae at 20°C and high relative humidity.

The efficacy of soil fumigants is related to their toxicity and the decline of the applied concentration. Apart from diffusion to the atmosphere, which is usually countered in practice by sealing the soil surface with a roller, the most immediate and direct losses are likely to be from physical sorption. It seems probable in our studies, done over only 24 h, that physical factors would have outweighed any microbiological effects that may also be expected to have the potential to transform the initial compounds applied. The high toxicity of methyl isothiocyanate to whitefringed weevil larvae may be reduced by its rapid and strong sorption by all soils tested, which encompassed an extreme range of characteristics. In practice, this limitation has been overcome empirically by applying sufficient amounts of the compound to generally achieve effective control of whitefringed weevil. However, a major consequence of compensating for high sorption is the economic problem of applying much larger quantities of an expensive agent than would otherwise be the case. About 100-fold more needs to be applied than theoretically required.

Reasons for instances of poor control of whitefringed weevil in southwestern Australia are unclear. On the basis of our data, application of 500 litres/ha of metham sodium may produce a dose of methyl isothiocyanate (toxic to at least first instar whitefringed weevils) in the extremes of soils encountered in the potato-growing districts of the region. The susceptibility of larger larvae, some of which occur all year in that environment (Matthiessen 1991), is worthy of investigation. This will require development of suitable rearing methods to produce them in large numbers as survival past first instar in laboratory rearing is low (Bartlett et al. 1967), and rearing times are long (Gough and Brown 1991).

Our results indicate that there would be little margin for concentration reduction from the use of 500 litres/ha of metham sodium before whitefringed weevil control efficacy would diminish. This study also indicated that temperature and soil moisture effects on the sorption of pure methyl isothiocyanate by soil are generally not great over ranges likely to be common in the field.

Temperature and moisture do, however, have substantial effects on the production of the active methyl isothiocyanate degradation product from the metham sodium precursor. The effect is direct in the case of temperature and inverse in the case of soil moisture (Turner & Corden 1963). Furthermore, high moisture can cause dilution and limit the rate of diffusion in soil (Leistra 1974). Dry soil enhances sorption of some chemicals (Hoffmann and Malkomes 1979), as we found with methyl bromide and carbon bisulfide. Turner and Corden (1963) also showed that pH influenced the nature of metham sodium decomposition, with more methyl isothiocyanate being produced under alkaline conditions, which was an attribute of only sand in our tests.

Carbonyl sulfide and carbon bisulfide, both of which are also breakdown products of metham sodium (Turner and Corden 1963, Kaufman 1967, Rosario et al. 1994), were 2-3 orders of magnitude, respectively, less toxic to whitefringed weevil larvae than methyl isothiocyanate. Both compounds were substantially less sorbed by soil than methyl isothiocyanate, but carbonyl sulfide was sufficiently removed by the high organic matter peat soil at higher moisture contents to reduce concentrations below levels toxic to whitefringed weevil larvae. Possibly, in field use of metham sodium, carbonyl sulfide could play a role in whitefringed weevil mortality, either directly, or synergistically with methyl isothiocyanate. Such possibilities are speculative and are likely to be soil-specific; their elucidation would require assessment of the chemistry of metham sodium decomposition under various soil conditions. Low carbon bisulfide toxicity combined with sorption by all soils maintained the vapour below toxic

levels. Methyl bromide was both highly toxic and little sorbed, attesting to its well-known status as a potent soil fumigant. The low sorption, however, suggests that a large proportion of applied methyl bromide would be released undesirably into the atmosphere.

These experiments provide a benchmark for the relative susceptibility of first instar whitefringed weevil to four fumigants. The agent of particular interest is methyl isothiocyanate which, through a growing use of metham sodium in cropping systems that have the capacity to sustain the cost, is seemingly the most effective synthetic control agent for whitefringed weevil currently available. Use of such potent chemicals is, however, coming under increasing environmental scrutiny. Alternative and less costly ways to deliver isothiocyanates for alleviating soil-borne pest and disease problems are now being investigated. Foremost among these is the potential use of selected cruciferous species or cultivars that are natural sources of isothiocyanates (Brown et al. 1991), as break or rotation crops for 'biofumigation' (Kirkegaard et al. 1993, Mojtahedi et al. 1993).

Toxicity and sublethal deleterious effects of allyl isothiocyanate, which can be derived from *Brassica* spp., has been demonstrated against the sugarbeet wireworm *Limonius californicus* (Mannerheim) (Williams et al. 1993). Notably, Borek et al. (1995) have recently found that aromatic isothiocyanates are considerably more toxic to black vine weevil, *Otiorhynchus sulcatus* (F.), than aliphatic forms such as methyl and allyl isothiocyanate. Studies such as these, and our study, are providing baseline information that is encouraging for the development and effective implementation of alternative biologically based and less costly management strategies for soil-dwelling pest insects.

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Biofumigation potential of brassicas. I. Variation in glucosinolate profiles of diverse field-grown brassicas.

John Kirkegaard & Muhammad Sarwar

CSIRO Plant Industry, GPO Box 1700 Canberra, ACT 2601

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Abstract

Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds released in soil when glucosinolates (GSL) in *Brassica* green manure or rotation crops are hydrolysed. We investigated the potential to enhance biofumigation by considering the variation in GSL production in the roots and shoots of 76 entries from 13 *Brassica* and related weed species grown in the field. Total plant GSL production on a ground area basis at mid-flowering ranged from 0.8 to 45.3 mmole m^{-2}. The variation derived equally from differences in biomass and GSL concentration, which were not correlated in either root or shoot tissues. Roots (0 - 0.15 m) contributed an average of 23.6 % (range 2 to 81%) of the total plant GSLs, their contribution limited by low biomass rather than GSL concentration, which was usually similar or higher than that of shoots. The GSL concentrations in root and shoot tissues did not correlate significantly with seed levels in any of the species, so selection for higher plant GSL cannot be based on seed GSL levels. The types of GSLs present in the tissues varied considerably between species but were consistent within species. In contrast, the concentration of individual and total GSLs in both root and shoot tissues varied four to ten-fold both between and within all species. Shoots contained predominately aliphatic GSLs, while aromatic GSLs, particularly 2-phenylethyl GSL, were dominant in the roots of all entries. Indolyl GSLs were present in all tissues but at low concentrations (< 1 μmole g^{-1}). The variation in the biomass, GSL profiles and concentrations in both roots and shoots provide significant scope to select or develop brassicas with enhanced biofumigation potential. Further studies on the efficacy of the various GSL hydrolysis products to suppress target organisms in soil are required to fully exploit biofumigation as a part of integrated pest management.

Introduction

Brassica species and other members of Cruciferae contain significant quantities of the thioglucoside compounds known as glucosinolates (GSLs) in their tissues. GSLs are hydrolysed by the myrosinase enzyme (present endogenously in *Brassica* tissues) to release a range of hydrolysis products including oxazolidinethiones, nitriles, thiocyanates and various forms of volatile isothiocyanates (ITCs). These hydrolysis products, in particular the ITCs, are known to have broad biocidal activity including insecticidal, nematicidal, fungicidal, antibiotic and phytotoxic effects (reviewed by Brown and Morra 1997; Chew 1988; Fenwick *et al.* 1983; Rosa *et al.* 1997;). Accordingly *Brassica* green manures, rotation crops or seed meal amendments have been reported to suppress pest and disease organisms when grown or incorporated in the soil (e.g. Chan and Close 1987; Mojtahedi *et al.*, 1991), and the ITCs are usually considered to be the most active compounds (Brown and Morra 1997)

'Biofumigation' is a term recently used to describe the suppression of soil-borne pests and pathogens by *Brassica* rotation or green manure crops (Angus *et al.*, 1994; Kirkegaard *et al.*, 1993). Interest in biofumigation has increased recently in horticultural industries due to prohibition of several synthetic pesticides and soil fumigants (e.g. methyl bromide, ethylene dibromide). In broad-acre cropping, biofumigation of intractable soil-borne fungal pathogens by *Brassica* rotation crops such as canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) is thought to contribute to their superiority as break crops for cereals (Angus *et al.* 1991; Kirkegaard *et al.* 1996). Strategies to enhance the biofumigation potential of brassicas in both horticulture and broad acre cropping systems are of interest.

There are about 20 different types of GSLs commonly found in brassicas which vary in their structure depending on the type of organic side chain (aliphatic, aromatic or indolyl) on the molecule. The profile, concentration and distribution of these GSLs varies within and between *Brassica* species and in different plant tissues, and consequently the concentration and type of biocidal hydrolysis products evolved also varies (Mithen 1992). Several studies have related the effectiveness of disease suppression by *Brassica* amendments to the concentration or type of GSLs present in the tissue (Kirkegaard *et al.* 1996; Mojtahedi *et al.* 1991). Among the major hydrolysis products, ITCs are generally considered the most toxic, however individual ITCs also vary in their toxicity to different organisms (reviewed by Brown and Morra 1997). For example, ITCs derived from aromatic GSLs have been found to be forty times more toxic to eggs of black vine weevil (*Otiorhynchus sulcatus* F.) than the aliphatic moiety (Borek *et al.* 1995). The range in GSL profiles, the differential toxicity of the ITCs evolved to different pests of plants, and the wide range of phenological and morphological diversity of brassicas provides scope to select or breed brassicas with enhanced biofumigation potential for particular target organisms.

Few studies have compared the GSL production of diverse, field grown brassicas with respect to biofumigation potential, and the role of root GSLs in suppression by green manure crops has often been overlooked. This paper is the first in a series which investigates the factors influencing the biofumigation potential of brassicas, and reports the GSL production in the roots and shoots of a diverse set of field grown *Brassica* and related species. The aim is to assess the potential to select or develop brassicas with enhanced biofumigation potential. Subsequent papers report environmental factors which influence biofumigation potential (Sarwar and Kirkegaard 1997) and the relative toxicity of the major GSL hydrolysis products to a range of soil-borne cereal pathogens (Sarwar *et al.* 1997).

Materials and methods

Brassica *collection*

Seeds of a diverse set of *Brassica* and related species representing oilseeds, fodders, vegetables and weeds were collected for the study (Table 1). The majority of the seed samples were obtained from the Australian Temperate Field Crops Collection at the Victorian Institute for Dryland Agriculture (VIDA), Horsham, Australia. Fodder brassicas were obtained from Wrightson Seeds while *B. juncea* lines came from the CSIRO Plant Industry breeding program which aims to develop low glucosinolate, low erucic acid (double-low) *B. juncea* for production of canola quality oil (Oram and Kirk 1992). For the oilseed species, representatives of winter and spring types with both low and high seed glucosinolate contents were included.

Crop management

Individual seeds of each entry were planted into cells (3 cm x 3 cm) of trays containing moist vermiculite/peat potting mix on 23 May 1995 and left to germinate indoors at 20°C for 3-4 days. After seedling emergence the trays were laid out in open cold frames, watered daily and covered at night to prevent frosting until the seedlings were ready for transplanting (June 18 to 30, 3 leaf stage). Seedlings were transplanted into cultivated field plots at CSIRO Ginninderra Experiment Station near Canberra, Australia. The plots were 0.5m x 1m and seedlings were planted with 0.1m inter-row and intra-row spacing (100 plants m^{-2}) with individual plots 1m apart. Three replicates of each of the 75 entries were arranged in a randomised complete block design. The plots were topdressed with fertilizer on 26 July to supply 20 kg ha^{-1} N, 20 Kg ha^{-1} P and 18 kg ha^{-1} S. The area was hand-weeded and irrigated occasionally to prevent water stress. Details of the site, soil type and environmental conditions during the growing season are shown in Table 2.

Table 1. *Brassica* and related Cruciferae used in the study.

Entry no.	Species/Cultivar	Origin	source	Description/use[B]
	B. napus (Winter rape)			
	ssp ***oleifera biennis***			
(1)	Ridana	GFR	H	Oilseed (High)
(2)	Lirakotta	GFR	H	Oilseed (High)
(3)	Korina	GFR	H	Oilseed (High)
(4)	Tamara	GFR	H	Oilseed (High)
(5)	Bienvenu	FRN	H	Oilseed (High)
(6)	Libravo	GFR	H	Oilseed (Low)
(7)	Rangi	NZ	W	Fodder
(8)	Arran	SCOT	W	Fodder
(9)	Wairoa	NZ	W	Fodder
(10)	Striker	NZ	W	Fodder
(11)	K488IR	NZ	W	Fodder
(12)	Hobson	SCOT	VS	Fodder
(13)	IDH	USA	IU	Cover crop
	ssp. ***oleifera annua*** (Spring rape)			
(14)	Cyclone	CAN	H	Oilseed (Low)
(15)	Oscar	AUS	H	Oilseed (Low)
(16)	Dunkeld	AUS	H	Oilseed (Low)
(17)	Lirawell	GFR	H	Oilseed (Low)
(18)	Cresus	FRA	H	Oilseed (High)
(19)	Norin 16	JAP	H	Oilseed (High)
(20)	Midas	CAN	H	Oilseed (High)
	ssp. ***rapifera*** (Swede)			
(21)	TNG 15	NZ	W	Fodder
(22)	TNG 16	NZ	W	Fodder
(23)	Doon Major	NZ	W	Fodder
	B. campestris			
	ssp. ***oleifera biennis*** (Winter turnip rape)			
(24)	Duro -	SWD	H	Oilseed (High)
	ssp. ***oleifera annua*** (Spring turnip rape)			
(25)	Arlo	CAN	H	Oilseed (High)
(26)	Bunyip	AUS	H	Oilseed (Mod)
(27)	Tyko	SWD	H	Oilseed (High)
(28)	Jumbuck	AUS	H	Oilseed (Mod)
(29)	Kova	SWD	H	Oilseed (Low)
	ssp. ***rapifera*** (Fodder turnip)			
(30)	K4880FB	NZ	W	Fodder
(31)	Pasja	NETH	W	Fodder (Hybrid turnip/rape)
(32)	GRes	NZ	W	Fodder
(33)	Barkant	NETH	W	Fodder
(34)	Simax	NETH	W	Fodder (Hybrid turnip/chinese cabbage)
	B. oleracea			
	var ***gemmifera*** (Brussel sprout)			
(35)	Drumtight	AUS	Y	Vegetable
	var ***capitata*** (Cabbage)			
(36)	Earliball	AUS	Y	Vegetable
	var ***botrytis***			
	subvar ***cauliflora*** (Cauliflower)			

(37)	Broccoflower	AUS	Y	Vegetable
	subvar ***cymosa*** (Broccoli)			
(38)	Winter Harvest	AUS	Y	Vegetable
	var ***acephala*** (Kale)			
(39)	Kapeti	NZ	W	Fodder
(40)	Grenadier	NZ	W	Fodder
	B. carinata (Abyssinian mustard)			
(41)	BRA 1028/79		H	Condiment/oilseed
(42)	Brown Raya	PAK	H	Condiment/oilseed
(43)	054100	ETH	H	Condiment/oilseed
(44)	054108	ETH	H	Condiment/oilseed
(45)	PI209023	PRI	H	Condiment/oilseed
(46)	PI360882	SWE	H	Condiment/oilseed
	B. nigra (Black mustard)			
(47)	91046	FRA	H	Condiment/oilseed
(48)	Junius 91072	DEU	H	Condiment/oilseed
(49)	91073	IND	H	Condiment/oilseed
(50)	91080	SUN	H	Condiment/oilseed
(51)	91131	YUG	H	Condiment/oilseed
	B. juncea (Brown, yellow or Indian mustard)			
(52)	Cutlass	CAN	H	Condiment
(53)	Can. Low (23896)	CAN	C	Oilseed (Low)
(54)	(651-2-5-1)	AUS	C	Oilseed (Low)
(55)	(651-2-5-7)	AUS	C	Oilseed (Low)
(56)	(690-13-2)	AUS	C	Oilseed (Mod.)
(57)	(689-27-1+-2)	AUS	C	Oilseed (High)
(58)	(683-18-1+-2)	AUS	C	Oilseed (High)
(59)	(347-6-1-1$M_3$4-4&5-2)	AUS	C	Oilseed (High)
(60)	Domo	CAN	C	Condiment
(61)	99Y-1-1	AUS	C	Oilseed (High)
(62)	Siromo	AUS	C	Oilseed (High)
(63)	Jap. mustard No.16	_	C	Leaf vegetable
(64)	Jap. mustard No.14	_	C	Leaf vegetable
(65)	Jap. mustard No. 7	_	C	Leaf vegetable
	Sinapis alba (White mustard)			
(66)	Kirby	GRB	H	Condiment
(67)	Ochre	CAN	H	Condiment
(68)	Tilney	GBR	H	Condiment
(69)	BHL6-3548	_	H	Condiment
(70)	SIN 2/77	_	H	Condiment
	B. fruticulosa			
(71)	BFR5 94719	AUS	H	Weed
	B. tournefortii (Wild turnip)			
(72)	BTO 13C 95072	AUS	H	Weed
	Sisymbrium orientale (Indian hedge mustard)			
(73)	SOR27 95035	AUS	H	Weed
	Sinapis arvensis (charlock)			
(74)	SAR 3 94959	AUS	H	Weed
	Diplotaxis tenuifolia (Lincoln weed, sand rocket)			
(75)	DTE 1	AUS	H	Weed
	Eruca sativa (salad rocket)			
(76)	90300		H	Leaf vegetable

[A]Sources of seed : H - Horsham Temperate Crop Collection, VIDA Horsham Victoria Australia; W-Wrightson Seeds; C- CSIRO Division of Plant Industry; VS - Valley Seeds; IU - University of Idaho; Y - Yates Seeds.

[B]Seed glucosinolate levels in oilseeds from Sernyk (1995) or provided by seed suppliers: Low = <30 μmole g^{-1}., Mod. = 30-80μmole g^{-1}, High = >80 μmole g^{-1}

Table 2. Description of field site and seasonal conditions at Ginninderra Experiment Station Canberra ACT.

Site description

Location	149° 06' long, 35° 12' lat: 600 m a.s.l.
Soil type	Yellow podzolic (GN 3.85 Northcote *et al* 1971)
Texture of surface soil	Fine sandy loam
pH (water)	5.9
EC (μS cm^{-1})	85
Total C (%)	1.4
Total N (%)	0.11
Total S (%)	0.01
CEC	64 meq kg^{-1}
Sulphate S (mg kg-1) (KCl)	
0-10 cm	7
10-30 cm	17

Seasonal conditions

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Temperature (°C)							
Average Max.	12.0	9.9	16.1	16.0	19.3	21.7	24.0
Average Min.	2.2	0.7	1.8	4.1	7.7	9.9	10.1
Rainfall (mm)	39	53	5	43	87	138	61
Daylength (h)	9.7	9.9	10.7	11.7	12.8	13.8	14.3

Sampling and sample preparation

GSL concentration is known to vary with plant ontogeny (e.g. Smith and Griffiths 1988) and above ground GSLs in vegetative material usually reach a maximum around flowering (Fieldsend and Milford 1994). Accordingly, each entry was sampled at mid-flowering, before pods had developed, corresponding to growth stage 3.1-3.2 of Berkenkamp (1973). Ten bordered plants from the inner three rows were dug from each plot to a depth of 0.15 m and taken to the laboratory with the soil surrounding the intact roots. The soil was washed from the roots, and a subsample of 8-10 plants of each entry was selected by combining plants from each of the three replicates. The plants were immediately separated into root and shoot tissue and frozen at -20°C. The samples were freeze-dried, and the material weighed, ground using a Wiley mill with 1 mm screen and stored in sealed bottles at -20°C prior to their extraction for HPLC analysis. Shoot and root biomass (g m^{-2}) and shoot index (shoot biomass/total plant biomass) were calculated for each entry.

The large number of entries prohibited separate analysis of individual replicates of all entries. In order to estimate Least Significant Differences to assess the variation in individual and total GSL concentration of the entries, a representative selection of 10 entries were harvested and analysed as separate replicates.

GSL extraction and analysis

GSLs from 300 mg of freeze-dried root and shoot tissues were extracted and transformed to desulphoglucosinolates

according to the method described by Magrath *et al.* (1993) with modifications as follows. The poly-prep columns (Biorad Laboratories, CA, USA) used for desulphation of GSLs were end-capped prior to introduction of sulphatase on the exchange resin. The end-caps were taken off momentarily to allow the enzyme to percolate into the resin and displace the extra buffer between the resin particles. The column ends were then recapped and left overnight for desulphation.

Desulphoglucosinolates were separated using a gradient HPLC method on a Waters HPLC System (Waters Inc., Milford, MA, USA) equipped with a Maxima 820 Chromatographic Workstation (Dynamic Solutions, CA, USA). The System consisted of a 600E multisolvent delivery system, a model 717 autosampler and a model 486 tunable absorbance detector set to a wavelength of 229 nm. A spherisorb (C-18) reversed-phase column (ODS2, 5μm, Alltech) with dimensions of 250 by 4.6mm (i.d.) was used for separation. The mobile phase consisted of two reservoirs: A - 100% Milli-Q reagent water (Millipore, MA, USA) and B - 50% acetonitrile: water passed through 0.2 μm nylon membrane (Schleicher & Schuell, NH, USA). Both the reservoirs were deaerated by sparging with helium. The solvent flow rate was 1 ml min^{-1} except for an initial reduction from 1 ml min^{-1} to 0.97 ml min^{-1} over 20 minutes. The programme consisted of 99% A + 1% B for 1 minute and a linear gradient over 20 minutes to 1% A + 99% B, held for 3 minutes, during which the desulphoglucosinolates were separated; the programme returned to the starting conditions (99% A + 1% B) by a linear gradient over 1 minute followed by 11 minutes equilibration. The desulphoglucosinolates were quantified using 2-propenyl (Sigma, St. Louis, MO, USA) and benzyl GSL (Canola Council of Canada, Winnipeg, Canada) as internal standards and response factors for desulphoglucosinolates published by European Economic Community (Commission Regulation (EEC) No 1864/90). The GSLs identified, their retention times and major hydrolysis products are shown in Table 3. Peaks were identified using pure standards either purchased (above), or kindly supplied by Dr R Mithen, John Innes Centre, Norwich U.K. and Dr R. Wallsgrove, Rothamsted U.K.

Seed was collected from the entries at maturity and total seed glucosinolate concentration determined using the X-ray Fluorescence Spectrometric Analysis technique described by Pinkerton *et al.* (1993).

Results

Growth and biomass production

There were no major constraints to growth during the season, although growth was slow during the cold, wet winter period. The species in the study included a wide range of oilseed, fodder, vegetable (root and leaf) and weed types and accordingly there was large variation in shoot and root morphology, phenology and biomass production (Table 4). Days to reach mid-flowering in the *Brassica* species varied from 102 days in *B. campestris* to 189 days in *B. nigra*. The *B. oleracea* vegetables did not flower and were harvested on day 186 to avoid excessive senescence and insect damage to the shoots. In species which had more than three entries, mid-flowering time varied by 20 to 50 days, and later flowering within species generally led to taller plants and greater biomass. Biomass varied within most species from three to ten fold and regression analysis within species indicated that flowering time accounted for most of this variation (r^2 = 0.58 to 0.95) in all species except *B. oleracea*, where the relationship was poor. When analysed as 75 separate entries, time to mid-flowering accounted for 60% of the variation in total biomass. The remainder of the variation in biomass was due to morphological differences. For example the short leafy fodder *B. napus* entry 22 produced the same biomass as the tall (>2m) *B. nigra* entry 51 despite flowering 30 days earlier. Shoot biomass generally accounted for at least 80% of total plant biomass, although this varied from 60 to 92%. Root and shoot biomass were significantly positively correlated in all species (r^2 = 0.60 to 0.96) except the *B. oleracea* vegetables.

Table 3. Glucosinolates identified in *Brassica* samples, their HPLC retention times and major hydrolysis products.

Number	Chemical name	Trivial name	Retention time	Hydrolysis products[A]
Aliphatic				
1	3-Methylthiopropyl	Glucoiberverin	16.80	ITC, nitriles
2	3-Methylsulphinylpropyl	Glucoberin	7.27	ITC, nitriles
3	2-Propenyl	Sinigrin	12.53	ITC, nitriles
4	4-Methylthiobutyl	Glucoerucin	19.79	ITC, nitriles
5	4-Methylsulphinylbutyl	Glucoraphanin	13.51	ITC, nitriles
6	3-Butenyl	Gluconapin	16.52	ITC, nitriles
7	2-Hydroxy-3-butenyl	Progoitrin	9.51	Oxazolidine -2-thiones
8	5-Methylsulphinylpentyl	Glucoalyssin	11.96	ITC, nitriles
9	4-Pentenyl	Glucobrassicanapin	18.83	ITC, nitriles
10	2-Hydroxy-4-pentenyl	Gluconapoleiferin	15.24	Oxazolidine -2-thiones
Aromatic				
11	2-Phenylethyl	Gluconasturtiin	21.42	ITC
12	2-Hydroxy-2-phenylethyl	Glucobarbarin	17.82	ITC
13	Benzyl	Glucotropaeolin	19.11	ITC
14	*p*-Hydroxybenzyl	Glucosinalbin	15.58	ITC
Indolyl				
15	3-Indolylmethyl	Glucobrassicin	20.20	Indolyl-3-carbinol
16	4-Hydroxy-3-indolylmethyl	4-Hydroxyglucobrassicin	17.56	Thiocyanate
17	4-Methoxy-3-indolylmethyl	4-Methoxyglucobrassicin	21.04	Auxins?
18	1-Methoxy-3-indolylmethyl	Neoglucobrassicin	23.09	Phytoalexins?

[A] from Mithen (1992) and Rosa *et al.* (1997)

GSL profiles

Shoot

Aliphatic GSLs dominated the shoot profiles of all *Brassica* species while some weed species contained higher concentrations of aromatic GSLs (Table 5 and 6). Indolyl GSLs were ubiquitous, but present in relatively low concentrations (< 1 umole g^{-1}) except in some of the *B. oleracea* vegetables. The concentration of major individual GSLs generally varied from two to ten fold within species (Table 5) as did total glucosinolates (Table 6). These differences were significant based on the LSDs calculated for individual or total GSLs in the representative entry selection (Table 5, 6).

B. napus and *B. campestris* entries had similar shoot GSL profiles dominated by 3-butenyl GSL, 4-pentenyl GSL and their hydroxy-substituted forms, and also contained significant levels of the aromatic 2-phenylethyl GSL. Maximum concentrations of these glucosinolates were two to ten times higher in *B. napus oleifera* biennus than *B. napus oleifera* annua while *B. napus rapifera* was intermediate.

The *B. oleracea* vegetables had lower concentrations of the three alkenyl aliphatic GSL dominant in other species, but higher concentrations of the methylthio and methylsulphinyl analogues as well as significant concentrations of the indolyl GSLs particularly 3-indolyl-methyl GSL and 1-methoxy-3-indolyl methyl GSL. The dominant GSL types varied within the subspecies (Table 5) although the total GSL concentrations were similar (Table 6).

Table 4. Range in mid-flowering date and plant biomass in field grown *Brassica* and related species.

Species	No. of Entries.	Days to mid-flowering	Height (cm)	Biomass (g m^{-2}) Shoot	Root (0-0.15m)	Shoot index
B. napus oleifera						
biennis	13	127-172	53-146	208-2662	42-649	0.60-0.89
annua	7	118-126	43-76	108-500	34-100	0.78-0.84
ssp. *rapifera*	3	131-167	117-125	1415-1868	239-623	0.75-0.86
B. campestris oleifera						
biennis	1	119	58	214	34	0.88
annua	5	102-120	28-57	56-248	17-46	0.75-0.83
ssp. *rapifera*	5	133-152	84-133	755-2080	93-942	0.69-0.89
B. oleracea						
var. *gemmifera*	1	186*	33	1202	216	0.85
var. *capitata*	1	186*	26	1589	220	0.88
var. *botrytis*						
svar. *cauliflora*	1	186*	32	1024	117	0.89
svar. *cymosa*	1	186*	39	2892	259	0.92
var. *acephala*	2	159-190*	52-55	1636-2063	327-649	0.72-0.86
B. carinata	6	139-162	76-138	435-2417	66-480	0.86-0.91
B. nigra	5	124-189	67-215	296-1630	33-322	0.84-0.91
B. juncea	14	116-148	63-118	205-1792	33-191	0.78-0.91
S. alba	5	125-126	42-66	140-387	30-78	0.81-0.92
E. sativa	1	116	36	157	24	0.89
B. fruticulosa	1	148	39	83	49	0.62
B. tournefortii	1	120	50	100	23	0.81
S. orientale	1	126	18	60	20	0.75
S. arvensis	1	106	18	66	12	0.88
D. tenuifolia	1	203	64	1075	141	0.89

* did not flower - harvested on days shown.

B. carinata and *B. nigra* entries had simple profiles dominated by 2-propenyl GSL and a similar range in total GSL concentration was evident in the two species. In *B. juncea*, while 2-propenyl GSL was the major GSL detected, significant levels of 3-butenyl GSL and 4-pentenyl GSL were also present in some entries. However, unlike *B. napus* and *B. campestris*, the OH-substituted forms of these alkenyl GSL were not present in appreciable quantities in *B. juncea*. *B. carinata* and *B. nigra* entries contained minimum 2-propenyl GSL concentrations of 10 μmole g^{-1}, while some *B. juncea* entries had concentrations as low as 0.1 μmole g^{-1}.

S. alba and weed species tended to have relatively simple profiles dominated by one or two GSLs in high concentrations. *S. alba, S. arvensis* and *D. tenuifolia* had high concentrations of the aromatic GSLs, benzyl GSL and *p*-hydroxybenzyl GSL, which were absent from the shoots of the *Brassica* species. *B. tornefortii, B. fruticulosa* and *S. orientale* were dominated by 3-butenyl GSL while the latter also contained 3-methylthiopropyl GSL, which was not found in appreciable quantities in any of the other entries. Despite the high concentrations of the individual aliphatic and aromatic GSLs in the weed species, the concentration of the indolyl forms was low.

Table 5. Range in the concentration of individual glucosinolates μmole g^{-1}) found in shoots of field grown *Brassica* and related species at flowering. Glucosinolates are numbered as in Table 3.

Species	No. of Entries	Aliphatic 2	3	5	6	7	9	10	Aromatic 11	Indolyl 15	16	17	18
B. napus oleifera													
biennis (oil)	6	-	-	0.2-0.3	0.5-3.3	1.6-9.2	0.8-7.8	0.6-1.9	0.6-2.3	0.2-0.4	-	0.1	0.1-0.3
biennis (fodder)	7	-	-	0.5-0.8	0.3-8.7	0.5-5.9	0.5-4.5	0.8-2.9	0.4-0.9	0.1-0.6	0.1	0.1	0.1-0.4
annua	7	-	-	0.1-0.5	0.1-0.8	0.2-3.8	0.1-1.8	0.1-1.5	0.1-1.3	0.1-0.6	0.1	0.1	0.1
B. napus rapifera	3	-	-	-	0.4-0.6	0.5-5.1	0.6	0.5-3.7	0-1.4	0.1-0.4	-	-	0.1-0.4
B. campestris oleifera													
biennis	1	-	-	-	1.6	1.7	3.8	1.8	1.6	0.2	0.1	-	-
annua	5	-	-	-	0.4-1.3	0.4-3.0	1.3-3.8	1.1-5.6	0.8-3.6	0.2-1.7	0.1-0.5	0.1-0.3	0.1-0.5
B. campestris rapifera	5	-	-	-	0.7-4.0	0.9-4.6	1.9-6.5	1.1-2.1	0.6-1.7	0.1-0.2	0.1	-	0.1-0.3
B. oleracea													
var. *gemmifera*	1	2.2	1.3	1.0	0.5	0.5	-	-	-	2.9	0.2	-	0.3
var. *capitata*	1	3.2	1.3	-	-	-	-	-	-	3.0	0.1	0.1	0.6
var. *botrytis*													
sv. *cauliflora*	1	1.4	-	-	-	-	-	-	-	1.6	0.1	0.7	3.2
sv. *cymosa*	1	-	-	2.4	-	-	-	-	-	1.3	-	0.1	4.1
var. acephala	2	1.4-1.5	2.2-3.2	0.5-1.1	0.5-1.6	0-1.4	-	-	-	3.1-4.0	0.2-0.3	-	1.0-1.1
B. carinata	6	-	10.0-20.2	-	-	-	-	-	-	0.4-0.9	0-0.1	-	0-0.1
B. nigra	5	-	10.7-26.4	-	-	-	-	-	0.3-0.4	0.1	-	-	-
B. juncea	14	-	0.1-18.7	-	0-7.5	-	0.1-2.0	0.1-0.3	0-1.3	0-0.2	0-0.1	-	0.1-0.2

Species	No.	Aliphatic 1	5	6	7	8	9	10	Aromatic 11	13	14	Indolyl 15	16	17	18
S. alba	5	-	-	-	0.4-0.5	-	1.0-3.4	-	0.3-0.4	1.9-9.6	9.0-14.4	0-0.1	-	-	0
E. sativa	1	-	4.4	-	-	-	-	-	-	-	-	0.5	-	-	
B. fruticulosa	1	-	-	33.8	-	-	-	0.6	0.4	-	-	-	-	-	-
B. tournefortii	1	-	-	34.8	-	0.8	0.7	-	0.9	-	-	-	-	0.1	-
S. orientale	1	13.2	-	30.2	-	-	-	-	-	-	-	-	1.5	-	-
S. arvensis	1	-	-	-	0.6	-	-	-	1.4	-	15.3	-	-	0.7	6
D. tenuifolia	1	-	8.1	1.2	-	-	-	-	1.1	12.0	-	2.1	0.7	0.5	-

LSD's ($P < 0.05$) for individual glucosinolate concentrations: 3 - 1.0; 5 - 3.4; 6 - 2.1; 9 - 1.2; 10 - 0.4; 11 - 0.6; 15 - 0.2; 16 - 0.1; 17 - 0.5; 18 - 0.5

(-) = not detected (<0.01 umole g^{-1})

Entries containing total GSL concentrations > 20 μmole g^{-1} were represented in *B. napus oleifera* biennis, *B. carinata, B. nigra, B. juncea, S. alba* and all of the weed species, although the ranges within species were large (Table 6). Maximum total GSL concentrations in *B. napus oleifera* annua, *B. campestris* and *B. oleracea* were < 15 μmole g^{-1}. In all *B. napus*, *B. campestris* and *B. oleracea* species only ~50% of the total GSLs were ITC liberating, while for *B. nigra, B. juncea, B. carinata, S. alba* and the weed species almost all of the GSLs contained in the plant were ITC liberating (Table 6).

Table 6. Range in concentration of glucosinolate classes found in the shoots of field grown *Brassica* and related species at flowering.

Species	No. of Entries	Glucosinolate concentration (umole g^{-1}) Total Aliphatic	Total Aromatic	Total Indolyl	Total	Total ITC liberating
B. napus oleifera						
biennis (oil)	6	3.5-22.2	0.6-2.3	0.2-0.6	4.6-23.8	1.9-12.3
biennis (fodder)	7	4.1-17.2	0.4-0.9	0.1-0.7	5.5-18.3	1.3-12.8
annua	7	0.1-8.4	0.0-1.3	0.0-0.7	0.2-10.4	0.1-4.4
ssp.*rapifera*	3	1.0-10.0	0.0-1.4	0.2-0.8	1.2-12.2	0.1-2.6
B. campestris oleifera						
biennis	1	8.4	1.6	0.3	10.3	7.0
annua	5	3.4-8.6	0.8-3.6	0.3-2.5	5.3-14.7	2.8-7.0
ssp. *rapifera*	5	6.4-13.4	0.6-1.7	0.2-0.6	7.8-15.5	3.6-9.7
B. oleracea						
var. *gemmifera*	1	5.5	-	3.4	8.9	5.0
var. *capitata*	1	4.5	-	3.8	8.3	4.5
var. *botrytis*						
svar. *cauliflora*	1	1.4	-	5.6	7.0	1.4
svar. *cymosa*	1	2.4	-	5.5	7.9	2.4
var. *acephala*	2	4.7-8.7	-	4.5-5.2	9.2-13.9	4.7-7.3
B. carinata	6	10-20.2	-	0.3-0.9	10.5-21.1	10.0-20.2
B. nigra	5	10.7-26.4	0.0-0.4	0.0-0.1	11.2-26.4	11.1-26.4
B. juncea	14	1.1-20.5	0.0-1.3	0.1-0.4	1.4-21.7	1.2-21.6
S. alba	5	1.4-3.8	12.6-24.0	0.0-0.1	15.8-25.6	15.7-25.6
E. sativa	1	4.4	-	0.5	4.9	4.4
B.fruticulosa	1	34.3	0.5	0.3	35.1	34.2
B. tournefortii	1	36.3	0.9	0.1	37.3	37.2
S. orientale	1	43.4	-	1.5	44.9	43.4
S. arvensis	1	0.6	16.8	2.3	19.7	17.4
D. tenuifolia	1	9.3	13.0	2.8	25.1	21.7
LSD (P<0.05)		3.2	5.3	0.3	6.6	4.3

(-) = not detected (<0.01 μmole g^{-1})

Roots

In contrast to the shoots, the roots of all *Brassica* species contained significant concentrations of aromatic GSLs, predominately 2-phenylethyl GSL (Table 7, 8). Significant quantities of benzyl GSL and *p*-hydroxybenzyl GSL were also present in the roots, while they were not detected in the shoots. In common with the shoots, indolyl GSLs were ubiquitous but in low concentrations (<2 μmole g^{-1}). Aliphatic GSLs were present in appreciable quantities in most species, and in general the profile in the shoots and roots was similar (c.f. Tables 5 and 7). As for the shoots, the concentrations of individual GSLs varied within species by two to ten fold (Table 7) while total

GSLs in roots varied by two to five fold (Table 8). These differences within species were significant based on the LSDs constructed from the representative entry selection (Table 7, 8).

B. napus profiles were dominated by 2-phenylethyl GSL although significant quantities of the aliphatic GSLs found in the shoots (3-butenyl GSL, 4-pentenyl GSL and their OH-substituted forms) were also present. Although the maximum concentrations of some individual aliphatic GSLs were similar in the roots and shoots (e.g. 3-butenyl GSL, 4-pentenyl GSL) (c.f. Table 5 and 7), total aliphatic GSL concentrations were generally higher in the shoots (see Tables 6 and 8). Consistent with the shoots, the maximum observed concentrations of individual and total root GSLs within *B. napus* sub-species followed the pattern biennis > rapifera > annua, although the range within sub-species was considerable.

In common with their shoot GSL profiles, *B. napus oleifera* annua and *B. campestris oleifera* had similar root profiles although 3-butenyl GSL and 2-Hydroxy-3-butenyl GSL were absent from the latter. *B. campestris* ssp *rapifera* had significantly higher individual and total concentrations of aliphatic GSLs in the roots than ssp. *oleifera.*

B. oleracea vegetables had similar profiles of aliphatic GSLs in roots and shoots, lower concentrations of indolyl GSLs in the roots but higher levels of the aromatic 2-phenylethyl GSL.

As for *B. napus* and *B. campestris,* 2-phenylethyl GSL was the dominant GSL found in the roots of *B. carinata, B. nigra* and *B juncea.* These species also had significant concentrations of 2-propenyl GSL in their roots although the maximum concentrations were approximately half of that found in the shoots (cf Table 5 and 7). Consistent with shoots, 3-butenyl GSL was also found in the roots of some *B. juncea* entries. The low levels of 2-propenyl GSL observed in the shoots of double low *B. juncea* lines were also observed in the roots, some of which had no detectable level present (Table 7).

In *S. alba* and the weed entries, the dominant GSLs found in shoots also dominated in roots, but as for all the other species, this was in association with high levels of 2-phenylethyl GSL. In some species the concentrations of individual GSLs were higher in the roots than the shoots (e.g. *D. tenuifolia, S. arvensis*). The high concentration of 3-butenyl GSL in the shoots of *B. fruiticulosa, S. orientale* and *B. tournefortii* were also present in roots, along with similarly high concentrations of 2-phenylethyl GSL. The roots of the weed species contained the highest concentrations of both individual and total GSLs measured in the study. A greater portion of the total root GSLs were ITC liberating compared to the shoots due to the dominance of 2-phenylethyl GSL in the roots (Table 8).

Significant positive correlations between total root and total shoot GSL concentrations were only found within *B. napus* and *B. carinata* entries (both $r^2=0.81$) while there were neither significant nor consistent correlations within the other species.

Seeds

There was a significant ($P<0.05$) positive correlation between total seed and total shoot GSL concentration for the 65 entries for which seed was collected, although the relationship accounted for only 22% of the variation. There were no significant or consistent relationships within species, with the exception of *B. juncea* where seed GSL and shoot GSL were positively correlated ($r^2=0.56$). There was no relationship between seed GSL concentration and root GSL concentration generally, or within any of the individual species.

GSL production

GSL production on a ground area basis (mmole $^{-2}$) is the product of the biomass and GSL concentration of a particular tissue and is shown for shoot and root tissues and whole plants for individual entries in Fig 1. Isolines of GSL production on a ground area basis are shown for combinations of GSL concentration and biomass production. In shoot tissues (Fig 1a) there was large variation in GSL production both within and between species. High shoot GSL production (> 20 mmole $^{-2}$) arose from high biomass (e.g. *B. oleracea*), from high

Table 7. Range in the concentration of individual glucosinolates (μmole g^{-1}) found in roots of field grown *Brassica* and related species at flowering. Glucosinolates are numbered as in Table 3.

Species	No. of Entries	Aliphatic 2	3	5	6	7	9	10	Aromatic 11	13	Indolyl 15	16	17	18
B. napus oleifera														
biennis (oil)	6	-	-	0.4-0.7	0.4-1.0	0.3-3.7	0.4-4.4	0.7-2.6	7.9-19.3	1.0-2.7	0.3-0.5	-	0.2-0.7	0.1
biennis (fodder)	7	-	-	0.5-0.9	0.7-10.2	1.3-5.0	0.6-7.5	0.8-2.0	7.1-19.5	1.0-3.4	0.2-1.1	-	0.4-1.1	0.1-0.4
annua	7	-	-	0.1-0.8	-	0.3-0.7	0.4	0.5-1.5	2.3-11.6	0.2-1.3	0.2-0.4	0.1	0.2-0.4	0.1-0.4
B. napus rapifera	3	-	-	-	-	0.6-1.4	-	0.6-1.6	1.5-12.8	0.4-1.3	0.3-0.7	0.1-0.2	0.1-0.2	0.2-1.1
B. campestris oleifera														
biennis	1	-	-	-	-	-	0.3	0.6	9.4	-	0.3	-	0.4	0.3
annua	5	-	-	-	-	-	0.3	0.4-0.6	2.1-9.0	0.2	0.2-0.4	0.1-0.8	0.2-0.8	0.2-1.0
B. campestris rapifera	5	-	-	-	0.3-2.0	0.3-4.5	0.5-2.7	0.9-1.8	7.1-11.9	-	0.2-0.6	-	0.1-0.2	0.1-0.5
B. oleracea														
var. *gemmifera*	1	0.3	1.2	0.4	0.7	0.5	-	-	2.5	-	0.1	0.3	0.1	0.3
var. *capitata*	1	1.3	6.2	-	-	-	-	-	3.3	-	0.5	2.0	0.5	0.4
var. *botrytis*														
sv. *cauliflora*	1	0.7	-	-	-	-	-	-	1.7	-	0.7	0.8	0.7	1.1
sv. *cymosa*	1	-	-	1.5	0.3	0.3	-	-	3.1	-	0.2	0.2	0.2	0.4
var. *acephala*	2	0.3	1.1-2.3	0.6-0.8	0.4	0-0.9	-	-	4.7-5.2	2.2-3.0	0.2-0.4	0.6-0.7	0.2-0.4	0.3-0.4
B. carinata	6	-	2.2-10.4	-	0-0.4	0-1.0	-	-	3.9-12.2	-	0.1-0.4	0-0.2	0.1-0.4	0.1-0.6
B. nigra	5	-	0.5-5.1	-	-	-	-	-	0.9-8.8	-	tr-0.1	tr-0.2	0.1-0.4	tr-0.1
B. juncea	14	-	0-4.8	-	0-1.9	-	0-0.5	-	2.5-12.5	0-0.6	0-0.9	0-0.2	0.1-1.1	0-0.7

Species	No.	Aliphatic 2	5	6	7	8	Aromatic 11	13	14	Indolyl 15	16	17	18
S. alba	5	0-0.2	0.9-1.7	-	0-0.4	0-0.7	2.1-4.1	2.0-5.3	2.7-3.9	0-0.2	-	0.1-0.9	0.2-1.2
E. sativa	1	-	0.7	-	-	-	1.8	-	-	-	-	-	-
B. fruticulosa	1	-	-	12.9	-	1.9	18.5	-	-	0.5	-	0.3	0.4
B. tournefortii	1	-	-	21.7	-	7.1	26.4	-	-	-	-	0.3	-
S. orientale	1	-	-	13.7	-	-	25.7	-	5.3	0.7	-	0.8	1.3
S. arvensis	1	-	-	-	0.9	-	19.0	-	23.1	-	0.2	-	0.1
D. tenuifolia	1	-	14.7	-	-	-	1.7	50.0	2.0	0.5	-	-	-

LSD's for individual glucosinolate concentrations: 5 - 1.5; 6 - 1.1; 8 - 1.0; 11 - 2.3; 13 - 3.6; 14 - 0.4; 15 - 0.1;17 - 0.2; 18 - 0.2.
(-) = not detected (<0.01 μmole g^{-1})

GSL concentration (e.g. *B. napus oleifera* biennis) or from combinations of moderate to high levels of each (e.g. *B. nigra*). Despite the large variation some general patterns emerged. All of the *B. napus oleifera* annua and *B. campestris oleifera* entries had shoot GSL production which fell on or below the 2 mmole m^{-2} isoline while no other species had all entries in this low category. All of the other species, with the exception of *S. alba*, had entries with > 10 mmole m^{-2} and six species had entries with > 20 mmole m^{-2}. There was no significant correlation found between shoot biomass and shoot GSL concentration in any of the species, indicating that high biomass production did not lead to a dilution of GSL concentration, although no entries had both high GSL concentration and high biomass.

Table 8. Range in concentration of glucosinolate classes and total glucosinolates found in the roots of field grown *Brassica* and related species at flowering.

Species liberating	No. of Entries.	Glucosinolate concentration (μmole g^{-1}) Total Aliphatic	Total Aromatic	Total Indolyl	Total	Total I T C
B. napus oleifera						
biennis (oil)	6	1.8-12.1	8.9-21.4	0.3-1.2	11.5-33.9	9.7-27.0
biennis (fodder)	7	4.6-21.7	9.0-21.3	0.7-2.2	20.7-34.9	13.8-29.7
annua	7	0.1-3.2	2.5-12.5	0.5-1.2	3.2-15.9	2.7-13.7
ssp.*rapifera*	3	1.2-3.0	1.9-14.1	0.6-2.2	3.7-19.3	1.9-14.1
B. campestris oleifera						
biennis	1	0.9	9.4	1.0	11.3	9.7
annua	5	0.0-0.9	2.1-9.4	0.7-1.9	3.8-11.3	2.1-9.0
ssp. *rapifera*	5	2.8-8.5	7.1-11.9	0.6-0.9	13.2-21.2	8.3-14.8
B. oleracea						
var. *gemmifera*	1	3.1	2.5	0.8	6.4	5.1
var. *capitata*	1	7.5	3.3	3.4	14.2	10.8
var. *botrytis*						
svar. *cauliflora*	1	0.7	1.7	3.3	5.7	2.4
svar. *cymosa*	1	2.1	3.1	0.9	6.1	4.9
var. *acephala*	2	2.6-4.5	6.9-8.2	1.3-1.9	12.1-13.3	10.5-10.8
B. carinata	6	2.2-10.9	3.9-12.2	0.5-1.2	8.0-24.3	7.1-23.1
B. nigra	5	0.5-5.1	0.9-8.8	0.2-0.6	4.0-11.1	1.4-10.5
B. juncea	14	0.4-4.8	2.5-12.8	0.1-1.2	4.6-14.5	4.1-11.9
S. alba	5	0.9-2.4	7.1-12.3	0.3-1.9	8.8-16.5	8.0-14.7
E. sativa	1	0.7	2.5	nd	3.2	2.5
B.fruticulosa	1	14.8	18.6	1.0	34.4	33.5
B. tournefortii	1	28.8	26.4	0.3	55.5	55.2
S. orientale	1	13.7	31.0	2.8	47.5	44.7
S. arvensis	1	0.9	42.1	0.3	43.3	43.0
D. tenuifolia	1	14.7	53.6	0.6	68.9	68.2
LSD ($P<0.05$)		2.7	3.3	0.2	4.9	3.7

GSL production in roots was lower than that of shoots primarily due to the lower root biomass, while the range in GSL concentrations in roots and shoots was similar (Fig 1 a,b). High levels of root GSL production resulted from high biomass in some entries (e.g. *B. campestris rapifera*) and from high glucosinolate concentration in others (e.g. *D. tenuifolia*), while most of the *B. napus oleifera* biennis combined moderate to high levels of biomass and GSL concentration. GSL production of all *B. napus oleifera* annua, *B. campestris oleifera* and *S. alba* entries fell below the 1 mmole m^{-2} isoline while *B. napus oleifera* biennus entries were predominately above the 5 mmole m^{-2} isoline. The entries of the other species/subspecies fell into the low (< 1 mmole m^{-2}) or moderate

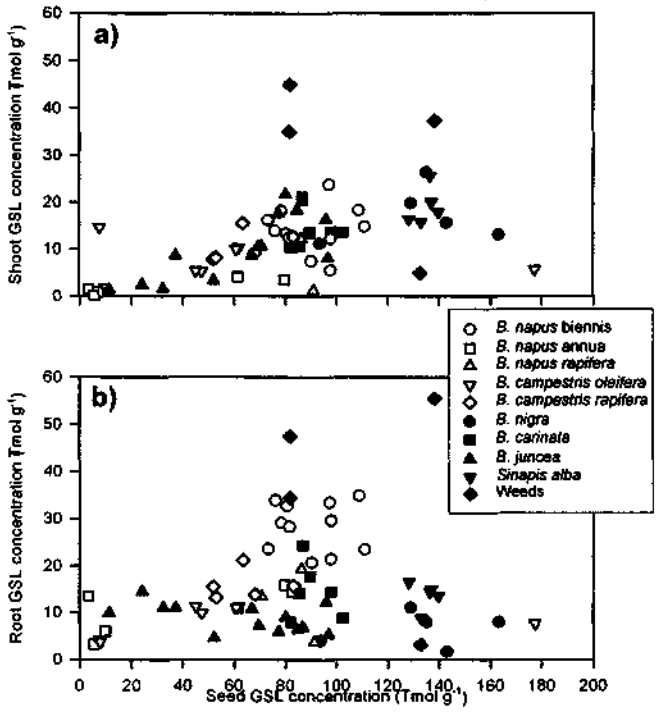

Figure 1. Relationship between seed glucosinolate concentration of (a) shoot tissue and (b) root tissue for *Brassica* and related weed species.

(<5 mmole m^{-2}) categories. Despite the high GSL concentrations found in weed species, low root biomass resulted in low levels of GSL production with the exception of *D. tenuifolia* which fell into the > 5 mmole m^{-2} category. Interestingly, there were significant ($P < 0.05$) negative correlations between root biomass and root GSL concentrations in *B. napus oleifera* annua ($r^2=0.47$), and *B. campestris oleifera* ($r^2=0.75$), the two species with the lowest GSL concentrations, but no significant relationships within any of the other species.

The pattern of total plant GSL production generally followed that of the shoots due to the relatively large contribution of the shoots to total plant biomass (Fig 1c). All entries of *B. napus oleifera* annua and *B. campestris oleifera* produced < 5 mmole m^{-2} GSL, as did several of the *B. juncea* entries, and the *S. alba* entries fell just above the 5 mmole m^{-2} isoline. Entries from all other species were distributed within the 5-30 mmole m^{-2} range. Entries from *B. napus oleifera* biennis, *B. campestris rapifera, B. oleracea, B. nigra, B. carinata* were represented in the > 30mmole m^{-2} category along with the weed species *D. tenuifolia.*

The ranking of entries for total plant GSL production changed when only the ITC liberating GSLs were considered (Fig 2). Only half of the total GSLs present in *B. napus, B. campestris* and *B. oleracea* species yield ITCs upon hydrolysis due primarily to significant concentrations of OH-substituted aliphatic GSLs (GSL 7, 10) or indolyl GSLs in their tissues, which do not yield ITCs upon hydrolysis (Table 3). Despite several entries within these species producing high total GSLs, only one entry within these species produced > 20 mmole m^{-2} of ITC liberating GSL (Fig 2). In contrast, *B. nigra, B. carinata, B. juncea, S. alba* and the weeds species contained predominately ITC liberating GSLs and consequently several entries within these species produced > 20 mmole m^{-2} of ITC liberating GSLs (Fig 2).

Discussion

The results from this experiment demonstrate the diversity of GSL profiles, concentrations and production within and between *Brassica* and related species when grown in the same environment. The variation was evident in both root and shoot tissues, and derived from differences in both GSL concentration and biomass production, which

were not significantly correlated. These results suggest that where pest or disease suppression by *Brassica* rotation or green manure crops can be linked to particular GSL hydrolysis products, opportunities will exist to select or

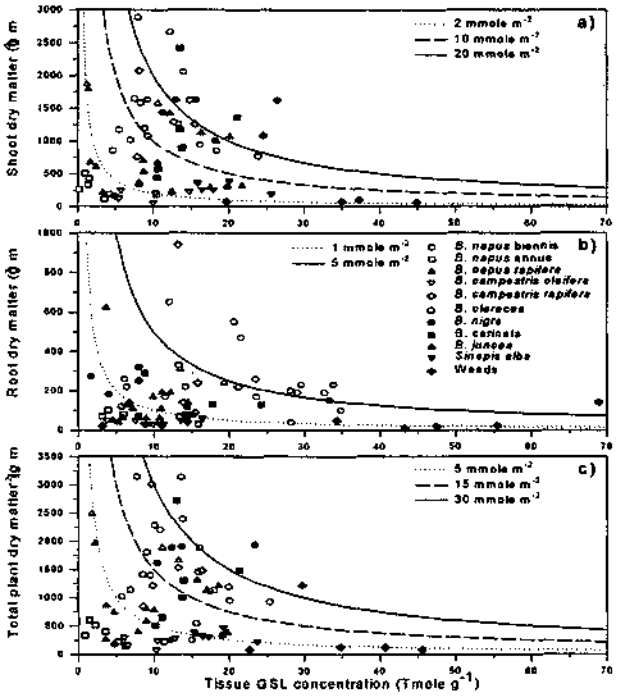

Figure 2. Relationship between tissue glucosinolate concentration and biomass for (a) shoot tissue, (b) root tissue and (c) total plant for *Brassica* and related weed species.

develop brassicas with enhanced biofumigation potential.

The factors which determine the biofumigation potential (BP) of a particular *Brassica* genotype for a given target organism can be considered in the following equation:

$$BP = TOTAL_{biomass} \times S_{i=1\ to\ n} [HI_i \times TOTAL_{GSi} \times S_{j=1\ to\ p} (Ai_{ij} \times TOXICITY_j)]. \ldots . (1)$$

where:
BP = Biofumigation potential for a particular target organism
$TOTAL_{biomass}$ = biomass of whole plant
HI_i = [biomass plant part i] / [$TOTAL_{biomass}$]
$TOTAL_{GSi}$ = Total glucosinolate concentration in plant part i
Alij (Active Index) = [Concentration of glucosinolate j in plant part i] / [$TOTAL_{Gsi}$]
$TOXICITY_j$ = Toxicity of hydrolysis products of glucosinolate j to target organism.

In the current study, all of the components of BP shown in equation (1) except $TOXICITY_j$ were estimated in a diverse set of entries grown in one environment. The plant parts considered were root and shoot tissues, and BP was assessed at one phenological stage. Ontogeny, environment and management factors also influence the components of BP, and this will be considered for a subset of the entries in a subsequent paper (Sarwar and Kirkegaard 1997). $TOXICITY_j$ of the major hydrolysis products to soil-borne cereal fungal pathogens is considered by Sarwar *et al.* (1997). This discussion will consider variation in the components of BP in (1) and the potential to manipulate them to enhance BP for particular applications. The variation in GSL production (0.8 to

43.5 mmole m^{-2}) was derived equally from variation in $TOTAL_{biomass}$ and $TOTAL_{GSi}$. The lack of correlation between these components of BP means that they are independent variables in (1) each capable of manipulation.

Biomass production ($TOTAL_{biomass}$) and partitioning (HI_i)

Significant variation within and between species for both $TOTAL_{biomass}$ and HI_i (e.g. shoot index) was evident in the experiment. Biomass at mid-flowering was highly correlated with time to flowering so that phenological development in the target environment will be a key determinant of the potential vegetative biomass production. Phenological development in *Brassica* species is considered to be primarily dependant on photoperiod, with general shortening of phases as daylength increases. (e.g. Nanda *et al.* 1996). However, many species also develop more rapidly when minimum temperatures are lower (Nanda *et al.* 1996), and some biennial species have an obligate vernalisation requirement as seedlings (e.g. Hodgson 1978 for *B.napus*). These temperature, photoperiod and vernalisation requirements vary within and between species and result in significant variation in the duration of developmental phases (Nanda *et al.* 1996). In the current study, daylength during the growing period ranged from 9.7 (sowing) to 14.3 h while minimum temperatures during winter were -5 to -2 °C. All species with the exception of *B. oleracea* vegetables had flowering requirements satisfied and flowering time varied from 102 days (*B. campestris*) to 189 days (*B. nigra*). This large variation in phenological adaptation combined with specific morphological adaptations (e.g. short leafy habit) provides significant scope to select or develop brassicas adapted to specific local requirements for particular purposes as well.

Green manure crops are usually required to grow vigorously in a short period of time, produce a large vegetative biomass, and be readily incorporated and decomposed prior to growing a subsequent crop. Thus, *Brassica* types which remain vegetative in the target environment and produce many leaves rapidly would be desirable. Options include brassicas insensitive to photoperiod, with a long juvenile phase, or biennial types with obligate vernalisation requirements grown during warmer times of the year. The former include leafy Asian vegetable types (e.g. mustard spinach) while the latter include the fodder rapes (*B. napus oleifera* biennis) although many related species such as fodder radish (*Raphanus sativa*) and *S. alba* are also grown as trap or cover crops in Europe for nematode control (Müller 1991). Many of these *Brassica* types have been commercially selected for vigorous leaf production and may offer immediate opportunities for biofumigation in some horticultural applications. Despite the generally larger contribution of shoots to total GSL production, the contribution of root GSL should not be overlooked. In some entries roots accounted for 40% of total plant biomass at mid-flowering and this ratio is likely to be higher prior to flowering particularly in root vegetables (e.g. turnips, swedes, radish). In addition to GSLs in the root biomass, exudation of ITCs from growing roots is known to occur (Elliot and Stowe 1971) and may represent a further source of "in-crop" suppression. Root GSLs would also be the primary source of GSLs for biofumigation by companion crops where shoot material is not incorporated and pathogen suppression relies on GSL exudation from roots (e.g. Akhtar and Alam 1991).

In *Brassica* oilseed rotation crops such as canola (*B. napus*) or Indian mustard (*B. juncea*), crops are grown to maturity for seed production and shoot biomass, if incorporated, is generally mature and low in GSL concentration (Kirkegaard *et al.* 1996). Under these circumstances, the roots may provide the principal source of GSLs for biofumigation in the soil, either in-crop or during post-harvest decomposition of the root tissues. Opportunities to enhance root biomass through later flowering are unlikely since flowering time is a key yield determinant in seed crops (e.g. Hodgson 1978). Reducing shoot index may be possible (see range in Table 4) although altering root/shoot ratios may also influence seed yield. It is likely that manipulating $TOTAL_{GSroot}$ or AI_{rootj} would be better strategies to enhance BP in harvested oilseed rotation crops.

Glucosinolate concentration ($TOTAL\ GSL_i$) and profile (A_{ij})

The GSL profiles detected in the entries here are similar to those reported in previous studies using HPLC analysis (e.g. Sang *et al.* 1984), which supports the general conclusion of Rosa *et al.* (1997) that the major GSLs and their relative proportions are relatively stable and predictable for particular species and subspecies. Comparison of GSL concentrations reported from different studies is generally more difficult. However the profiles and production of GSLs reported in this experiment are similar to those reported elsewhere (Booth and Walker 1991; Fieldsend

and Milford 1994 for *B. napus oleifera* biennis in the UK; Clossais-Besnard and Larher 1991 for *B napus oleifera* annua in France). The magnitude of the inter and intra-specific variation in $TOTAL_{GSi}$ in both root and shoot tissues (~ 1 to 50 μmole g^{-1}) provides significant scope to select or develop brassicas with higher BP for both green manuring (where root and shoot tissue is incorporated) or rotation and companion planting (where only root tissues are present in the soil). In addition, the variation in the dominant GSL types found in different species provides further scope to increase BP by selecting brassicas high in specific GSLs known to release hydrolysis products most toxic to target organisms (e.g. ITCs). The variation in the relative proportion of ITC-liberating GSLs shown in Figure 2 indicates there is significant scope to increase BP without necessarily increasing total GSL concentration. Several *in vitro* studies have demonstrated the differential toxicity of different GSL hydrolysis products (predominately ITCs) to target organisms (e.g. Borek *et al.* 1995 for soil insects; Drobnica *et al.* 1967 and Mari *et al.* 1993 for soil fungi; Brabbam *et al.* 1995 for bacteria). Up to 30 fold differences in toxicity of individual ITCs to some organisms have been reported in these studies and this is discussed further in relation to suppression of cereal fungal pathogens in Sarwar *et al.* (1997).

The results of this study illustrate the need to consider the contribution of root GSLs to the suppressive potential of green manure crops and as the principal contributor in rotation or companion crops where shoot material is not incorporated. The universal presence of 2-phenylethyl GSL in significant concentrations in the roots of brassicas raises questions regarding its function. The ITCs produced by hydrolysis of aromatic glucosinolates such as 2-phenylethyl are generally less volatile than aliphatic types and may therefore persist for longer in the soil. This persistence may have provided greater suppression of soil-borne pests, pathogens and weeds and led to selection pressure for higher levels of aromatic types in root tissues in the same way that selection for particular glucosinolates from pest pressure in the aerial environment has lead to selection for specific aliphatic types in the leaves (Mithen *et al.* 1995). Relatively little is known about the activity of 2-phenylethyl ITC in the soil since many previous studies have concentrated on aliphatic types such as methyl ITC (a commercial soil fumigant) or 2-propenyl ITC (allyl) due to its early recognition as the active constituent of mustard oils. However 2-phenethyl ITC has been shown to be significantly more toxic than 2-propenyl ITC *in vitro* to fungi (Drobnica *et al.* 1967, Sarwar *et al* 1997), insects (Borek *et al.* 1995) and germinating wheat seeds (Bialy *et al.* 1990). The predominance in roots of the aliphatic GSLs, particularly 2-phenylethyl revealed in this study suggests that their activity and persistence in soil warrant further investigation.

Effectiveness of biofumigation

Brown *et al.* (1991) have estimated that the amount of methyl ITC used commercially for soil sterilization ranges from 517 to 1294 nmole g^{-1} of soil depending upon the specific crop and control required. The maximum total plant GSL at mid-flowering measured in this experiment was 45.3 mmole m^{-2} which is equivalent to 324 nmole g^{-1} of soil assuming a soil bulk density of 1.4 g cm^{-3} and incorporation to 10 cm. In a subsequent spring-sown experiment at the same site, GSL production was doubled in some entries (Sarwar and Kirkegaard 1997). The potential production of ITCs by hydrolysis of GSLs in brassicas (up to 700 nmoles g^{-1}) is therefore in a range likely to contribute to pathogen suppression, considering that commercial rates of methyl ITC are aimed at total control.

The effectiveness of brassicas for biofumigation will ultimately depend on many factors beside the BP of the particular *Brassica* used. The timing of incorporation or exudation of the GSL containing tissue must coincide with a susceptible stage in the life cycle of the pest organism and the suppression must persist to provide protection for the crop of interest. In addition, effectiveness of biofumigation will be influenced by efficiency of incorporation, activity of the hydrolysing myrosinase enzyme, and losses due to volatilisation, sorption onto clay and organic matter, leaching, and microbial degradation (reviewed by Brown and Morra 1997).

Equation (1) provides a framework with which to consider strategies to manipulate the components of biofumigation potential most likely to enhance the suppression for particular pest control scenarios. The diversity of GSL profiles, concentrations and production within and between *Brassica* and related species demonstrated in this experiment suggest that where pest or disease suppression by *Brassica* rotation or green manure crops can be linked to GSL hydrolysis products, opportunities will exist to select or develop brassicas with enhanced biofumigation potential. In addition to the existing genetic variation in glucosinolate production demonstrated here,

further genetic manipulation of glucosinolate concentration, profile and distribution is possible using wide crossing (e.g. Giamoustris and Mithen 1995), interspecific crosses (e.g. Chopra *et al.* 1996) and molecular genetic techniques (Mithen and Campos 1997).

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Biofumigation potential of brassicas. II. Effect of environment and ontogeny on glucosinolate production and implications for screening.

Muhammad Sarwar & John Kirkegaard

CSIRO Plant Industry, GPO Box 1600, Canberra, ACT

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Abstract

Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds released by Brassicaceous green manure and rotation crops when glucosinolates in their tissues are hydrolysed. We investigated the effect of environment and ontogeny on the glucosinolate production of eight entries from five *Brassica* species. The environments included autumn and spring sown field plots (FA and FS) and potted plants grown under ambient conditions (PAM) or in a temperature controlled glasshouse at 20°C/12°C (PTC). Glucosinolate concentration was measured in the root and shoot tissue at buds-raised, flowering and maturity. Of particular interest was the suitability of the pot-grown plants for screening large numbers of brassicas for glucosinolate production. The type of glucosinolates present in the tissues and their relative proportions remained relatively constant across environments and at different growth stages, with the exception of an increase in indolyl glucosinolates in the FS environment suspected of being induced by insect attack. Total glucosinolate concentration generally declined from buds-raised to flowering in all environments, and was lowest at maturity. The exceptions were *B. campestris*, which had higher glucosinolate concentration at buds-raised than at flowering, and the PTC environment in which most species also showed an increase at flowering. The total glucosinolate concentration in the root and shoot tissue of all entries varied significantly with environment (3 to 10-fold) and was generally ranked FS > PAM > FA > PTC. Interactions between species and environments meant that the ranking of the *Brassica* entries for total shoot and root glucosinolate concentration changed with environment. However within three entries from *B. napus*, the ranking was consistent across the environments. The added effect of environment on phenological development and biomass production further influenced glucosinolate production on a ground area basis. The results suggest that glasshouse environments can be used to determine the types and proportions of glucosinolates present, and to rank entries within, but not between species for the total concentration in the tissues. However the influence of the environment on both glucosinolate concentration and biomass production suggests that an accurate estimate of glucosinolate production on a ground area basis to assess biofumigation potential will require measurement in the target environment.

Introduction

Brassica green manure, rotation crops or seed meal amendments have been reported to suppress pest and disease organisms when grown or incorporated in the soil (e.g. Chan and Close 1987; Mojtahedi et al. 1991). These effects are generally attributed to biocidal compounds released into the soil when glucosinolates (GSLs) in the incorporated tissues are hydrolysed. Isothiocyanates (ITCs) are the most toxic of several hydrolysis products and are known to have broad biocidal activity (reviewed by Brown and Morra 1997; Chew 1988; Fenwick et al. 1983; Rosa et al. 1997). 'Biofumigation' is a term recently used to describe this suppression of soil-borne pests and pathogens by *Brassica* rotation or green manure crops (Angus et al., 1994; Kirkegaard et al. 1993). Interest in biofumigation has increased recently in horticultural industries because of restrictions on several synthetic pesticides and soil fumigants (e.g. methyl bromide, ethylene dibromide) and in broad-acre cereal cropping for suppression of soil-borne fungal pathogens (Angus et al. 1991; Kirkegaard et al. 1996). Enhancing biofumigation will require identification of brassicas which produce sufficient quantities of GSLs so that the ITCs released are effective in pest suppression.

There are about 20 different types of GSLs commonly found in brassicas. These vary in their structure depending on the type of organic side chain (aliphatic, aromatic or indolyl) on the molecule. The profile, concentration and

distribution of these GSLs is known to vary within and between *Brassica* species, and with plant age and growing conditions. Climatic, edaphic and biotic factors have all been reported to influence the glucosinolate concentration in *Brassica* tissues (Rosa et al. 1997). Environmental factors such as daylength and temperature also influence the phenology and biomass production of brassicas (Nanda et al. 1996). As a result, the total production of GSL on a ground area basis, and therefore biofumigation potential, will be significantly influenced by growing conditions.

These effects have implications for the selection of a suitable screening environment to assess the GSL production and biofumigation potential of a large number of diverse *Brassica* entries. Glasshouse studies using potted plants have the advantage that large numbers of entries can be screened under uniform and repeatable conditions. However it is important that the environment chosen for screening the brassicas gives results consistent with that in the intended field situation. Depending on specific objectives of the investigations, this may mean either a qualitative assessment of the glucosinolate profiles, a ranking of entries for GSL concentration, or an accurate estimate of GSL production.

In the first paper of this series (Kirkegaard and Sarwar 1997), we reported the range of mid-flowering GSL production in the roots and shoots of 75 diverse *Brassica* and related species grown at one field site. In this study, a subset of those lines was grown in different environments, including pot-grown plants in the glasshouse, to determine the effect of environment and ontogeny on GSL production. The objectives were (1) to determine if the GSL production of pot-grown plants was consistent with that of the field environments, and (2) to investigate the effect of different seasons at the field site on GSL production. A subsequent paper reports the relative toxicity of the major GSL hydrolysis products to a range of soil-borne cereal pathogens (Sarwar et al. 1997).

Materials and methods

Brassica *species*

The brassicas used in the study are listed in Table 1. Most of the seed was obtained from the Australian Temperate Field Crops Collection at the Victorian Institute for Dryland Agriculture (VIDA), Horsham, Australia. The *B. juncea* lines came from the CSIRO Plant Industry *B. juncea* breeding program which has produced lines with low seed GSL levels (Oram and Kirk 1992). The eight brassicas included *B. napus olifera* annua (spring canola) and *B. juncea* lines with high and low seed GSL content.

Growing environments

The four growing environments were: (1) Field - autumn sown (FA), (2) Field - spring sown (FS), (3) Pots in open cold frames (PAM), and (4) Pots in a temperature-controlled glasshouse (PTC). The details of these environments are summarised in Table 2.

Field site

Characteristics of the soil at the field site and the agronomic management of the field plots are given in the previous paper (Kirkegaard and Sarwar 1997). Briefly, the seedlings were established in trays of moist vermiculite/peat until the 3rd leaf stage, and then transplanted into field plots 0.5m x 1m with 0.1m inter-row and intra-row spacing (100 plants m^{-2}). Three replicates of each entry were arranged in a randomised complete block design. The autumn and spring sowings were on adjacent blocks and were transplanted on 18 June and 9 October 1995, respectively. The plots were topdressed with fertilizer (20 kg ha^{-1} N, 20 kg ha^{-1} P and 18 kg ha^{-1} S) during the vegetative stage (prior to budding) and the area was hand-weeded and irrigated when necessary to prevent water stress. In the spring sowing, an infestation of striped flea beetle (*Phylotreta undulata*) during the early vegetative phase was controlled with endosulphan.

Pots

The potting mix used was a sterilised recycled loam/peat potting mix amended with a complete fertilizer and adjusted to pH 6.5. Brassicas were sown into 4.3 l pots (17.5 cm diameter and 18 cm deep) on 4 July 1995. Four uniform plants were established in each pot by oversowing and thinning plants following establishment. Six pots of each entry were established so that duplicate pots could be harvested at each of three growth stages. The temperature controlled environment (PTC) was within the Canberra Phytotron (see Morse and Evans 1962). Temperature was controlled between 20°C maximum and 12°C minimum on a 12 hour sine wave. Natural photoperiod was extended to 16 h by low intensity incandescent light. Pots were watered daily with full strength Hoagland solution (am) and water (pm).

The pots in the open cold frames (PAM) received natural rainfall, supplemented with regular watering when required to avoid water stress and full strength Hoagland solution was applied every 2 weeks. The PAM plants received ambient daylength and temperature except that the minimum temperature within the frames did not fall below 0°C. No diseases developed on the plants during the experiments. Insects were completely excluded from the PTC environment, and while present on PAM plants, there were no serious infestations that required spraying.

Sampling and analysis

Plants grown in each environment were sampled at buds-raised, mid-flowering and maturity, which correspond to growth stages 2.2, 3.1 and 5.0 of Berkenkamp (1973). In the field plots, five bordered plants from the inner three rows were dug from each plot to a depth of 0.15 m and taken to the laboratory with soil surrounding the intact roots. The soil was washed from the roots, the plants immediately separated into root and shoot tissue, and frozen at - 20°C. The plants in pots were removed intact from the pot, and soil washed free of the roots before separation from shoots and freezing. All samples were freeze-dried, ground using a Wiley mill with 1 mm screen, weighed, and stored in sealed bottles at -20°C prior to GSL analysis.

GSLs 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 (1997). The desulphoglucosinolates were then separated and quantified using the HPLC method described in detail by Kirkegaard and Sarwar (1997). The GSLs identified and their retention times are given in Table 3. Peaks were identified using pure standards either purchased (2-propenyl - GSL Sigma, St Louis, MO USA: benzyl GSL - 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.

Table 1. Details of the *Brassica* entries used in the study.

Species	Common name	Cultivar	Code	Origin	Description/Use
B napus olifera biennis	Winter Rape	Tamara	BnaW	GER	Oilseed High GSL
B napus olifera annua	Spring Rape	Midas	BnaSH	CAN	Oilseed High GSL
B napus olifera annua	Spring canola	Oscar	BnaSL	AUS	Oilseed Low GSL
B campestris olifera	Spring rape	Arlo	Bcam	CAN	Oilseed High GSL
B juncea	Indian mustard	CSIRO651	BjunL	AUS	Oilseed Low GSL
B juncea	Indian mustard	CSIRO99Y-1	BjunH	AUS	Oilseed High GSL
B carinata	Abyssian mustard	94044	Bcar	ETH	Condiment/Oilseed
B nigra	Black mustard	91080	Bnig	SUN	Condiment/Oilseed

Table 2. Summary of environments used in the experiment and seasonal conditions in Canberra in 1995.

Environment	Code	Growing medium	Sowing date	Temperature	Daylength (h)
Field autumn sown	FA	Field soil	18 June	Ambient	Ambient (9.7 - 14.3)
Field spring sown	FS	Field soil	9 October	Ambient	Ambient (12.8 - 14.3)
Pots - open cold frames	PAM	Potting mix	4 July	Ambient (no frost)	Ambient (9.9 - 14.3)
Pots - Temperature control	PTC	Potting mix	4 July	20/12°C	16

Canberra 1995

Month	J	F	M	A	M	J	J	A	S	O	N	D
Av. Max. Temp. (°C)	25.1	26.4	23.7	18.3	15.6	12.0	9.9	16.1	16.0	19.3	21.7	24.0
Av. Min. Temp.(°C)	14.0	12.3	9.4	4.4	6.7	2.2	0.7	1.8	4.1	7.7	9.9	10.1
Daylength (h)	14.1	13.2	12.2	11.1	10.1	9.7	9.9	10.7	11.7	12.8	13.8	14.3

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Table 3. Glucosinolates identified in *Brassica* samples, their HPLC retention times and major hydrolysis products.

Number	Chemical name	Trivial name	Retention time (min)	Hydrolysis products[A]
Aliphatic				
1	3-Methylthiopropyl	Glucoiberverin	16.80	ITC, nitriles
2	3-Methylsulphinylpropyl	Glucoberin	7.27	ITC, nitriles
3	2-Propenyl	Sinigrin	12.53	ITC, nitriles
4	4-Methylthiobutyl	Glucoerucin	19.79	ITC, nitriles
5	4-Methylsulphinylbutyl	Glucoraphanin	13.51	ITC, nitriles
6	3-Butenyl	Gluconapin	16.52	ITC, nitriles
7	2-Hydroxy-3-butenyl	Progoitrin	9.51	Oxazolidine-2-thiones
8	5-Methylsulphinylpentyl	Glucoalyssin	11.96	ITC, nitriles
9	4-Pentenyl	Glucobrassicanapin	18.83	ITC, nitriles
10	2-Hydroxy-4-pentenyl	Gluconapoleiferin	15.24	Oxazolidine-2-thiones
Aromatic				
11	2-Phenylethyl	Gluconasturtiin	21.42	ITC, nitriles
12	2-Hydroxy-2-phenylethyl	Glucobarbarin	17.82	ITC, nitriles
13	Benzyl	Glucotropaeolin	19.11	ITC, nitriles
14	*p*-Hydroxybenzyl	Glucosinalbin	15.58	ITC, nitriles
Indolyl				
15	3-Indolylmethyl	Glucobrassicin	20.20	Indolyl-3-carbinol
16	4-Hydroxy-3-indolylmethyl	4-Hydroxyglucobrassicin	17.56	Thiocyanate
17	4-Methoxy-3-indolylmethyl	4-Methoxyglucobrassicin	21.04	Auxins?
18	1-Methoxy-3-indolylmethyl	Neoglucobrassicin	23.09	Phytoalexins?

[A] from Mithen (1992) and Rosa et al. (1997)

Results

Glucosinolate concentration

Total GSL concentration at each sampling and for each environment averaged for all entries is given in Table 4. Total GSL concentration in both root and shoot tissues decreased with plant age in all environments. The reduction in total GSL levels from buds-raised to flowering was generally less in the pot-grown plants than in the field-grown plants. In the field environments, the GSL concentration was just detectable at maturity while somewhat higher levels remained in the tissues of pot-grown plants. The mean GSL concentration in both root and shoot tissues at buds-raised decreased in the order FS > PAM > FA > PTC, while at flowering the maintenance of GSL levels in the PTC environment resulted in higher concentrations than those in the FA environment. In all cases at buds-raised and flowering, the mean GSL concentration in the roots exceeded that of the shoots.

The total shoot GSL concentration for individual species in each environment at buds-raised and flowering is shown in Fig. 1. Data for BjunH and Bnig in the FS environment are unavailable due to a freeze-drier malfunction, and BnaW did not flower in the FS and PTC environments due to a lack of vernalisation. The data for 'buds-raised' in this entry were from vegetative harvests on day 147 and 119, respectively. In the FA, FS and PAM environments, total shoot GSL concentrations generally decreased from bud-raised to flowering with the exception of Bcam which increased in the FA and PAM environments. In contrast, the concentration of most entries increased from bud-raised to flowering in the PTC environment. There was a large effect of environment on the total GSL concentration in the shoot tissues of all entries. The magnitude of the response varied from 3-fold for Bcar, to 10-fold for BnaW at bud-visible, and a similar range of response existed at flowering. In addition to the variation in concentration within individual entries, the ranking of the entries was also influenced by the environment. For example, BnaW had relatively low shoot GSL concentration in the FA environment compared to the other entries (ranked 4th), but was ranked highest in all of the other environments. Despite this effect of environment and sampling time on the overall ranking of the entries, the ranking of entries within the same species was consistent. For example, the ranking within the three *B. napus* entries was BnaW > BnaSH > BnaSL in all environments and at both sampling times, and the same was true for BjunH and BjunL.

Table 4. Mean total glucosinolate concentration (μmole g^{-1}) in shoot and root tissue of all entries at three phenological stages in each environment. (FA-Field autumn, FS- Field spring, PAM-Pots ambient, PTC-Pots temperature controlled)

Time of	Shoot				Root			
Sampling	FA	FS	PAM	PTC	FA	FS	PAM	PTC
Bud raised	20.5	35.2	21.7	12.5	31.0	71.7	37.5	28.5
Flowering	9.2	18.8	17.6	12.9	11.5	28.3	24.4	20.3
Maturity	0.7	0.2	1.8	4.0	0.8	0.7	7.5	2.3
LSD (P=0.05)								
sample time			7.4				9.2	
environment			n.s.				10.6	
interaction			n.s.				18.4	

Although total root GSLs were generally higher than the shoot, the same general influence of environment and ontogeny was observed (Fig. 2). Total GSL concentration in root tissues (1) declined from buds-raised to flowering (with the exception of Bcam in the PAM environment), (2) varied with environment for individual species from 3 to 6 fold, (3) was ranked differently across environments among species (e.g. Bnig) (4) was ranked consistently across environments within species (eg BnaW > BnaSH > BnaSL in all environments). The differences between BjunH and BjunL in root tissues were not significantly different in any environment so no assessment of ranking is possible.

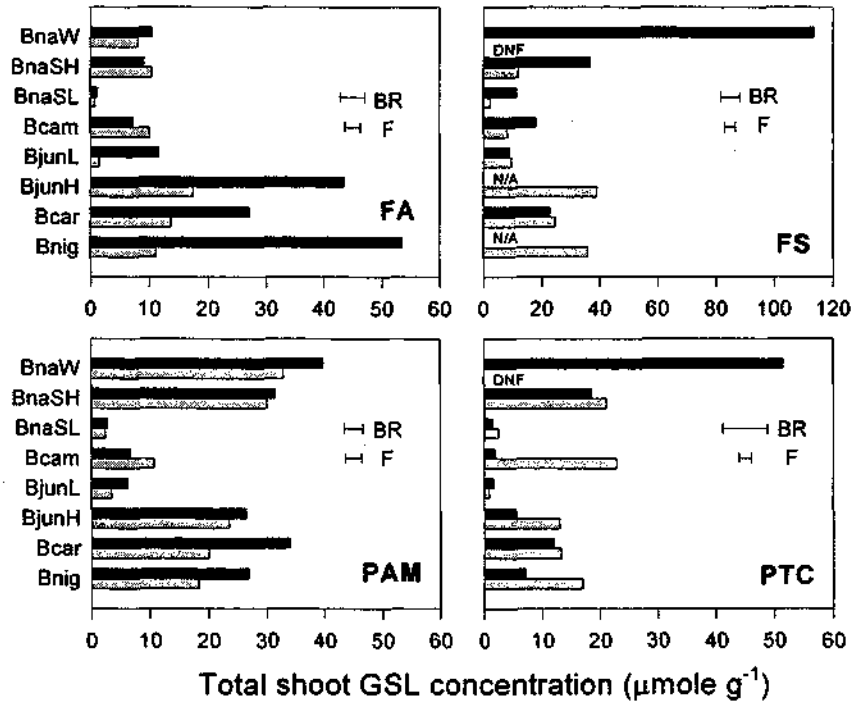

Figure 1. Total shoot glucosinolate concentration in eight *Brassica* entries at buds-raised (solid bars) and flowering (hatched bars) in four environments; (FA-Field autumn sowing, FS- Field spring sowing, PAM- Pots ambient, PTC-Pots temperature controlled). The horizontal lines represent LSDs (P=0.05) for comparison of entries within each environment (BR-Buds-raised, F-flowering). N/A-not available due to freeze-drier malfunction. DNF - Did not flower, sampled for BR on day 147 in FS and 119 in PTC.

Glucosinolate profiles

The glucosinolates identified in the root and shoot tissues are given in Table 5. The types of glucosinolates identified in the tissues were consistent across environments and harvests. The only exceptions were in cases where the total GSL concentration was low (e.g. at maturity harvest) and some minor GSLs were undetectable (<0.1 μmole g^{-1}). Aliphatic GSLs were dominant in the shoots of all species except for the BnaSL in which indolyl GSLs were dominant. The *B. napus*, Bcam and BjunL entries were dominated by 4-butenyl GSL, 5-pentenyl GSL and their hydroxy analogues while BjunH, Bcar and Bnig were dominated by 2-propenyl GSL. The aromatic 2-phenylethyl GSL was dominant in the roots of all entries, but usually in association with many of the GSLs present in the shoots.

The relative proportions of various GSL classes in shoot and root tissues remained relatively consistent across environments although some changes were evident (shown for the shoot and root tissue at buds-raised in Figs 3 and 4 respectively). In the shoot tissues, there was a greater proportion of indolyl GSLs in the FS environment in *B. napus* entries and in Bcar. The apparent changes in the GSL proportions in BnaSL are exaggerated somewhat due to the low total GSL in that entry which are close to the error of detection. In all *B. napus* entries, the aliphatic component was comprised of 4-butenyl GSL, 5-pentenyl GSL and their hydroxy analogues. The proportions of these individual aliphatic GSLs remained relatively consistent across environments (data not shown). The exception was the absence of OH-pentenyl GSL in the FA environment. In BjunH, BjunL, Bcar and Bnig, the large aliphatic component was comprised solely of 2-propenyl GSL and its proportion remained relatively stable across environments.

Aromatic glucosinolates were dominant in the roots of all entries, (principally 2-phenylethyl GSL) (Fig. 4) and the proportion within each entry remained relatively constant across environments. The exception was the reduction in the proportion of aromatics and increase in the proportion of aliphatics in BnaW and BnaSH in the pot environments. A small increase in the proportion of indolyl GSLs in the *B. napus* entries in the FS environment was evident, consistent with that observed in the shoots.

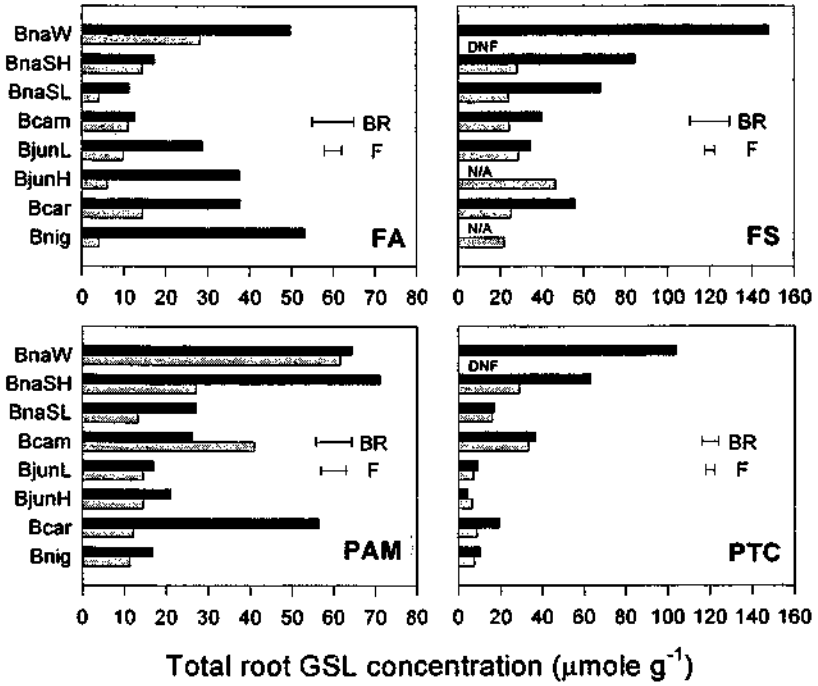

Figure 2. Total root glucosinolate concentration in eight *Brassica* entries at buds-raised (solid bars) and flowering (hatched bars) in four environments; (FA-Field autumn sowing, FS- Field spring sowing, PAM- Pots ambient, PTC-Pots temperature controlled). The horizontal lines represent LSDs (P=0.05) for comparison of entries within each environment (BR-Buds-raised, F-flowering). N/A-not available due to freeze-drier malfunction. DNF - Did not flower, sampled for BR on day 147 in FS and 119 in PTC.

Glucosinolate production

In addition to effects on GSL concentration, environmental conditions also influenced the phenology and biomass production of the entries. To demonstrate the range in responses to environment, Table 6 summarises the components of total plant glucosinolate production for three entries in the field environments where meaningful comparisons of biomass on a ground area basis can be made. The 20-fold increase in GSL production in BnaSH between FA and FS resulted from an increase in both biomass production and glucosinolate concentration in root and shoot tissues. A smaller increase (3-fold) in GSL production was observed in BnaSL where, despite the increase in GSL concentration in root and shoot tissues, biomass production was reduced. In BjunL, both root and shoot biomass and shoot GSL concentration was less in FS than FA, so that GSL production was reduced. Fig. 5 shows GSL production for all entries on a ground area basis for FA and FS environments at buds-raised and flowering. Generally, GSL production increased from buds-raised to flowering, and with the exceptions of BjunL at buds-raised and Bnig at flowering GSL production was greater in FS than in FA. Roots contributed less to the total GSL production in most entries due to their lower biomass.

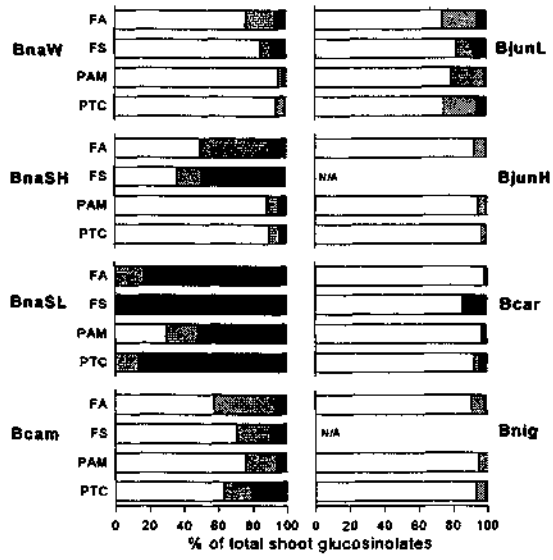

Figure 3. Proportion of different glucosinolate types (white-aliphatic, grey-aromatic, black-indolyl) in the shoot tissues of eight *Brassica* entries sampled at buds-raised in four environments; (FA-Field autumn sowing, FS- Field spring sowing, PAM- Pots ambient, PTC-Pots temperature controlled). N/A as for Fig. 1.

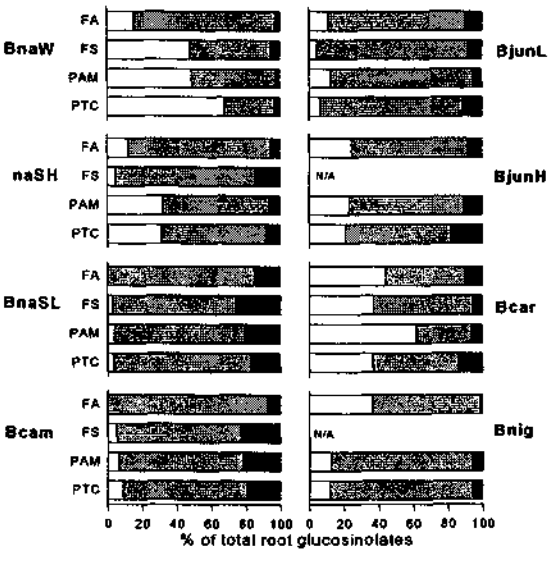

Figure 4. Proportion of different glucosinolate types (white-aliphatic, grey-aromatic, black-indolyl) in the root tissues of eight *Brassica* entries sampled at buds-raised in four environments; (FA-Field autumn sowing, FS- Field spring sowing, PAM- Pots ambient, PTC-Pots temperature controlled). N/A as for Fig. 1.

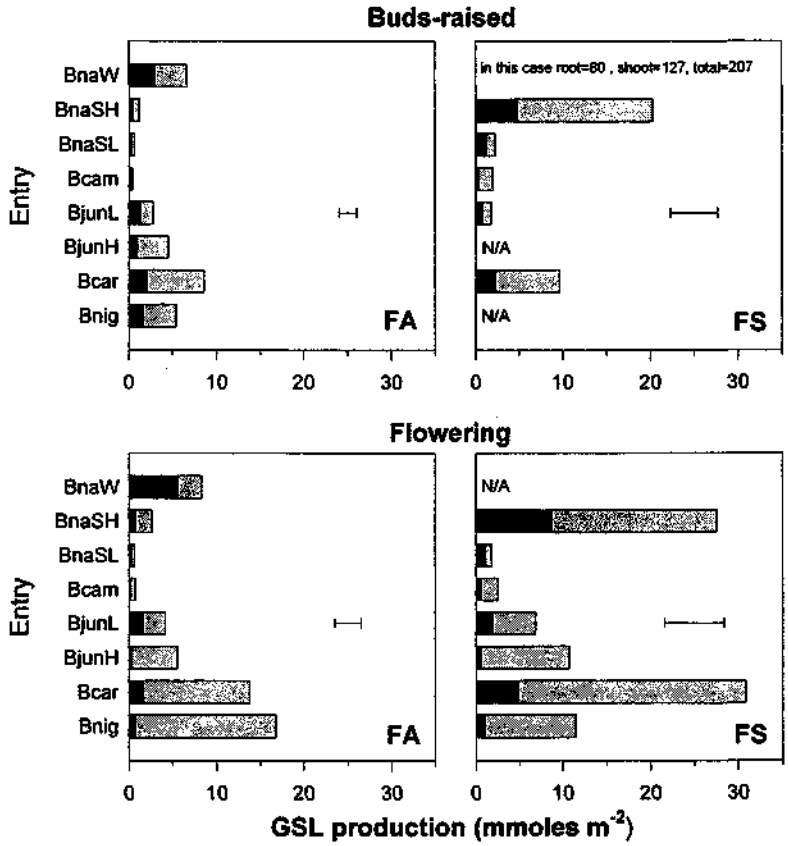

Figure 5. Glucosinolate production on a ground area basis for eight *Brassica* entries at buds-raised and flowering in two environments; (FA-Field autumn sowing, FS- Field spring sowing). Solid -roots; hatched - roots. The horizontal lines represent LSDs (P=0.05) for comparison of total GSLs of entries within each environment. Data for BnaW in the FS environment are written in text. N/A as in Fig. 1.

Discussion

GSL profiles

The GSL profiles detected in the entries here are similar to those reported for the same species in previous studies using HPLC analysis (Sang et al. 1984). The lack of large environmental and ontogenic effects on GSL profiles in this study supports the general conclusion of Rosa et al. (1997) that the major GSLs and their relative proportions are relatively stable and predictable for particular species and subspecies. The exception in the current study was the increase in the proportion of indolyl GSL in the FS environment. The factor most likely to have contributed to this was the greater prevalence of insect pests in the FS environment, including striped flea beetle (*Phylotreta undulata*), which caused some leaf damage during early vegetative growth before control with endosulphan. An increase in GSL concentration, in particular indolyl GSLs, has been associated with attack by flea beetles in a previous study (Koritsas et al. 1989 for *B. napus*).

Other changes in the proportion of GSL types may have been associated with the effects of the environment on the relative proportion of different tissues within the root or shoot. Van Etten et al. (1979) showed that the cambial cortex of *B. oleracea* tops had twice the total GSL levels as the leaves or pith, and the ratio of individual

Table 5. Glucosinolates identified in *Brassica* tissues (numbered as in Table 3). The glucosinolates are listed in order of abundance in each tissue: major GSLs (>50% of total) are underlined, while minor GSLs (<2% of total) are shown in italics.

Entry	Shoot	Root
BnaW	7,9,10,6,11,*15,17,18*	11,7,9,13,10,6,17,*15,18*
BnaSH	7,10,9,6,11,15,*5,17,18*	11,7,13,9,10,6,17,*15,18*
BnaSL	15,18,*17,10,11,5*	11,18,15,13,17,5,*16*
Bcam	10,9,7,6,11,*15,17,18,16*	11,18,10,15,9, *13,16*
BjunL	6,9,3,*10,11,15*	11,3,6,9,*18,17,15*
BjunH	3,11,6,*16,18*	11,3,*15,16,17,18*
Bcar	3,*15,18,11*	11,3,18,6,*17,15,16*
Bnig	3,*11*	11,3,*15,17,18*

Table 6. Components of total GSL production in selected entries in the FA and FS environments at buds-raised.

Entry /Environment			Root			Shoot			Total plant
		Harvest Time (d)	Biomass ($g\ m^{-2}$)	GSL conc. ($\mu mole\ g^{-1}$)	GSL Prod. ($mmole\ m^{-2}$)	Biomass ($g\ m^{-2}$)	GSL conc. ($\mu mole\ g^{-1}$)	GSL Prod. ($mmole\ m^{-2}$)	GSL Prod. ($mmole\ m^{-2}$)
BnaSH	FA	104	25	17.3	0.43	82	9.1	0.8	1.2
	FS	77	57	84.5	4.82	425	36.5	15.5	20.3
BnaSL	FA	99	31	11.2	0.35	111	1.2	0.3	0.7
	FS	64	18	67.9	1.22	94	11.3	1.1	2.3
BjunL	FA	112	47	28.7	1.35	127	11.6	1.5	2.8
	FS	70	24	34.1	0.82	113	8.9	1.0	1.8

GSLs was also different. Effects of environment on the ratio of these tissues in the plant may therefore influence the proportion of individual GSLs.

Total GSL concentration

Ontogeny

The general reduction in the total GSL concentration in above ground tissues with ontogeny in this experiment is consistent with the results of several previous experiments using field-grown plants (Fieldsend and Milford 1994, Clossais-Besnard and Larher 1991, Jürges 1978). The major exception in this experiment was in the PTC environment, in which most of the entries showed an increase in GSL concentration from buds-raised to flowering. This has been observed in other controlled environment studies. For example Booth et al. (1991) showed that in *B. napus olifera* biennis (oilseed rape) grown in the glasshouse, the total GSL concentration remained relatively constant from the beginning of stem elongation (immediately after buds-raised) until midway to flowering before declining in the usual way. In another study using three forage *B. napus* varieties grown in the glasshouse, Smith and Griffiths (1988) reported a 20-fold increase in GSL concentration of above ground tissues during the period 9 to 21 weeks after sowing. In both studies, the regular application of nutrients may have contributed to the maintenance of higher GSL concentrations, although in the later study, the failure of the plants to vernalise may have prevented the normal decline that accompanies the initiation of flowering. The results suggest that controlled environment studies with regular application of water and nutrients may result in an abnormal pattern of GSL accumulation in above ground tissue, particularly if conditions fail to satisfy the vernalisation requirements usual in the field.

In this study, the effects of ontogeny on the root tissues were similar to that on the shoots, with a general decline in concentration from buds-visible to flowering and low concentrations remaining at maturity. The increase in GSL concentration from buds-visible to flowering observed in the shoots in the PTC environment was not observed in the roots. The higher concentrations in the mature tissue of pot-grown plants compared with field grown plants was presumed to be due to the reduced decay of the root tissues due to the rapid drying of soil in the pots once watering ceased. There have been few previous studies on the ontogenic effects on root tissues in the species used here, although Clossais-Bersnard and Larher (1991) showed that the level of 2-phenylethyl GSL (the dominant form in roots) in *B. napus olifera* biennis increased up to buds-visible and remained relatively constant to the green pod stage.

Although there was an overall decline with ontogeny, species differed in their response. The most obvious was Bcam, in which shoot GSL concentration increased from buds-visible to flowering in the FA, PAM and PTC environment and in root concentration in the PAM environment. Within the FA environment, the magnitude of decline in shoot and root tissue was higher in Bjun, Bcar and Bnig than in the *B. napus* entries. Interestingly, these two groups contain different major GSL types; the latter contains predominantly 3-butenyl, 4-pentenyl and their OH-analogues, while the former contains predominantly 2-propenyl. It is possible that some GSL types are more subject to change as plants develop than others, and this may be influenced by the environment.

Environment

Total GSL concentration is known to be influenced by climatic (temperature, daylength, radiation, water stress), edaphic (soil-type, nutrients) and biotic (pests and diseases) factors (reviewed by Rosa et al.1997). The lower GSL content of plants in the FA environment compared with the FS environment in this experiment is consistent with previous observations that short days, lower radiation, and cooler temperatures accompanied by frost, induce lower levels of glucosinolates in vegetative material (Ishii and Saijo 1987). In addition, both insect attack (Koritsas et al. 1989) and water stress (Freeman and Mossadeghi 1973), more prevalent in the FS environment, can increase total GSLs. Comparisons with the pot-grown environments is more difficult since climatic, edaphic and biotic factors varied simultaneously. Despite the generally warmer conditions in the PTC environment and regular nutrient additions, there was also less insects, less water stress, lower radiation and no gradual change in daylength. For most species, the PTC produced the lowest GSL concentration, the exception being BnaW which

did not vernalise as discussed previously.

The importance of individual factors responsible for differences in the overall GSL concentration between environments is of less interest than the comparison of the ranking of the entries within the different environments. In this experiment, the ranking of entries changed with environments due to differences in the magnitude of the response of species to different environments. For example the leaf GSL concentration in BnaWH was 10 times higher in the FS environment than in the FA environment, while the concentration in Bcar decreased. Such species x environment interactions make it unlikely that accurate predictions of the relative GSL concentrations of diverse species in the field can be made from pot-studies carried out in the glasshouse. However, the consistent ranking across environments within species in this experiment suggests that glasshouse screening studies of lines within a species for GSL concentration may be possible. This conclusion is based on only three *B. napus* entries and the shoot tissue of the two *B. juncea* entries and would need to be verified for other species with more entries. The consistent ranking within species probably arises because the major GSL types and the environmental effects on phenology and physiology will be more similar within, than between species.

Glucosinolate production

Despite the fall in GSL concentration with ontogeny, the accompanying increase in biomass usually resulted in an increase in the GSL production on a ground area basis from buds-raised to flowering. In previous studies, the maximum GSL production in vegetative tissues has also coincided with flowering (Fieldsend and Milford 1994, Clossard-Besnard and Larher 1991). The very low concentrations in mature tissue result in low GSL production in the mature tissue. Overall, the entries in this experiment produced more GSLs on a ground area basis in the FS environment than in the FA environment because the increase in the GSL concentration in spring outweighed any reduction in biomass which occurred due to shorter developmental phases. The temperature, photoperiod and vernalisation requirements varied within and between species as previously reported (Nanda et al. 1996), and added to the effects of environment on GSL concentration. Consequently, there was a range of potential responses to changes in environmental conditions as demonstrated here in Table 6. These interactions will be specific to particular combinations of daylength, temperature and sowing times, so that although a general increase in GSL production was observed here in the spring sowing, no generalisation can be made with respect to effects of environment on GSL production.

Implications for assessing biofumigation potential

In the first paper of this series, Kirkegaard and Sarwar (1997) provided the following equation as a framework to consider factors influencing the biofumigation potential of brassicas:

$$BP = TOTAL_{biomass} \times S_{i=1\ to\ n} [HI_i \times TOTAL_{GSi} \times S_{j=1\ to\ p} (Ai_{ij} \times TOXICITY_j)]. \ldots . (1)$$

where:
BP = Biofumigation potential for a particular target organism
$TOTAL_{biomass}$ = biomass of whole plant
HIi = [biomass plant part i] / [$TOTAL_{biomass}$]
$TOTAL_{GSi}$ = Total glucosinolate concentration in plant part i
AIij (Active Index) = [Concentration of glucosinolate j in plant part i] / [$TOTAL_{Gsi}$]
$TOXICITY_j$ = Toxicity of hydrolysis products of glucosinolate j to target organism.

The results of this experiment indicate that both environment and ontogeny can influence all of the plant-related components of the equation ($TOTAL_{biomass}$, HIi and $TOTAL_{GSi}$), with the exception of AIij which remained relatively constant. Thus, the GSL production and biofumigation potential will vary significantly, as has been demonstrated here in the comparison of eight entries in the FA and FS environments. Generally, GSL production increased from buds-raised to flowering, and was higher in the FS environment than FA environment, although the range in responses discussed above makes general predictions on environmental effects difficult. In the most extreme case, BnaW grown in the FS environment failed to vernalise and so continued to produce vegetative

biomass of high GSL concentration resulting in a 20-fold increase in GSL production compared with the FA environment. This may provide an excellent biofumigation option for green manuring since by remaining vegetative there is; (1) no risk of seeding, (2) an apparent accumulation or at least maintenance of GSL concentration in the tissue, (3) large biomass accumulation, (4) a long window of opportunity for incorporation of high GSL vegetative material. Other considerations for biofumigation potential include the time available for growth, and the most desirable GSL profile in terms of toxicity to target organisms (Sarwar and Kirkegaard 1997).

The results of this experiment indicate that glasshouse screening can be used to identify the major GSL types and relative proportions in *Brassica* tissues, and potentially to rank entries within species for $TOTAL_{GSi}$. However, where a ranking of diverse species is required, or an accurate estimate of the actual GSL production on a ground area basis, measurements will be necessary in the intended field environment, and under seasonal conditions likely to be encountered.

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Biofumigation potential of brassicas. III. *In-vitro* toxicity of isothiocyanates to the mycelial growth of soil-borne pathogens.

Muhammad Sarwar[A], John Kirkegaard[A], Percy Wong[B], Jim Desmarchelier[C].

[A]CSIRO Plant Industry, GPO Box 1600 Canberra ACT 2601
[A]Agricultural Research Institute, NSW Agriculture, Wagga Wagga NSW 2650
[B]CSIRO Entomology, GPO Box 1700, Canberra 2601

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Abstract

Isothiocyanates (ITCs) released from *Brassica* crops or seed meal amendments incorporated into soil have the potential to suppress pest and disease organisms in soil. We investigated *in vitro* toxicity of six ITCs to the mycelial growth of five cereal root pathogens (*Gaeumannomyces graminis* var. *tritici, Rhizoctonia solani, Fusarium graminearum, Bipolaris sorokiniana, Pythium irregulare*) by either adding them to the headspace above, or dissolving them in the growing media. Four alkenyl aliphatic ITCs (methyl-ITC, propenyl-ITC, butenyl-ITC, pentenyl-ITC) and two aromatic ITCs (benzyl-ITC and 2-phenylethyl-ITC) were tested. Aromatic ITCs were less toxic in the headspace experiments due to their lower volatility which reduced the headspace concentration, but were more toxic than the aliphatic ITCs when dissolved in the agar. In both experimental methods, the toxicity of the aliphatic ITCs decreased with increasing length of the side chain although there was little difference between methyl-ITC and propenyl-ITC in the headspace experiment. The fungi differed in sensitivity to the ITCs. *Gaeumannomyces* was the most sensitive, *Rhizoctonia* and *Fusarium* intermediate and *Bipolaris* and *Pythium* the least sensitive. *Pythium* was 2-4 times more resistant than the other fungi to the ITCs dissolved in agar and, in contrast to the other fungi, was more sensitive to the aliphatic ITCs than the aromatic ITCs. Suppression of some fungi by propenyl ITC and 2-phenylethyl ITC, principal products of glucosinolate hydrolysis in *Brassica* tissue, was superior to that of the synthetic fumigant methyl-ITC, suggesting an important role for these compounds in the pest suppression potential of brassicas.

Introduction

Brassica and other members of Brassicaceae contain significant quantities of thioglucoside compounds known as glucosinolates (GSLs) in their tissues (Kjaer 1976). Upon tissue disruption, GSLs are hydrolysed by endogenous myrosinase (thioglucoside glucohydrolase EC3.2.3.1) to release isothiocyanates (ITCs), thiocyanates, nitriles or oxazolidinethiones. The nature of the hydrolysis products depends upon the type of organic side chain (which can be aliphatic, aromatic or indolyl) on the parent molecule and the environmental conditions (Rosa et al. 1997). GSLs are relatively inactive against microorganisms, but their hydrolysis products, particularly ITCs, are highly biocidal to a diverse range of organisms including nematodes, bacteria, fungi, insects and germinating seeds (Fenwick et al. 1983, Rosa et al. 1997, Brown and Morra 1997). Accordingly, ITCs released from *Brassica* rotation and green manure crops or seed meal amendments incorporated into soil have the potential to suppress pest and disease organisms (Smolinska et al 1997). As many ITCs are volatile, biofumigation is a term recently used to describe the suppression of soil-borne pests and pathogens by *Brassica* crops (Kirkegaard et al. 1993; Angus et al. 1994, Kirkegaard and Sarwar 1997) and there is considerable interest in biofumigation as an alternative to synthetic soil fumigants in horticulture, and for control of soil-borne pathogens in broad acre agriculture (Brown and Morra 1997).

The antifungal effects of pure ITCs have long been reported (Walker et al. 1937) and vary with different organic side chain structures (Drobnica et al. 1967). Fungicidal concentrations of ITCs are also known to differ by an order of magnitude for different fungal species (Brown and Morra 1997). However, generalising with regard to the toxicity of different ITCs is made difficult by the different experimental approaches used in previous studies. Some studies have investigated the toxicity of pure ITCs in headspace experiments where the volatility of the

compound may influence its activity (Angus et al. 1994), while others have used ITCs dissolved in the growing media (Drobnica et al. 1967). Although inhibitory effects have been reported in studies involving release of volatiles from *Brassica* tissues (Kirkegaard et al. 1996; Mayton et al. 1996) these assessments may be complicated by the contribution of non-ITC compounds. In addition, spores, conidia and mycelia have all been used to assess the toxicity of the compounds and these may differ in their sensitivity (Mari et al. 1993).

The superior growth and yield of wheat following *Brassica* crops such as canola (*B. napus L.*) and Indian mustard (*B. juncea* (L.) Czern & Coss) in Australia is thought to be due to suppression of soil-borne fungal pathogens by ITCs released from the *Brassica* crop residues (Angus et al. 1991, 1994, Kirkegaard et al. 1994, 1996). Several fungal root pathogens including take-all (*Gaemannomyces graminis* (Sacc.) Arx&Olivier var. *tritici* Walker), *Rhizoctonia solani* Kuhn, *Bipolaris sorokiniana* (Sacc.) Subram. & Jain, *Pythium irregulare* Buisman, and *Fusarium graminearum* Schwabe, cause significant losses in wheat yield worth $223 M pa (ABARE, 1996) in Australia alone. Previous studies have established the toxicity of volatiles released from *Brassica* tissues to these fungi (Angus et al. 1994; Kirkegaard et al. 1996) but the contribution of individual ITCs is not known, and is important in order to develop selection criteria to develop brassicas with enhanced biofumigation potential.

This study reports the *in vitro* toxicity of pure ITCs on the mycelial growth of five soil-borne fungal root pathogens of cereals when presented in both the headspace and dissolved in the growing media. The selection of ITCs (2-propenyl-, 3-butenyl-, 4-pentenyl-, benzyl- and 2-phenylethyl ITC) for this study was based upon their dominance in the tissues of several field grown *Brassica* species reported in a previous paper (Kirkegaard and Sarwar 1997). The synthetic soil fumigant methyl-ITC was included in the headspace experiment for comparison. Specific objectives of this investigation were to determine (1) relative toxicity of the ITCs to a range of soil-borne cereal pathogens (2) comparison of a headspace toxicity method with that of dissolving the ITCs in the growing media.

Materials and methods

Preparation of ITC solutions

Pure ITCs were obtained from Sigma Chemical Company (St. Louis, MO) or kindly provided by Dr. Alastair Hick (IACR-Rothamsted, UK) (Table 1). Methyl-ITC (MtITC), propenyl-ITC (PrITC), butenyl-ITC (BtITC) and pentenyl-ITC (PtITC) represent a series of alkenyl aliphatic ITCs with increasing side chain length, while benzyl-ITC (BzITC) and phenylethyl-ITC (PeITC) have aromatic side chains (Table 1). Stock solutions of each ITC were initially prepared in methanol and stored covered at -19°C prior to use. Further dilutions were prepared in methanol according to experimental requirements in such a way that the required amount of ITC was delivered in ≤5 ml of methanol. A preliminary experiment showed that ≤ 12 ml of methanol added to the 250 ml Erlenmeyer flasks did not influence fungal growth.

Fungal cultures

The fungi, *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani*, *Bipolaris sorokiniana*, *Pythium irregulare* and *Fusarium graminearum* were originally isolated from field-grown wheat or grass roots from various locations in southern Australia (Kirkegaard et al. 1996). The major characteristics of these fungal species have been summarised in Table 2. The fungal isolates were grown in petri plates (85mm diameter) prepared with full strength potato dextrose agar (PDA). The plates were kept refrigerated (4 °C) and periodically subcultured for experimental use.

Table 1. Characteristics and occurrence of isothiocyanates used in the experiments.

Isothiocyanates	Trivial name of parent glucosinolate	Structural formula	Mol. Wt.	Common occurrence
Methyl	Glucocapparin	CH_3-NCS	73.11	Capparales, Metham sodium (synthetic fumigant)
2-Propenyl	Sinigrin	CH_2=CH-CH_2-NCS	99.15	*Brassica juncea, B. carinata, B nigra*
3-Butenyl	Gluconapin	CH_2=CH-$(CH_2)_2$-NCS	113.18	*B. napus, B. campestris*
4-Pentenyl	Glucobrassinapin	CH_2=CH-$(CH_2)_3$-NCS	127.20	*B. napus, B. campestris*
Benzyl	Glucotropaeolin	◇-CH_2-NCS	149.20	*Sinapis* spp.
2-Phenylethyl	Gluconasturtiin	◇-CH_2-CH_2-NCS	163.23	*Brassica* roots

(◇ -benzene ring)

Table 2. Characteristics of the fungal pathogens and their contribution to the estimated annual loss in wheat yield in Australia.

Common name of disease	Pathogen	Taxonomic Subdivision	Growth rate as colony dia. in control (mm/h)	Host	Annual loss of wheat yield * ($M)
Take all	*Gaeumannomyces graminis* var. *tritici*	Ascomycotina	0.7	Gramineae	101.2
Fusarium crown rot	*Fusarium graminearum*	Deuteromycotina	0.8	Gramineae	44.2
Common root rot	*Bipolaris sorokiniana*	Deuteromycotina	0.5	Gramineae	18.5
Rhizoctonia bare-patch	*Rhizoctonia solani*	Deuteromycotina	0.7	wide range	58.2
Pythium root rot	*Pythium irregulare*	Mastigomycotina	2.2	wide range	0.9

*: ABARE, Grain Statistics, 1996.

Headspace toxicity of ITCs

Volatilisation of the ITCs

An experiment was conducted to determine the behaviour of the ITCs when introduced into the headspace of 250 ml Erlenmyer flasks. The flasks had glass stoppers customised with a half-hole cylindrical (8mm x 6mm OD) rubber septum (Alltech, Deerfield, IL) at the top, and an internal glass hook protruding from the side of a stopper towards the centre. The glass hook was used as a site for dispensing the ITC solutions. Flasks were prepared with and without 20 ml of sterile PDA in the bottom so that the sorption of ITCs by the agar could be estimated. Flasks were sealed by applying a thin layer of high vacuum grease around the stopper and were wrapped with aluminium foil prior to injection of ITCs.

Approximately 100 mM of each ITC in 5 ml of methanol was introduced in the head space of the flasks using a 10 ml syringe to dispense the ITC solution onto the glass hook inside the stopper. Flasks were incubated at room temperature (24±1 °C). Head space concentration of the ITCs was monitored at 1 min after injection and then every 5 mins by taking a 50 ml sample from the flask and injecting it into a gas chromatograph (GC-Varian 3300). The GC was equipped with a fused silica capillary column (BP624, 50 m x 530 mm, 0.25 mm film, J&W Scientific, Folsom, CA) and a Thermionic Specific Detector (TSD) with injector and detector temperatures set at 200 and 300 °C, respectively. ITCs were eluted using a programme to increase column temperature from 170 to 200 °C so that the peak was emitted within 5 min. Headspace concentrations were calculated using standard curves prepared for each ITC. Duplicate flasks were measured for propenyl ITC to determine the level of error associated with this procedure. The time taken for the ITC droplet to completely disappear from the site of dispensing was recorded, and the headspace concentration of the ITCs was calculated over time as a percentage of the initial amount injected.

In-vitro *headspace toxicity*

Experimental flasks were prepared by transferring 20 ml of cooled (~50 °C) sterile PDA to sterilised 250 ml Quickfit Erlenmeyer flasks. When the agar had solidified, each flask was inoculated centrally with a single 6mm diameter agar plug, taken from the margins of actively growing fungal colonies using a cork borer. The plug was placed with its mycelial side down on the agar surface to maximise contact. High pressure vacuum grease was applied to the outer surface of the stopper, which was sealed firmly. The flasks were incubated at 25°C for 24 h (8 h for *Pythium* due to its rapid growth), and the margins of the fungal colony were then marked prior to the addition of the ITCs.

A range of concentrations of each ITC (0 to 5 mM) was dispensed onto the hook inside the flask with a syringe through the septum. Selection of the concentration range for each fungus was based on the results of preliminary experiments to estimate approximate fungicidal doses. All concentrations were applied in duplicate, with controls receiving 5 ml of methanol only. Flasks were placed back in the incubation chamber after ITC injection and allowed to incubate for a further 24 h (8h for *Pythium*) after which time the diameter of the fungal colonies was measured. Flasks with no fungal growth were left for a further 24 h, opened in the fume hood at room temperature and aired for 24 h. Treatments in which fungal mycelium then grew were termed as fungistatic, while those with no growth were considered fungicidal (LD_H). Fungicidal status of these treatments was further confirmed by transferring the original fungal plug from the treatment flasks onto freshly prepared agar petri dishes and incubating further to check for growth. Concentrations of ITCs responsible for 50% suppression in the fungal colony diameter relative to controls (SD_{50H}) were interpolated from the response curves for each fungus.

Toxicity of ITCs dissolved in agar

Agar flasks were prepared in a similar way to the headspace experiments except that the ITC solutions were injected into the bottom of the flask immediately prior to the addition of 20ml of cooled (50 °C), sterile PDA. ITC solutions were prepared in such a way that equimolar amounts of each ITC could be tested for fungal response. Methyl ITC was not included in this experiment. Flasks were sealed immediately and gently swirled for 2 min

to achieve uniform distribution of ITCs until the agar had solidified aseptically. A fungal plug was then transferred on to the agar before sealing the flask with vacuum grease. A range of concentrations of ITCs (0-90 mM agar) were added for each fungus based on the results of preliminary experiments to estimate approximate toxic doses and fungal response was compared with controls receiving 10 ml of pure methanol. The diameter of the fungal colonies was measured 48 h after inoculating the flasks (16 h for *Pythium*) and calculation of SD_{50D} and LD_D was identical to that described for the headspace experiment.

Data analysis

Variation in fungal response between duplicates was very low in both the headspace experiment and the experiment with ITCs dissolved in agar, and the magnitude of the variation did not change with increasing ITC concentration. As a result, ANOVA was carried out on the data for each fungus separately, considering each concentration of each ITC as a separate treatment to estimate an overall standard error of means. SD_{50} values were determined by linear interpolation of the response curves relating fungal suppression to ITC concentrations. Estimates of LD were $\pm$ 0.1 mmole l^{-1} in the headspace experiments and $\pm$ 5 mmole l^{-1} for the ITCs dissolved in agar.

Results

Headspace toxicity experiments

Pattern of ITC volatilisation

Table 3 summarises the pattern of ITC volatilisation within the flasks and its apparent sorption by the agar. The variation recorded for the duplicates of PrITC were low in relation to the differences between the ITCs. The aliphatic ITCs (MtITC, PrITC, BtITC, and PtITC) were more volatile than the aromatic ITCs (BzITC and PeITC) and within these categories, volatility decreased with increasing molecular weight. This is most simply demonstrated by the time taken for the ITC droplet to disappear from the glass hook, which ranged from 1.5 minutes for MtITC up to 120 minutes for BzITC, while the PeITC had not fully volatilised after 72 h. These observations are consistent with the levels of headspace concentrations of the ITCs after 1 minute (327% for MtITC compared with 0.8% for PeITC). There was a similar trend at 6 and 40 minutes after injection although the headspace concentrations of each ITC changed with time. The levels of ITC exceeding 100% presumably resulted from temporary concentration of the compounds in the upper part of the flask near the glass hook on which they were placed since the samples for analysis were withdrawn from this upper zone. The subsequent decline in concentration of the aliphatic ITCs over time may have been due to sorption onto the glass or settling of the compounds into the lower region of the flask. Degradation of the ITCs is unlikely as no additional peaks were evident on the chromatographs. The concentration of the aromatics was still increasing slowly after 40 minutes. The presence of 20 ml of agar in the flask resulted in reduced headspace concentrations for the aliphatic ITCs. This apparent sorption decreased with increased molecular weight from 40.9% for MtITC to 18.5% for PtITC. The very low level of aromatic ITCs measured in the headspace made it difficult to detect significant sorption by the agar. The failure of PeITC to volatilise from the hook after 72 h resulted in its omission from the headspace fungal toxicity tests and the inclusion of MtITC. PeITC was included in tests on take-all only to demonstrate the problems associated with its low volatility in headspace experiments.

Headspace toxicity of ITCs to fungal pathogens

All of the ITCs tested suppressed the fungi but the pattern of the response varied with ITC type and fungal species (Figure 1). Among the aliphatic ITCs, suppression generally increased with decreasing molecular weight, although MtITC and PrITC showed very similar response patterns for most of the fungi. BzITC showed a similar level of suppression as the aliphatic types to all fungi except *Pythium* at low concentrations, but at higher concentrations it was generally less effective. The response to PeITC is shown for take-all only (Fig 1a). The flattening of the response curve is consistent with its low volatility and can be explained by a saturated vapour phase occurring at a relatively low concentration. Consequently additional ITC failed to influence fungal

response. The SD_{50H} and LD_H values shown in Table 4 demonstrate the pattern of ITC suppression. PrITC was more suppressive than MtITC to most fungi as seen by the lower LD_H values for all fungi except *Pythium* and *Bipolaris*. Although BzITC had comparable SD_{50H} to longer side chain aliphatics (BuITC and PtITC) the LD_H, where calculated, were higher.

Gaeumannomyces and *Rhizoctonia* were generally the most sensitive fungi to the ITCs, *Fusarium* was intermediate while *Bipolaris* and *Pythium* were generally more resistant. The growth of *Bipolaris* was stimulated by low levels of all the ITCs (Fig 1). The suppression of *Bipolaris* at higher levels was associated with a change in the colour of the colony from black to pale white. *Pythium* was as sensitive to MtITC and PrITC as *Fusarium* and *Bipolaris* but BzITC had no effect on *Pythium* colony diameter up to the highest rate of ITC added. Despite the lack of impact on colony diameter, the density of hyphal growth of the *Pythium* colony was observed to be reduced and much less branched at high rates of all ITCs, and subsurface hyphal growth (into the agar) occurred in response to the higher levels of PtITC and BzITC. Such behaviour was not observed in the other fungi.

Toxicity of ITCs dissolved in agar

Results presented in Fig 2 and Table 5 summarise the activity of five ITCs against the fungi when dissolved in agar. All ITCs suppressed the fungi but, in contrast to the headspace experiment, the aromatic ITCs (BzITC and PeITC) were more suppressive than the aliphatic ITCs (PrITC, BtITC and PtITC) to all fungi except *Pythium* (Fig 2e). Within these classes, the lower molecular weight ITCs were more suppressive, consistent with the headspace experiment. The pattern of fungal sensitivity to the ITCs was generally similar to the headspace experiment. *Gaeumannomyces* was the most sensitive, *Fusarium*, *Rhizoctonia* intermediate, *Bipolaris* less sensitive, while *Pythium* showed a high degree of resistance compared to the other fungi. The stimulation of *Bipolaris* growth at low concentrations observed in the headspace experiment was apparent for BtITC and PtITC but not for the other ITCs, although it may have occurred at concentrations lower than those used in the experiment. Also noteworthy was the abrupt decline in the growth of *Pythium* when the concentration of BzITC exceeded 60 mM, which was in contrast to the response patterns observed for the other ITCs and for the other fungi.

Table 3. Volatility and pattern of headspace concentration of isothiocyanates (ITCs) injected in 250ml Erlenmyer flasks. Numbers in parenthesis are SEM for duplicates of propenyl ITC.

	Apparent Droplet volatilisation by Agar (%) time (mins)	Headspace concentration of ITCs at different time intervals after injection (% of total added) Without Agar 1 min	6 min	40 min	With Agar 40 Min	sorption
Aliphatic						
MtITC *	1.5	327	206	57.3	23.4	40.9
PrITC	5	77.1 (1.2)	154 (4.2)	58.1 (0.2)	22.3 (0.1)	38.4
BtITC	10	41.1	111	47.4	18.2	29.2
PtITC	10	10.4	40.1	30.0	11.5	18.5
Aromatic						
BzITC	120	1.0	2.1	3.3	5.5	0.0
PeITC	>72 h	0.8	1.1	2.0	2.6	0.0

*MtITC - methyl, PrITC - 3-propenyl; BtITC - 4-butenyl; PtITC - 5-pentenyl; BzITC - benzyl; PeITC - 2-phenylethyl

Table 4. Concentrations of ITCs causing 50% growth suppression (SD_{50H}) and death (LD_H) of five cereal fungal pathogens in headspace experiments. The concentrations are presented as μmoles l^{-1} added to headspace.

Fungus	MtITC		PrTC		BtITC		PtITC		BzITC	
	SD_{50H}	LD_V	SD_{50H}	LD_H	SD_{50H}	LD_H	SD_{50H}	LD_H	SD_{50H}	LD_H
Gaeumannomyces	0.81	1.8	0.55	1.6	1.13	2.3	1.36	3.4	1.23	4.1
Rhizoctonia	0.71	1.8	0.63	1.6	1.28	2.3	1.59	3.7	0.37	4.7
Fusarium	0.85	1.8	0.72	1.6	1.28	3.4	1.55	4.3*	1.62	4.1*
Bipolaris	0.73	3.6	0.79	3.9	1.78	4.5	2.26	6.2	1.64	4.1*
Pythium	0.77	1.8	0.73	2.0	1.72	5.1*	3.20	7.4*	NE	NE
LSD P=0.05	0.05		0.08		0.08		0.15		0.74	

LD_H: Lethal dose of ITCs for fungi; SD_{50H}: Concentrations of ITC vapour causing 50% reduction in the colony diameter
*: Concentration fungistatic only; NE: no effect

Table 5. Concentrations of ITCs dissolved in agar causing 50% growth suppression (SD_{50D}) and death (LD_D) of five cereal fungal pathogens. The concentrations are presented as μmoles l^{-1} of agar (mM).

Fungus	PrITC		BtITC		PtITC		BzITC		PeITC	
	SD_{50D}	LD_D	SD_{50D}	LD_D	SD_{50D}	LD_D	SD_{50D}	LD_D	SD_{50D}	LD_D
Gaeumannomyces	3.7	20	5.3	30	4.4	30	1.7	5	3.6	10
Rhizoctonia	7.1	30	7.6	40	8.4	40	1.8	10	3.3	10
Fusarium	4.3	40	7.6	60	10.8	70	3.8	10	3.9	20
Bipolaris	8.3	40	11.6	60	19.4	80	7.2	30	6.6	30
Pythium	14.0	40	20.6	60	41.0	90	56.2	80	34.2	60
LSD P=0.05	0.5		0.2		1		0.8		0.8	

LD_D: Lethal dose of ITCs for fungi, SD_{50D}: Concentrations of ITC causing 50% reduction in diameter of fungal colony

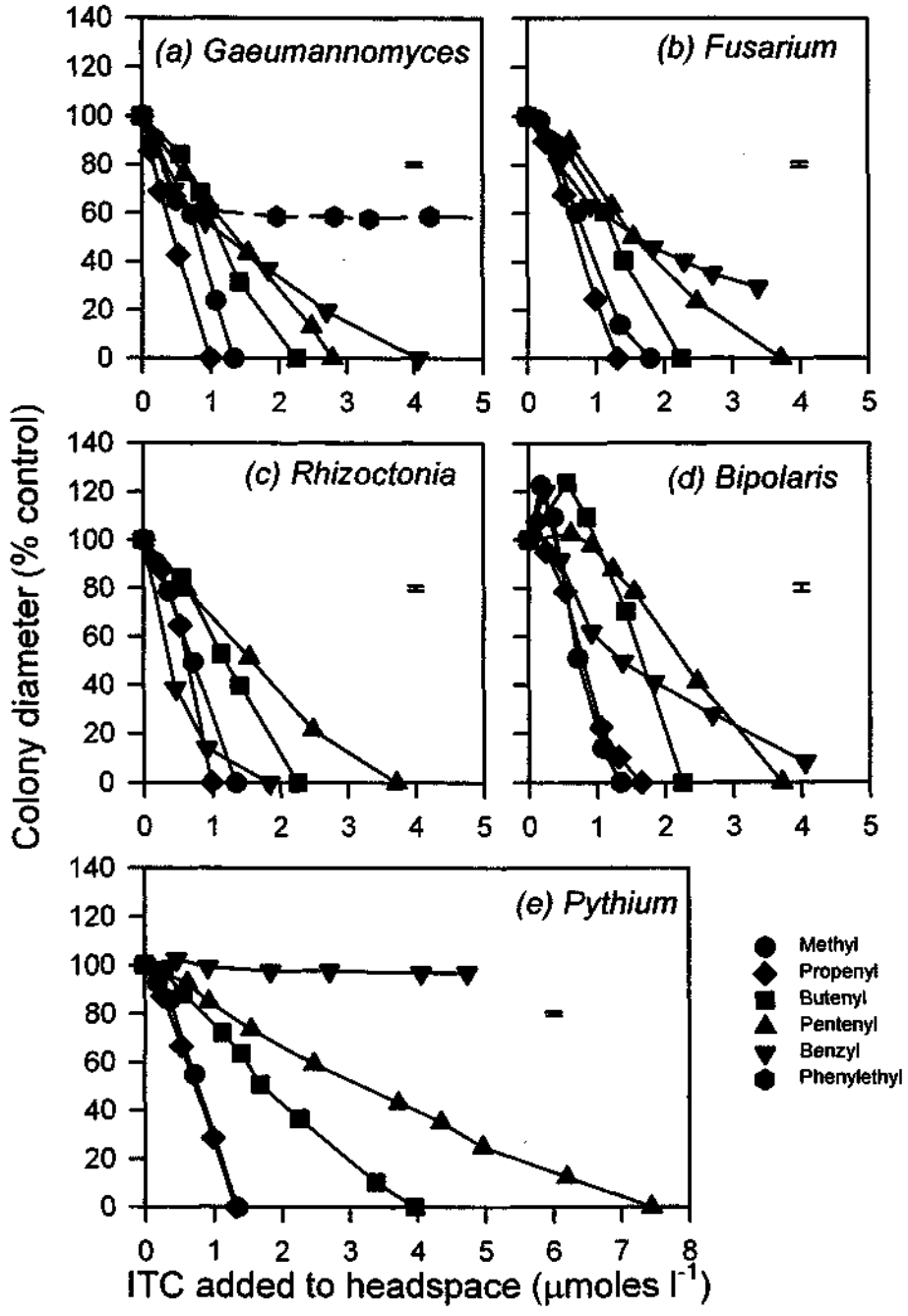

Figure 1. The *in-vitro* effect of six isothiocyanates: methyl, 3-propenyl, 4-butenyl, 5-pentenyl, benzyl and 2-phenylethyl added into the headspace on the mycelial growth of (a) *Gaeumannomyces* (b) *Fusarium*, (c) *Rhizoctonia* (d) *Bipolaris* and (e) *Pythium*. Vertical bars show standard error of the difference for the analysis of individual fungal species.

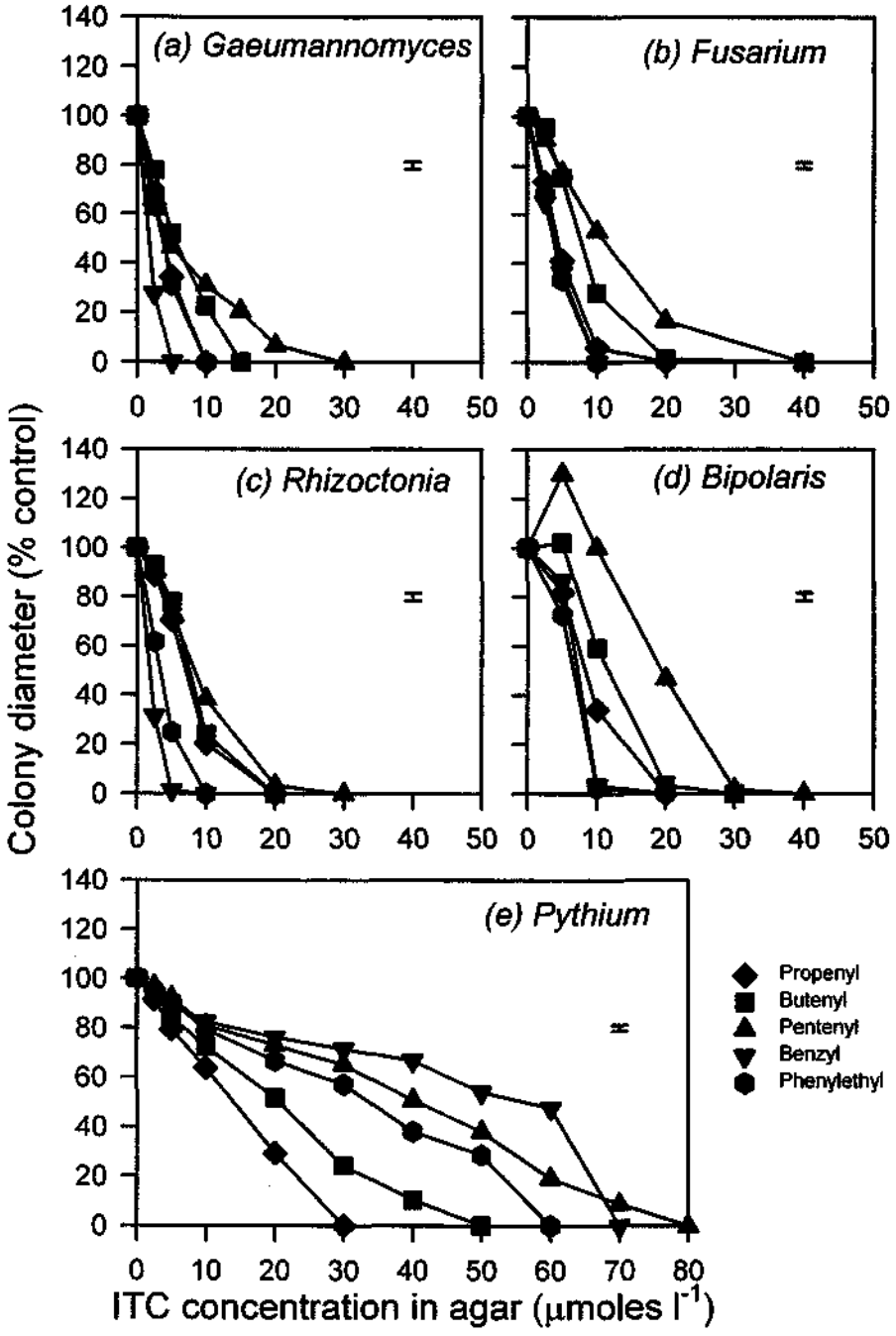

Figure 2. The *in-vitro* effect of five isothiocyanates: 3-propenyl, 4-butenyl, 5-pentenyl, benzyl and 2-phenylethyl dissolved in agar on the mycelial growth of (a) *Gaeumannomyces* (b) *Fusarium*, (c) *Rhizoctonia* (d) *Bipolaris* and (e) *Pythium*. Vertical bars show standard error of the difference for the analysis of individual fungal species.

Discussion

The results of these experiments confirm the antifungal effects of ITCs to cereal pathogens and demonstrate the significant variation which exists in both the toxicity of various ITCs and the sensitivity of the fungal species. Importantly, while the ITCs added to headspace or dissolved in agar provided some consistent trends in terms of fungal response, the two methods provided contrasting results in relation to the relative toxicity of the aliphatic and aromatic ITCs. This discrepancy may underlie some of the apparent contradictions from previous studies but is explicable in terms of the behaviour of the ITCs.

In the headspace experiment, the lower volatility of the aromatic ITCs (BzITC and PeITC) resulted in a slow rate of increase in ITC concentration in the headspace and a lower saturated headspace concentration. In the most extreme case, the droplet of PeITC was visible on the glass hook after 72 h, and, as a result, there was no response of the fungus to additional ITC (Fig 1a). For the aliphatic compounds, the time to complete volatilisation was small in relation to the experimental duration, although the impact of different volatility patterns on fungal response is uncertain. In addition to differences in volatility, the apparent sorption of the ITCs by the agar in headspace experiments varied from 40.9% for MtITC to 18.5% for PtITC although the role of these absorbed ITCs is not known. Sorption onto the agar surface may still expose the fungal mycelium to the ITCs as they grow across the surface but the capacity of some fungi for growth into the agar creates further uncertainties. It is difficult to measure the exact ITC concentration to which the fungus is exposed in the headspace experiments and thus, direct comparisons of SD_{50} and LD values from the two methods of application are difficult. The higher LD values for ITCs dissolved in agar are possibly due to the uniform distribution of ITCs throughout the agar after solidification, resulting in a constant concentration experienced by the mycelia as they first contact the agar. The diffusion of some ITCs into the headspace above the agar may also occur in this closed system. In contrast, the constant motion of ITCs in the headspace experiment, and settling and deposition of ITCs on the agar surface may at times expose the fungus to higher ITC concentrations then those assumed present in the overall headspace, and thus give a lower LD. More mycelial surface area is also likely to be exposed to the ITCs in the headspace experiments at any point in time.

Differences in absolute values of LD between experimental approaches are of less concern than the differences in the ranking of toxicity of aliphatic and aromatic ITCs which has been observed here. In these experiments the discrepancy can be explained in terms of the greater reliance on volatility for effective suppression of the fungi in the headspace experiments. Ranking of toxicity reported in previous studies has also depended upon the application method used in relation to the characteristics of the ITCs tested. In experiments where only the volatiles released from hydrolysis of *Brassica* tissues contact the test fungus, tissues dominant in more volatile types such as PrITC were more toxic (Angus et al. 1994, Kirkegaard et al. 1996, Mayton et al. 1996) while in experiments using ITCs dissolved in agar, aromatic ITCs were up to 20 times more toxic (Drobnica et al. 1967). Similarly, where aqueous extracts are tested, the activity of more soluble ITCs will be favoured and the toxicity of less soluble forms may be underestimated. The dependence of toxicity ranking on the application method must be considered when making inferences about the relative toxicity of individual ITCs.

Despite the differences in the results of the two methods (associated primarily with ITC volatility) some consistent trends were observed in these experiments. In both methods, the toxicity of the aliphatic compounds increased with decreasing side chain length (i.e. molecular weight). This trend was also evident for the aromatic ITCs dissolved in agar. This ranking of toxicity (MtITC=PrITC >BtITC > PtITC) and (BzITC > PeITC) of dissolved ITCs suggests that the shorter-chained ITCs within each class are more toxic, irrespective of their volatility. The cytotoxicity of ITCs is generally attributed to their reaction with sulfydryl, disulphide and amine groups present in proteins and amino acids (Zsolnai 1966, Kawakishi and Kaneko 1987) and the resultant inactivation of metabolic enzymes (Wood 1975). Differences in fungal sensitivity may not only relate to the cytotoxicity but to the ability of the ITCs to penetrate cells (Wood 1975). Nastruzzi et al. (1996) have shown that the octanol-water partition coefficient, a measure of the molecular lipophilicity and hydrophobicity, is an important parameter influencing the ability of the ITCs to penetrate cell membranes. In their study the most active ITCs were characterised by low partition coefficients. Those in common with this study support that assertion, as their partition coefficients were 0.006, 0.015 and 0.628 for benzyl ITC, propenyl ITC and butenyl ITC, respectively.

Manici et al (1997) have recently reported rankings of *in vitro* toxicity of some ITCs common to this study to *Fusarium culmorum* as benzyl > propenyl > butenyl, consistent with the results reported here. In the same study, the sulfinated aliphatic ITC 3-methylsulfinylpropyl was up to 10 times more toxic than benzyl ITC to some fungi including *Rhizoctonia solani* and *Pythium irregulare*. Although not present in high concentrations in *Brassica* tissues this compound is found in Iberus spp. which may have potential for use as biofumigant crops.

Differences in sensitivity of fungal species to pure ITCs have been reviewed by Fenwick et al. (1983), Rosa et al. (1997) and Brown and Morra (1997) and fungicidal concentrations of ITCs for fungi may differ by an order of magnitude. In these experiments, *Gaeumannomyces* and *Rhizoctonia* were the most sensitive to ITCs using both experimental methods, *Pythium* and *Bipolaris* were least sensitive, and *Fusarium* was intermediate. The most obvious difference among the fungi was the resistance of *Pythium* to the ITCs, particularly aromatic ITCs dissolved in agar, which were the most toxic to all the other fungal species. Although *Pythium* was the fastest growing fungus (2.2 mm/h), there was no significant correlation between the rate of fungal growth (as in control) and sensitivity to ITC. *Bipolaris* had the slowest growth rate (0.5 mm/h) but was relatively resistant to the ITCs compared to the other fungi. *Pythium* is a member of the Mastigomycotina and its cell wall composition and membrane structure differ from those of the other fungi (Bartnicki-Garcia 1987, Elliot 1977). It is possible that its membrane structure may reduce the efficiency of penetration of ITCs into the cells although the exact mechanism of resistance is not known. Stimulation of *Bipolaris* growth at low concentrations of all the ITCs in vapour phase was also notable, and is consistent with results observed for *Bipolaris* exposed to volatiles from *Brassica* tissues (Kirkegaard et al. 1996). The resistance of *Bipolaris* may result from melanisation of the hyphae, as loss of their black colour preceded the onset of fungal suppression, and melanin is thought to play a role in the resistance of hyphae and spores to lysis (Lockwood 1960, Bloomfield and Alexander 1967). *Bipolaris* was particularly resistant to lower concentrations of short-chained aliphatic ITCs (MtITC and PrITC) in the headspace experiments compared to other fungi including *Pythium*, and melanin may have been responsible for the resistance at these lower concentrations. Although the ranking of fungal sensitivity in these experiments is consistent with that reported by Kirkegaard *et al.* (1996) for the same fungi exposed to volatiles from *Brassica* tissues, significant differences in the sensitivity of different isolates within species were observed in that study. This variation in sensitivity within biotypes of the same species should be considered when generalising with respect to fungal sensitivity and may influence conclusions regarding the ranking of species for their sensitivity. Further research is required to investigate the possible roles of differences in the biochemistry and physiology of the fungi in their differential sensitivity to ITCs.

These results have implications for future investigations of the use of *Brassica* rotation and green manure crops for suppression of soil-borne diseases in agriculture and horticulture. The potential for suppression of cereal root fungal pathogens by *Brassica* oilseed rotation crops such as canola appears significant since the root tissues are high in 2-phenylethyl GSL (PeGSL) (Kirkegaard and Sarwar 1997) the precursor of PeITC which was highly toxic to the fungi. The roots are likely to provide the major source of ITCs since dry shoot residues left after harvest are low in GSL, often burnt or grazed and not incorporated. The low volatility of PeITC may also result in considerable persistence in soil (Gardiner et al. 1997) which may be necessary for significant suppression of diseases in a cereal crop sown 3-5 months after the *Brassica* crop is mature. Levels of PeGSL in roots of canola varieties have been shown to vary up to ten fold, and are not correlated with seed GSL levels (Kirkegaard and Sarwar 1997) so that potential exists to select or develop types with higher levels of aromatics in roots to enhance biofumigation.

The results of these experiments support previous data showing that some ITCs which are released by hydrolysis of *Brassica* root and shoot tissues, are more toxic than MtITC to a range of soil-borne fungal pathogens (Drobnica et al. 1967., Vaughn et al. 1993). Although the potential ITC production in the tissues of *Brassica* crops falls within the range used in commercial MtITC fumigation (400-1200 nM g^{-1} soil) (Kirkegaard and Sarwar 1997), recent studies have indicated that the efficiency of conversion of GSLs to ITCs in soil may be as low as 15% (Borek et al 1997). Predictions concerning the efficacy of ITCs based on *in vitro* studies will need to be confirmed with studies of *in vivo* effects in soil, which are currently not well characterised.

The relative insensitivity of *Pythium* to ITCs is consistent with the lack of suppression of this fungus by

incorporated *Brassica* residues in some previous studies (Stephens and Davoren 1997) and indicates that biofumigation using brassicas will not be effective for all pathogens. Enhancing biofumigation potential will require selection of brassicas high in GSLs which release the ITCs most toxic to the particular target organism. Further screening of ITC toxicity to different organisms should consider carefully the influence of the experimental method, and possible variation in isolates on the ranking of fungal sensitivity to ITCs.

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Biofumigation potential of brassicas. IV. Assessment of potential in contrasting environments.

John Matthiessen[A], John Kirkegaard[B] & Muhammad Sarwar[B]

[A]CSIRO Entomology, Private Bag PO Wembley, WA 6014
[B] CSIRO Plant Industry, GPO Box 1600 Canberra ACT 2601

Abstract

A range of brassicas was grown at Manjimup and Busselton, Western Australia to assess their potential for growth in potato-growing regions. Plants were sown in autumn and spring and samples were taken at mid-flowering. Height and biomass were measured and chemical assay results profiling the glucosinolate-producing isothiocyanates in the various species and cultivars from plants grown in Canberra were applied to the WA-grown plants to assess their biofumigation potential. Large biomass, especially for autumn-sown lines grown at the climatically milder coastal location of Busselton, produced a high potential production of isothiocyanates in several lines. Biomass tended to be greater for lines sown in spring compared to autumn at Manjimup. A generalised comparison of potential to produce isothiocyanates relative to production of methyl isothiocyanate by a typical application of metham sodium soil fumigant indicated that the brassicas had a high potential for biofumigation.

Introduction

The growth of brassicas with potential for biofumigation will vary in different environments. As part of the process of determining appropriate species and cultivars for different areas, seasons and production systems, it is necessary to determine growth patterns in the various locations. Biofumigation potential is related to the types and concentration of the various isothiocyanates in the tissue, which tissue within the plant that they are in, and the biomass which determines the overall amount likely to be present for a given area or volume of soil.

The main array of brassicas selected for chemical analysis to determine biofumigation potential was grown in autumn and spring in Canberra at CSIRO's Ginninderra Experiment Station (see above). This was convenient for ensuring that the plants could be quickly returned to the laboratory to be prepared for analysis. The Canberra environment is, however, limiting for growth during winter because of the cold, dry upland climate. To gauge the growth potential and in turn biofumigation potential, a selection of species and cultivars was grown in two contrasting locations.

Methods

Around 20-25 different *Brassica* species and cultivars that had been included in the original evaluation studies in Canberra (see above) were sown in small (1m x 0.5m) plots at Manjimup and Busselton in Western Australia, both potato-growing regions. Sowings were made in autumn and in spring. No irrigation was applied during growth. Manjimup is an inland area, whereas Busselton is near coastal. Manjimup is cooler and has a higher rainfall than Busselton. Annual rainfall averages 1055 mm at Manjimup and 838 mm at Busselton; mean annual maximum temperatures are 20.2°C and 22.1°, and mean minima are 9.7°C and 11.1°C, at Manjimup and Busselton, respectively.

At approximately mid-flowering, five plants from each plot were sampled. The height of each plant was measured, the roots separated from the tops and the material oven-dried and then weighed. The identity and concentration results of the glucosinolate analyses carried out on the Canberra-grown plants of each species or cultivar were applied to the biomass of the WA-grown material to estimate the biofumigation potential of the plants in the two environments and in the two seasons.

Results

Plant height and weight

Figures 1 and 2 show the mean height of the various autumn- and spring-sown brassicas, respectively. Sampling which coincided with flowering in those species that flowered; some species that did not flower because of insufficient vernalisation were sampled at a similar time to those that flowered, or when senescence of lower leaves began. Many of the species produced very tall plants. Autumn-sown plants at both sites were taller than those sown in spring (Figs. 1 & 2). The species numbers used in the figures match those assigned in the more extensive original sowings in Canberra for the glucosinolate profiling studies (see above).

Figures 3 and 4 show the mean dry weight of tops and roots for the autumn- and spring-sown brassicas, respectively. The biomass of the autumn-sown plants was greater at Busselton than at Manjimup, and greater than the spring-sown plants at Busselton, whereas the spring-sown plants at Manjimup were generally larger than their autumn-sown counterparts there (Figs. 3 & 4).

Estimated potential ITC production

In order to estimate the relative potential for biofumigation of the various species in each location, the isothiocyanate-producing glucosinolate (ITC-GS) concentration values obtained from the plants grown in Canberra in each season were applied to the biomass of the relevant species obtained at Manjimup and Busselton. To further relate this derived potential to the field, the estimated total amount of ITC produced per mass of soil was calculated, assuming a plant density of 100 m^{-2} and incorporation and total glucosinolate breakdown in the top 25 cm, and a soil bulk density of 1.35 g cc^{-1}. These represent typical values for the depth to which metham sodium fumigant is incorporated into soil and soil density in potato-growing regions.

Figures 5 and 6 show the estimates of the potential concentration of ITC's in soil for autumn- and spring-sown brassicas at Manjimup and Busselton. To obtain some indication of what these estimates of potential mean, the concentration of methyl isothiocyanate that would derive from complete breakdown of 500 l of metham sodium H. arator^{-1} with uniform dispersal through the top 25 cm of soil at the assumed bulk density is also shown in Figures 5 and 6.

Discussion

These analyses of potential concentration of ITC's in soil deriving from the brassicas grown at Manjimup and Busselton take account of seasonal variation of glucosinolate concentrations within the same species, but do not consider the different ITC profiles of the different species, nor the likely different toxicities of the different ITC's to various pests and diseases. As such, the results are simply an crude indication of potential for biofumigation effects. Nevertheless, they are encouraging in showing that several species or types would appear to have good potential for producing large biomass in potato-growing regions with climate and soils similar to Manjimup and Busselton.

The results also indicate types of brassicas that would appear to have the potential to grow well in such situations. It is likely that many of the commercial cultivars of those genera or types have been selected for reduced isothiocyanate production for oilseed or fodder purposes. These results could, therefore, be used as a guide to selecting alternative types that might have higher ITC production from, or related to those in the groups that grew well, in order to enhance biofumigation potential. These results will be linked with tests of the various types to determine their toxicity to soil pests, in order to help in the selection process for maximal biofumigation types.

Acknowledgements

Mark Shackleton and Diana Doyle for technical assistance. Keith Taylor (Busselton) and staff of the AgWA Horticultural Research Centre (Manjimup) for use and preparation of the land.

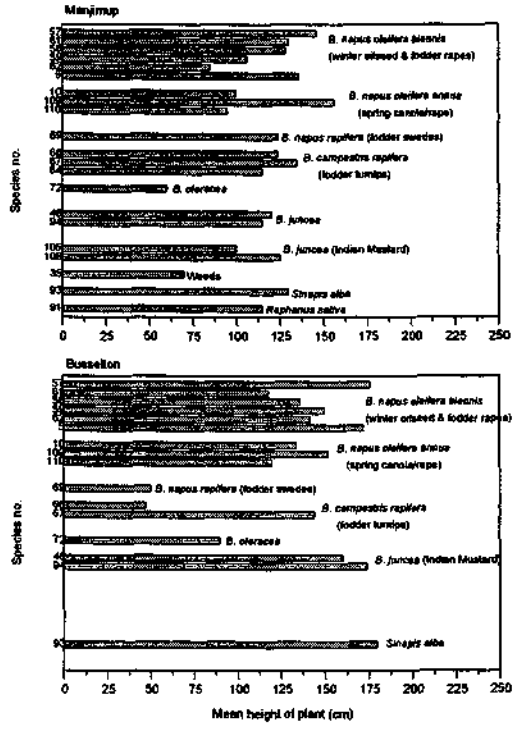

Figure 1. The mean height of various brassicas sown in autumn at Manjimup and Busselton, WA.

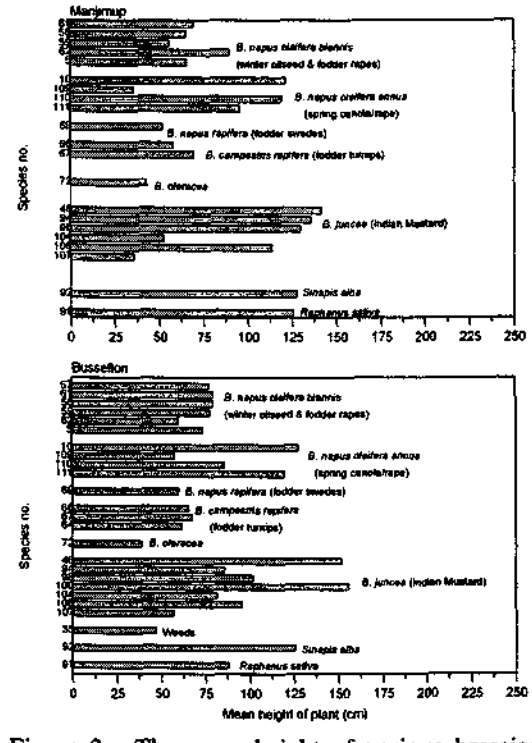

Figure 2. The mean height of various brassicas sown in spring at Manjimup and Busselton, WA.

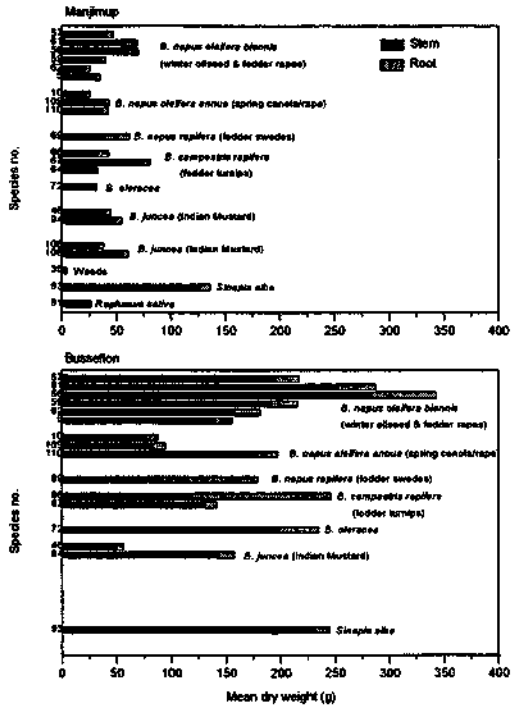

Figure 3. The mean dry weight of autumn-sown brassica tops and roots at Manjimup and Busselton, WA.

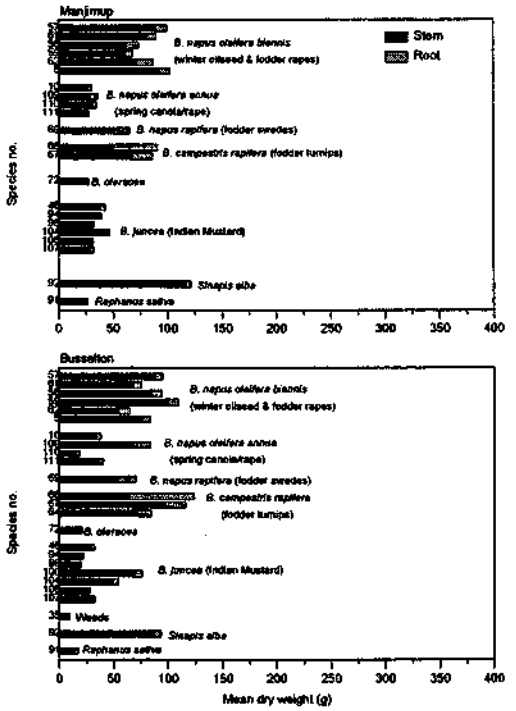

Figure 4. The mean dry weight of spring-sown brassica tops and roots at Manjimup and Busselton, WA.

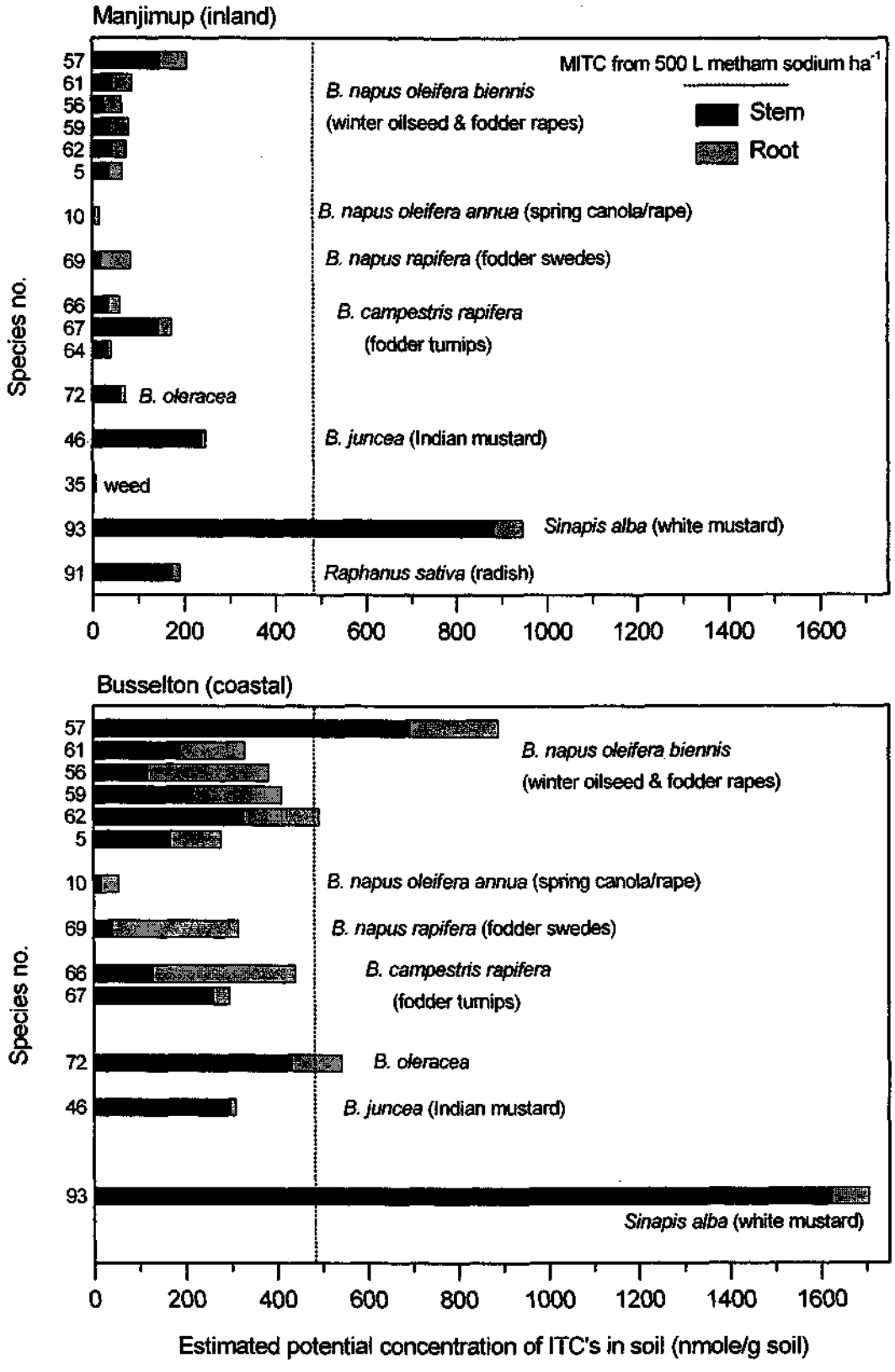

Figure 5. Estimated potential production of isothiocyanates by several brassicas sown in autumn at Manjimup and Busselton, WA, relative to the potential concentration of metham sodium from 500 l ha^{-1}.

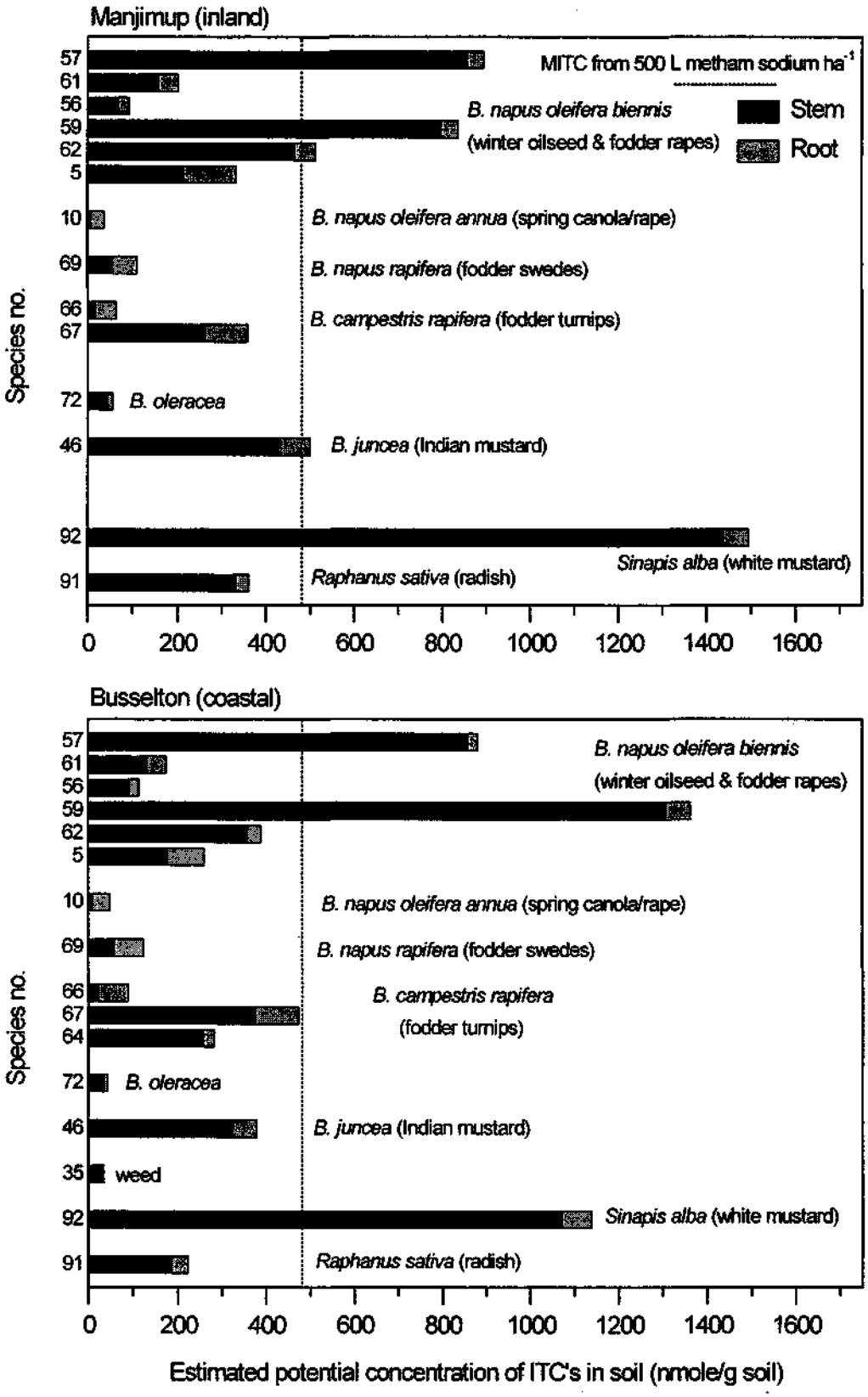

Figure 6. Estimated potential production of isothiocyanates by several brassicas sown in spring at Manjimup and Busselton, WA, relative to the potential concentration of metham sodium from 500 l ha^{-1}.

Whitefringed weevil as a bioassay tool, susceptibility to isothiocyanates and effects of temperature on toxicity.

John Matthiessen & Mark Shackleton

CSIRO Entomology, Private Bag PO Wembley, Western Australia 6014

Abstract

Whitefringed weevil eggs are readily obtained in large numbers and can be stored for long periods after providing appropriate conditions for embryogenesis. The fully-developed larvae remain in the egg until stimulated to hatch. Large numbers of larvae can be stockpiled and obtained as required for experimental studies. The first instar larva is a non-feeding stage capable of long survival with neither food nor shelter. Longevity varies with temperature, but readily accommodates the period required to conduct bioassays for susceptibility to fumigants and biofumigants without appreciable mortality of untreated controls. These attributes make first instar whitefringed weevil an unusually good bioassay model and tool, for a soil insect.

Bioassays of the effect of pure methyl isothiocyanate (MITC) and phenylethyl isothiocyanate (PeITC), exposed in the vapour phase to first instar whitefringed weevil showed MITC to be highly toxic, and varying little in its potency at temperatures ranging from 5-20°C. PeITC, which is markedly less volatile, was similar in toxicity to MITC at the higher temperatures but its toxicity diminished as temperature declined.

Introduction

Unlike the larvae of many insects that occur in foliage, the larvae of soil-dwelling insects in most cases are adapted to moist, protected environments typical of the soil in which they feed during their development. The adult stage of soil insects is usually more robust and able to tolerate desiccation as it is the adult that often comes to the surface of the ground or lives outside of the soil environment, feeding on foliage and moving about by walking or by flight.

Whitefringed weevil adults occur on the surface of the ground and lay their eggs into the soil or amongst litter on the surface. The larvae develop within the soil. An unusual characteristic of the larvae is that the first instar, which is about 1mm long, is a non-feeding stage. Field sampling has shown that in the cool conditions typical of southern Australia during winter first instar larvae are present in the field population for many weeks during winter. Progression of development into and beyond the second instar only occurs when conditions begin to warm during spring. The prolonged period that first instars are present suggests that they are capable of living a considerable time without feeding.

Soil-dwelling insects are often difficult to rear in the laboratory because of the need to house the larvae in soil. With that comes the problems of uniformity of the medium, disease outbreaks, the difficulty of observing growth and assessing survival without disruption of development that can come from disturbance of the soil, long development periods and decay of food sources. Whitefringed weevil has proven difficult and unpredictable to rear, and only small numbers have been successfully reared through to adult. Survival is low and the environmental cues that trigger emergence of the adults from the pupal stage have proved impossible to define, with sporadic transition into the pupal stage and sporadic adult emergence.

Because of these difficulties in rearing soil insects to obtain large numbers of individuals, and their sensitivity to desiccation, the larval stages are generally considered inappropriate organisms for carrying out classical *in vitro* dose-mortality studies of the effects of toxins. These typically require large numbers of individuals to enable tests to be properly assessed. Furthermore, the organisms need to be sufficiently robust and have adequate longevity in the absence of protective media so that untreated controls do not suffer unusually high levels of mortality that compromise evaluation of the response.

Whitefringed weevil has several attributes that make it an unusually suitable subject for conducting bioassays. Eggs are produced in large numbers over a long period provided that the adults are fed on a suitable nitrogen-rich food. Typically, legume foliage is a good food source. Lucerne, which has the advantages of being perennial and readily grown in pots is used. Adults are normally collected from the field during summer (January-March) and can be maintained for many weeks (until around June-July) on lucerne foliage, with steady egg production over that period.

A significant attribute of whitefringed weevil eggs is their capacity to be stored for long periods after initial maturation, enabling larvae to be obtained as required. Embryogenesis occurs in two weeks at 26°C and 95% RH, and the larvae do not hatch under those conditions but remain in the egg awaiting a stimulus. The eggs in this condition can be stockpiled for many months at 15°C and 95% RH with only a slow loss of viability. When larvae are required, it is only necessary to thoroughly wet the eggs and they hatch within about 24h at ambient laboratory temperature (~21°C).

With the ease of obtaining large numbers of larvae on demand, little seasonal constraint because of a long oviposition period and capacity to stockpile, and with a likely long survival without requiring food, it appeared that first instar whitefringed weevil larvae potentially offered a particularly good model soil insect for bioassays to determine effects of toxins. This would be particularly so if the larvae could survive in the absence of both food and a protective medium such as soil for a sufficient period to enable ready assessment of the effect of various treatments.

The aim of this study was to measure the longevity of first instar whitefringed weevil larvae in the absence of food or soil in order to formulate appropriate bioassay techniques. The effect of temperature on longevity was examined and studies of the toxicity of the vapour phase of two contrasting isothiocyanates were carried out to determine the baseline response of the larvae *in vitro*, also at a range of temperatures.

Methods

Longevity of first instar larvae

The longevity of newly-hatched whitefringed weevil larvae was determined by placing 30 larvae in each of five replicate containers at each of five constant temperatures (5, 10, 15, 20, 26°C). The temperature range was chosen to reflect likely The larvae were checked daily and the number still alive was recorded. Dead individuals were discarded. There was no food, moisture or soil provided in the containers. Records were taken until the last larva had died.

Toxicity of methyl and phenylethyl isothiocyanates

The toxicity of the aliphatic methyl isothiocyanate and the aromatic 2-phenylethyl isothiocyanate at different temperatures was measured by injecting known quantities into the headspace of five replicate sealed flasks each containing 20 hatchling whitefringed weevil larvae. The volume of the flasks was 140 ml, and the isothiocyanates were made up in methanol and injected through a rubber septum using a microlitre syringe. Contact of the material with the insects was only through the vapour.

The insects were exposed to the vapour for 24 h, after which time the flasks were opened and the gases allowed to evacuate in the fume cabinet, before the insects were transferred to a clean plastic vial. They were held at a constant 20°C for assessment of mortality at 96h after initial exposure. After counting the dead insects, the mortality was estimated by correcting for control mortality using Abbott's formula.

Results

Longevity of first instar larvae

The longevity of the hatchling whitefringed weevil larvae at each temperature is shown in Fig. 1. Longevity was greatest at 10°C, being around 35-40 days before 50% of the insects had died. At 10°C the inset of mortality was slow, with greater than 90% of the larvae surviving for 20 days, and mortality not accelerating until around 30 days (Fig. 1).

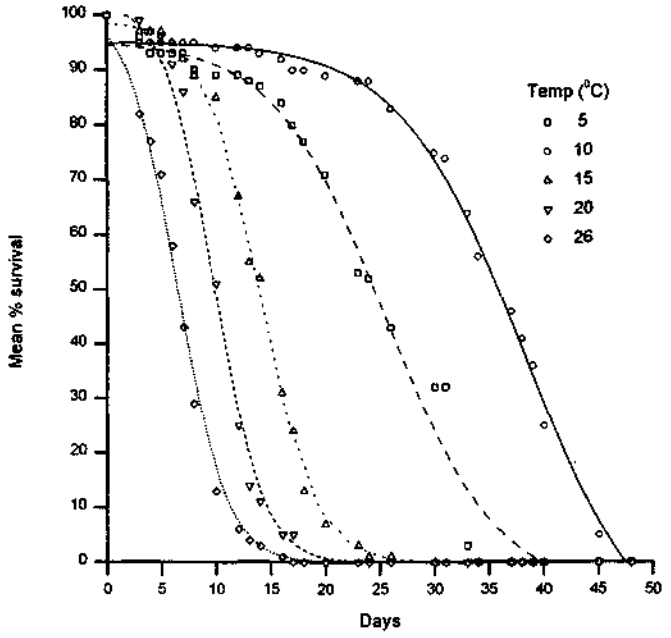

Figure 1. Longevity of first instar whitefringed weevil larvae maintained at a range of temperatures without food or shelter.

Mortality occurred at a faster rate at 5°C, especially after about 10 days, with a median longevity of around 25 days. The survival diminished uniformly as temperature was increased from 15-26°C, with median longevity falling from around 17 days at 15°C to 8 days at 26°C. At these higher temperatures larvae began dying quickly and the decline in survival was very steep, dropping from 90% to around 10% in little more than one week (Fig. 1).

Toxicity of methyl and phenylethyl isothiocyanates

Figure 2 shows the toxicity of methyl isothiocyanate (MITC) and 2-phenylethyl isothiocyanate (PeITC) vapour to first instar whitefringed weevil larvae at a range of temperatures (5, 10, 15, 20°C). Although the Abbott's formula correction for mortality in the controls was applied to all data, mortality in the controls was consistently low (< 10%).

The most striking aspect of the results is the very high toxicity of MITC at all temperatures and the very steep dose-response curve. Only at 5°C was there an appreciable reduction in the toxicity of MITC, the effect at all other temperatures being within a narrow concentration range (Fig. 2). The LD_{50} of MITC was in the range 0.030 - 0.040 mg l^{-1}, and the LD_{95} around 0.035 - 0.50 mg l^{-1}, from 20 -10°C, with the LD_{50} being around 0.050 mg l^{-1} at 5°C.

The toxicity of PeITC was affected much more by decrease in temperature than MITC, with the LD_{50} declining from around 0.070 mg l^{-1} at 20°C to approximately 0.14 mg l^{-1} at 5°C (Fig. 2). As temperature declined, the slope of the PeITC dose-response curve flattened markedly, such that the dose required to achieve an LD_{95} at 5°C was around 0.30 mg l^{-1}, about 2.5-fold greater than the 0.12 mg l^{-1} required to produce an LD_{95} at 20°C (Fig.2).

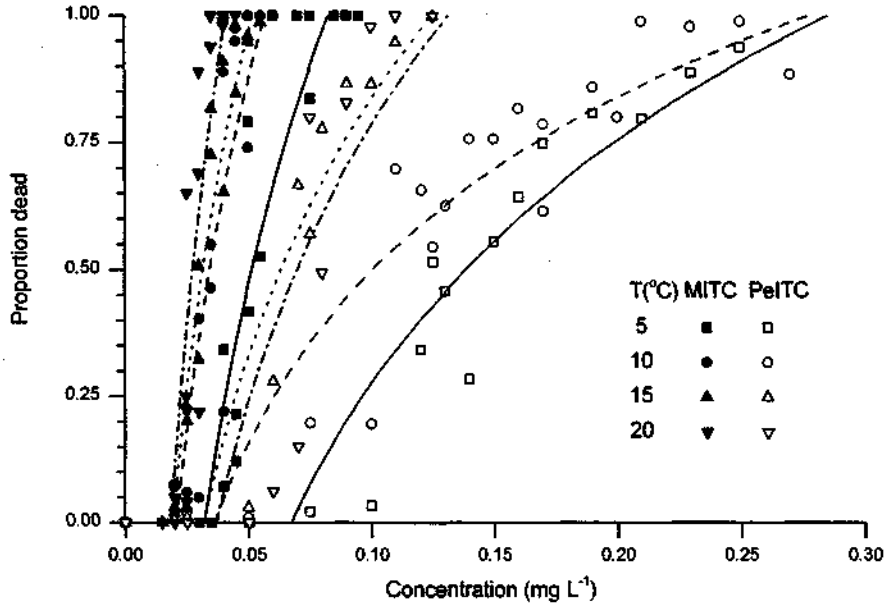

Figure 2. Toxicity of methyl isothiocyanate and 2-phenylethyl isothiocyanate, to first instar whitefringed weevil larvae exposed only to the vapour phase *in vitro* at a range of temperatures.

Discussion

Despite their small size (~1mm long), newly-hatched whitefringed weevil are remarkably robust and able to withstand long periods without food or shelter. For the immature stage of a soil insect, that would normally be associated with a need for protection from desiccation, this is an extraordinary attribute. It makes newly-hatched whitefringed weevil larvae a particularly useful model soil insect for the assessment of toxic effects in bioassays, especially the effect of volatile compounds such as fumigants and biofumigants. The first instar larvae are probably too small to be practical bioassay subjects for testing contact toxins.

A key additional attribute that makes hatchling whitefringed weevil larvae an especially good bioassay tool is the ability to stockpile pre-hatching eggs for long periods and hatch the larvae readily and quickly on demand. The high fecundity and longevity of the adults when fed on suitable food such as lucerne foliage makes collection of eggs easy. The requirements for maturation merely involve maintenance of a high, but not saturated, relative humidity and a warm temperature. This is easily maintained by holding the eggs over an appropriate concentration of sulphuric acid in a desiccator in a constant temperature room or cabinet. Long-term storage of the mature eggs is conveniently maintained at a similar RH at 15°C.

While laboratory rearing of whitefringed weevil through a complete developmental cycle has proven difficult and unpredictable, there have been occasional successes in bringing through new adults which can be quickly brought into oviposition. Generally, however, field collection of adults during summer provides an adequate source to provide large numbers of eggs to last until a new collection of adults can be made the following year. The major

risk with such a strategy is that the sporadic nature of whitefringed weevil occurrence can make it difficult to locate infestations of adults.

Bioassays for determining the effect of toxins on organisms are notable for requiring large numbers of subjects to achieve adequate results. Considerable numbers of insects often need to be used in 'range-finding' the crucial dose range, followed by a large requirement to finalise a rigorous dose-mortality relationship. In all respects whitefringed weevil has highly desirable attributes for such bioassays as the ability to stockpile eggs and hatch them as required means that the system is not bottlenecked by the often seasonal constraints in the availability of insects.

The high toxicity of methyl isothiocyanate at a range of temperatures probably reflects the high volatility of this, the simplest, shortest-chain aliphatic isothiocyanate. The aromatic 2-phenylethyl isothiocyanate is known to be markedly less volatile. Nevertheless, the PeITC showed similar toxicity to whitefringed weevil larvae exposed to headspace vapour. This contrasts with results of Borek *et al.* (1995) who showed that PeITC was approximately 80 times more toxic than MITC to eggs of black vine weevil. However, that study was carried out as contact toxicity tests by dipping the eggs in solutions of the isothiocyanates. It was clear from that study that aromatic ITC's were intrinsically more toxic than the aliphatic types.

Studies of the toxicity of PeITC vapour to fungi was found to be markedly less than that of the aliphatic ITC's and was ascribed to the low volatility of PeITC (see Sarwar & Kirkegaard 'Biofumigation potential of brassicas III.', above). The interesting comparison of those fungal tests with these insect tests was that the insects were essentially as susceptible to PeITC as MITC on a solution concentration injected basis. Such a result, in the knowledge that PeITC has poor volatility, suggests that it is probably intrinsically much more toxic to whitefringed weevil larvae than is indicated by the results given here.

References

Borek V, Elberson L R, McCaffrey J P and Morra M J (1995) Toxicity of aliphatic and aromatic isothiocyanates to the eggs of the black vine weevil (Coleoptera, Curculionidae). J. Econ. Entomol. 88, 1192-1196.

Biofumigation potential of brassicas. V. Determining toxicity of volatiles from *Brassica* tissue to whitefringed weevil larvae.

John Matthiessen & Mark Shackleton

CSIRO Entomology, Private Bag PO Wembley, Western Australia 6014

Abstract

A method was developed to bioassay for determining the toxicity of volatiles emanating from hydrolysing *Brassica* tissue, using first instar whitefringed weevil larvae as the test organism. Larvae were placed in a 140 ml Erlenmeyer flask fitted with a stopper from which a glass vial containing the test material and water was suspended, exposing the insect to only volatile compounds resulting from the hydrolysis. The method was developed and tested using mustard and canola seed meal. The insects were exposed for 24 h at 15°C and then removed into clean containers and held at 20°C until mortality was assessed at 96 h post-exposure. Mustard meal produced highly toxic compounds, and had a steep dose-response; canola seed meal had no effect at doses ranging up to 200 times that giving an effect from mustard seed meal.

Introduction

The high degree of susceptibility of whitefringed weevil to the aliphatic isothiocyanate methyl ITC (MITC), and the aromatic variant 2-phenylethyl ITC (PeITC) has been established (see above). The results with MITC reinforce the findings of farmers that metham sodium is an effective control agent for whitefringed weevil. Together with the results for PeITC, they also give encouragement to the notion that isothiocyanate-producing plants could offer a means of suppressing whitefringed weevil populations in the field.

It is known that the concentration and type of glucosinolates, and hence the isothiocyanates that can be produced by their breakdown, varies between *Brassica* species and cultivars, within plant tissues, and is influenced by growing conditions (see above). In order to make appropriate judgements about the potential for biofumigation effects of various brassicas it is necessary to determine that they can produce toxic amounts of ITC's. There is also the need to assess the relative toxicity of different types and plant tissues as a basis for selecting types that indicate greatest potential for use in the field.

In order to make objective assessments of the biofumigation capability of different brassicas and tissue, it was necessary to develop a suitable *in vitro* bioassay technique to allow exposure of subject organisms to the toxins being released by the plant material. As it was considered that the likely toxic agents were volatile, the need was for a method that allowed exposure of the organisms to the vapours emanating from the material. Whitefringed weevil has been shown to be an excellent test organism (see above). The development of a suitable *in vitro* bioassay system would allow measurements to be made under a set of defined conditions, and allow the influence of such factors as temperature to be evaluated, without potentially confounding influences of soil type effects. The purpose of these studies was to develop and evaluate a system suitable for bioassay assessment of the toxicity of *Brassica* tissue.

Methods

Bioassay vessels

The containers for the bioassays were 140 ml Erlenmeyer glass flasks fitted with ground-glass Quick-Fit stoppers. In order to ensure that the test insects were exposed only to the vapours emanating from plant tissue, the test material was placed in a 8 ml glass vial that was suspended within the flask on a piece of stainless steel wire attached to a hook on the stopper. After placing the subject insects in the bottom of the flask, the test material was placed in the vial and placed into the flask immediately after activation of the hydrolysis process which results in

release of ITC's.

Bioassay method

Twenty whitefringed weevil larvae were used in each flask. There were typically five replications of each treatment and controls. Initial tests were conducted using cold-pressed seed meal of mustard (*Brassica juncea*), as previous studies had shown it to produce toxic compounds. These first tests were used to define suitable exposure times and determine likely amounts of material that would need to be fitted into the vials. As a comparison with mustard meal, cold-pressed canola meal was also tested.

Quantities of meal were weighed out and placed in the vials. Deionised water was then added to the material and the vials suspended in the flask. The flasks were held at a constant 15°C for 24 h. This temperature was chosen as being representative of soil temperatures in potato-growing areas during early spring when first instar whitefringed weevil larvae occur. After 24 h exposure, the flasks were opened and the vial containing the test material was withdrawn. After aeration in a fume cupboard the weevil larvae were removed from the flask and placed in small plastic vials. These were held at 20°C and the larvae were checked for mortality at 24 h intervals for up to seven days. Mortality in the treatments was corrected for any mortality in the controls using Abbott's formula.

Results

Checking of the larvae daily showed that mortality rose with time, generally plateauing around 96 h, so this was adopted as a standardised end-point for the assays. Mortality in the untreated controls was negligible during that period, because of the intrinsic longevity of the larvae (see above).

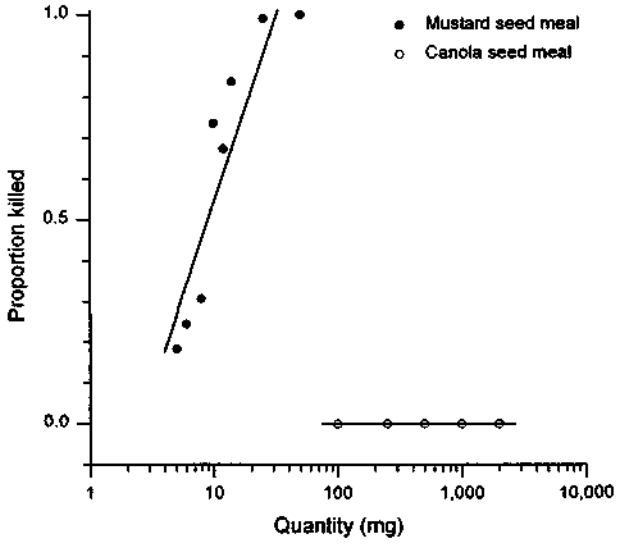

Figure 1. Toxicity of vapours from hydrolysing mustard and canola seed meal to first instar whitefringed weevil larvae.

The results of the test assays using both mustard seed meal and canola seed meal are shown in Fig. 1. Mustard seed meal released toxic vapours when present in very low amounts (milligram quantities). In contrast, it proved impossible to obtain any mortality of larvae from canola meal. Up to 2000 mg of canola meal was used, which reached the limits of the holding vial. The mortality response to increasing amounts of mustard seed meal was very steep, with mortality increasing from 50% to near 100% with a doubling of the amount from around 10 mg to little more than 20 mg (Fig. 1).

Discussion

A suitable technique for bioassaying volatiles released from hydrolysing *Brassica* plant tissue was developed and tested. It showed that mustard seed meal releases vapours highly toxic to whitefringed weevil larvae. Canola seed meal was ineffective, but this is of no surprise as canola is oilseed rape that has been specifically bred to have extremely low concentrations of isothiocyanate precursors in its seeds.

The technique will be used as a standard method for bioassaying to determine the toxicity of tissue from various brassicas in future studies. Such studies will be an essential part of selecting brassicas for biofumigation that have the greatest levels of biological activity. It is not yet possible to establish biological activity from the chemical analysis of *Brassica* tissue. The linking of the bioassay results with the chemical profiling will help in developing an understanding of which compound, or quite likely groups of compounds, give optimal activity. This will assist in the selection or breeding of superior biofumigation types.

INTEGRATED PEST MANAGEMENT

INTRODUCTION

Integrated Pest Management (IPM) is a concept for management of pests that seeks to utilise a diverse range of techniques and approaches to achieve control of pests or diseases without undue reliance on, or overuse of, chemical pesticides. It often seeks to combine, or integrate, chemical, biological and cultural control strategies in a package to achieve a pest management system that can be sustained in the longer term.

One of the key elements of IPM is to adequately understand the biology and ecology of the pest organism in order to identify critical parts of its life-system where control efforts are likely to be successful, and to enable pin-pointing of control strategies to the 'weakest links'. Very often there are subtleties in the biology of an organism that are only evident after detailed study, and these may be important pieces of information that allow a change or refinement of existing control tactics, or implementation of alternative methods.

IPM does not exclude the use of chemical pesticides. Rather, it embraces their use in the most efficient way possible in terms of impact on the target, while at the same time trying to minimise adverse impacts on beneficial organisms such as predators and parasites, and on the general biota, and adopting strategies that minimise the onset of such problems as resistance and enhanced biodegradation. It also seeks to utilise methods of applying pesticides that are least disruptive environmentally. Very often, it is important to adopt more 'rational' or better targetted use of chemical pesticides as the first element in the move towards adherence to the operative principles of IPM, or the establishment of a formal IPM program.

Traditional chemical methods for the control of soil insects are severe. They normally involve pre-planting incorporation of pesticides into the soil using such devices as structure-destroying rotary hoes. The approach of mixing the pesticide broadly through the soil suggests also that relatively large quantities need to be used.

The soil insect pests African black beetle and whitefringed weevil are particularly difficult to control because they occur patchily and they are damaging in low abundance. Biological controls do not seem feasible, as neither pest occurs at levels of abundance that appear sufficient to sustain natural agents, not does it appear likely that natural agents or biopesticides produced using current technology and marketing could suppress their populations adequately at economic cost.

The use of chemical pesticides to control soil pests and diseases is likely to remain a major method for some time. During this project particular attention was placed on seeking to improve the methods of chemical control for these two pest insects, through better understanding of their biology and population processes. The objective has been to develop control strategies and alternative approaches in the use of pesticides that are more focussed on the insect's population dynamics, or 'insect centric', rather than being related entirely to the cropping process.

Seasonally contrasting activity of African black beetle, *Heteronychus arator* (Coleoptera: Scarabaeidae): implications for pest status and management.

John Matthiessen[A] & Stewart Learmonth[B]

[A]CSIRO Entomology, Private Bag PO Wembley, Western Australia 6014
[B]Agriculture Western Australia, Manjimup, Western Australia, 6258

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Abstract

The relative levels of ground surface crawling and flight of the usually subterranean African black beetle, *Heteronychus arator* (Fabricius) were seasonally reversed. High surface activity relative to flight occurred in spring when beetles were mature, while the opposite occurred in autumn when they were immature. The sex ratio of beetles crawling on the surface in spring was male-dominated, but equivalently female-dominated in autumn. Flight, detected by light traps, was consistently female-dominated. The more active autumn flight was predominantly at an altitude greater than 1 m and, while appearing dispersive, was primarily localized to the pasture habitat. Some of the pest beetles flew into potato crops in autumn but the irrigated crops were not specifically attractive, as beetle abundance remained substantially less than in surrounding pasture. High surface activity in spring suggests that is the strategically optimal and least disruptive time for control measures to reduce the subsequent abundance of H. arator derived from resident breeding. Most of a population's seasonal cohort of eggs was laid by the time maximum surface activity occurred in mid-spring, making it imperative to target such adulticide control measures earlier to ensure maximum impact on the new generation's larval population.

Introduction

African black beetle, *Heteronychus arator* (Fabricius), is a pest of horticulture, pastures and turf in Australia, New Zealand and South Africa. It is univoltine and subterranean in all of its life-stages, but the adults fly actively at times. Captures in light traps indicate that flight activity predominantly occurs in autumn as sporadic massed events, with spring captures making up only a very small proportion of the annual total (Watson, 1979a,b). Newly-emerged adults occur in autumn, while those in spring are mature (Watson 1979a; Matthiessen & Ridsdill-Smith, 1991).

In south-western Australia *H. arator* is a particularly severe pest in the production of potatoes. Greatest risk of attack occurs in the crop planted in summer and harvested in late autumn-early winter. The conventional approach to controlling *H. arator* is to incorporate insecticide into the soil with a rotary hoe prior to planting the crop, in order to maximize the chances of the insecticide contacting the soil-dwelling insects. At that time of the year the *H. arator* population is comprised of late-instar larvae, pupae and newly-emerged adults in high abundance relative to that of their parental generation in the previous spring (Matthiessen & Ridsdill-Smith, 1991).

Matthiessen & Ridsdill-Smith (1991) showed a clearly-defined period in spring when oviposition occurred. Given that adults are subterranean, Learmonth & Matthiessen (1993) tested to what extent insecticide applied only to the surface of pasture prior to the commencement of oviposition, and with no physical or irrigation incorporation, could reduce subsequent abundance of *H. arator*. A single application of insecticide in late winter produced a large reduction in the larval population that persisted during spring and summer (Learmonth & Matthiessen, 1993). It suggested that adults were more accessible to surface insecticide treatment than had been considered possible.

Although the surface application of insecticide markedly reduced subsequent *H. arator* abundance, populations in the adjacent insecticide-treated and non-treated pasture areas returned to a uniform level in the following autumn, primarily through a large decline in abundance in the more heavily-infested non-treated areas, with a relatively smaller increase in the treated areas (Learmonth & Matthiessen, 1993). This suggested that the typical autumn

decline in abundance (Matthiessen & Ridsdill-Smith, 1991) may result from dispersal of newly-emerged adults (Matthiessen, 1993). While net reduction in areas of more successful breeding implied non-random movement, no attraction of adults to particular areas in the pasture such as moist patches with apparently more favourable perennial grass food were discernible (Matthiessen, 1993).

The only other major alternative habitat appeared to be potato crops that are grown under irrigation in the dry pasture during summer-autumn, but which form only a small portion of the total area. A small-scale study in two crops divided into insecticide-treated and untreated areas showed an upward trend in *H. arator* adult abundance during autumn when light traps recorded flights of *H. arator*. Density in the crops, however, remained less than in adjacent dry pasture (Matthiessen & Learmonth, 1995), suggesting that potato crops did not attract *H. arator* in unusually large numbers.

The association of autumn flight activity with the large decline in *H. arator* abundance in some areas, yet only small gains in others, such as in nearby insecticide-treated plots (Learmonth & Matthiessen, 1993) and potato crops (Matthiessen & Learmonth, 1995), implied that dispersive activity has some role in regulation of *H. arator* populations. Apart from the high levels of *H. arator* flight activity in autumn, the success of the late winter surface insecticide treatment in reducing subsequent abundance raised the question of whether adults show types of activity in spring that predispose them to management approaches that could be based on less toxic pesticides and less invasive methods than is currently practiced. The aim of this study was to examine the activity of *H. arator* adults throughout the year, to better determine the timing of key population events, how such factors may affect its populations and pest status, and whether such information could reveal alternative strategies helpful for its management in more benign ways.

Methods

Field studies were carried out in the Busselton (33° 39' S, 115° 21' E) district in south-western Australia. Soil sampling was used to assess *H. arator* population abundance. Light and window traps were used to measure flight, and pitfall traps measured surface activity. The seasonal pattern of oviposition was measured using a cohort of *H. arator* maintained under ambient conditions in the laboratory, and the rate of egg development with temperature was measured in controlled temperature cabinets.

Density estimates in pasture and potato crops

Estimates of the density of *H. arator* in pastures were obtained from 100 10 cm diameter x 15 cm deep soil core samples. The soil cores were broken up at the time they were taken and searched for the easily-seen large life-stages of *H. arator* that occur in the summer and autumn (Matthiessen & Ridsdill-Smith, 1991). In potato crops, the density of *H. arator* was estimated by searching the soil in 50 cm long portions of the hilled-up rows in which potatoes are grown (Matthiessen & Learmonth, 1993). The hills are typically 60 cm wide at the base, meaning each sample unit represented 0.3 m^2 of ground area, and 40 or 50 samples were taken on each occasion.

In one season, samples were taken on two farms in potato crops divided into two sections. One section had been treated with chlorpyrifos incorporated into the soil prior to planting to control *H. arator*, while the adjacent section was untreated (Matthiessen & Learmonth, 1995). During a second season, sampling was carried out in six commercial potato crops that had been treated with chlorpyrifos prior to planting. Pasture next to all crops was core sampled at the same time as the crops.

Light and window trapping

Light traps had as their light source a vertically-orientated 60 cm-long 20 watt fluorescent blacklight. The centre of the light was typically positioned approximately 1.5 m above ground. Four vertically-orientated 17.5 cm wide panels equi-radial from the light served as baffles to arrest the flight of insects attracted to the light, causing them to fall through a 21 cm diameter funnel below. The collecting container held an insecticidal vapour strip. A time switch controlled the operation of each trap, with the light being switched on daily from before sunset to after

sunrise.

Up to three light traps were operated simultaneously in different pasture locations in the district during various periods over several years. In addition, to determine *H. arator* flight activity in habitats other than pasture, one trap was placed 50 m from the waterline at the coast, in an open habitat several hundred metres from pasture, while another was located in forest about 200 m from the nearest pasture. The traps were cleared at intervals ranging from daily to one or two week intervals, with weekly being the most common. All insects caught were returned to the laboratory for separation, counting and sexing of *H. arator*.

At times, two light traps were operated in pasture on the same farm. One trap was positioned in the standard way described above, while the other trap was placed such that the bottom of the 60 cm light was at ground level, its collecting container fitting into a buried PVC pipe, from which the trap could be easily lifted for clearing the catch. A metal flange that served to brace the bottom of the interceptor panels was orientated upwards at 45° to reach 4.5 cm above ground at its outer edge. This ensured that beetles could not crawl directly into the collecting container, but were required to leave the ground slightly in order to enter the trap.

Window traps were made from sheets of clear perspex 123 cm high x 106 cm wide, mounted between two vertical timber frames. A 50 cm wide trough beneath the perspex sheet was filled with a water/detergent mixture to capture insects that collided with the perspex. One trap was mounted 1.5 m above ground at its bottom edge. The other was mounted with its bottom edge 10 cm above ground level, with the trough dug into the ground and surrounded by a 10 cm high vertical sheet metal barrier to prevent insects walking into the trough. The traps were orientated facing the prevailing wind.

Pitfall trapping

An initial type of pitfall trap, used only on one farm (site 1), was made from a 21 cm diameter plastic funnel fitted at ground level into a buried PVC cylinder. The collecting container was a 2 L plastic jar and 500 ml of a 1:1 mixture of ethylene glycol and water was used as a preservative. Mesh panels on the upper sides of the collecting jar allowed excess water from heavy rain to drain away.

Subsequent traps, used at all other sites, were made from a 10 cm diameter plastic funnel glued into the screw-top lid of a 250 ml plastic jar. These smaller traps (12.5 cm high compared to 34.5 cm for the larger trap type) had the advantage of being more easily placed in pasture by creating a hole with the 10 cm diameter corer used for estimating *H. arator* populations, and inserting the whole trap assembly. The small size also reduced the chance of damage from livestock. No preservative was used, and small holes in the collecting jar prevented flooding when it rained.

A preliminary period of pitfall trapping was carried out from February to July 1993 using ten of the large traps spaced at 10 m intervals. In August 1993, in preparation for a full year of trapping, 38 trap cylinders were buried in groups of four or five spaced 10 m apart at eight locations around a farm. At any one time, 20 of the 38 cylinders had traps installed for catching *H. arator*, while the others were covered. The 20 'active' traps were allocated at random to cylinders on each occasion that the traps were collected and reset. The intention of the trap rotation was to minimize the possibility of local extinctions from the long period of trapping because *H. arator* density is typically low (Matthiessen & Ridsdill-Smith, 1991). Collections were made usually weekly but up to three weekly when captures were very low. The trap contents were returned to the laboratory for separating, counting and sexing the *H. arator*.

When the small pitfall traps were used, typically ten traps were deployed, located 5 m apart in each of two lines 10 m apart. Captures were assessed usually weekly. Because of the absence of preservative, beetles were counted and sexed on site and live beetles were released. On occasions, the density of *H. arator* in the soil of the areas where pitfall traps were placed was estimated from 100 10 cm diameter x 15 cm deep soil cores uniformly spaced over an area 20 m wide x 100 m long, in which the grid of pitfall traps was centrally located.

Oviposition pattern and egg development

The pattern of oviposition by *H. arator* was determined by collecting from the field immature beetles in autumn and holding them as a group in boxes of soil in an outside laboratory at ambient temperature. Fresh carrots were provided as food. During winter the boxes were checked regularly, with dead beetles being discarded. Periodically, the beetles were shaken through a mixture of dry sand and sulphur to remove any mites. With the approach of spring, the boxes of soil were checked weekly for eggs which were counted. Dead beetles were discarded and the remaining beetles counted and sexed. This procedure was continued until all beetles had died.

For determining the temperature-specific duration of egg development, samples of eggs collected were placed individually on synthetic sponge moistened with 4% sodium hypochlorite solution as a fungistat, in the wells of microtitration plates and kept at constant temperatures ranging from 5-26°C. The eggs were checked frequently and the time taken to hatch was recorded. In order to estimate in conjunction with the laboratory development data the likely time for egg development in the field at different times, the temperature at 10 cm depth in what was considered to be an average loam soil for the district was measured periodically during field visits between winter and summer.

Results

Light, pitfall and window trapping

The captures of *H. arator* in light traps operated in various habitats in the Busselton area during the period February-June, 1993 are given in table 1. A consistently large number of beetles was caught in the three traps operated in pasture. Many fewer beetles were caught in the trap located in the open habitat at the coast, and very few in the trap located in the forest. At every site, almost all of the beetles captured between February and June in 1993 were trapped within a two week period in mid-April (table 1).

Table 1. Captures of *H. arator* in light traps at various locations in the Busselton area between February and June, 1993.

Site	Total no. beetles caught	% of catch in period 8-20 April
Pasture 1	13901	93
Pasture 2	12348	97
Pasture 3	10366	98
Coast	860	95
Forest	73	96

Figure 1 shows the mean number of *H. arator* captured in pitfall traps and standard above-ground light traps during 1993-1996. Captures are expressed as mean number caught per trap per day because the traps were serviced at varying intervals. Data from the initial set of ten large pitfall traps at site 1 during the first half of 1993 has been plotted overlaying the light trap captures during the same period at the nearest pasture light-trapping site, only 4 km away (site 3 in fig. 1, and pasture site 1 in table 1). From August 1993 to June 1994 the twenty large pitfall traps and a light trap were operated simultaneously at site 1. Captures in the small pitfall traps which were situated at site 2, approximately 15 km from site 1, are shown for comparison of phenology. In 1995-96, a light trap and pitfall traps were operated at farm 4 and pitfall traps at the neighbouring farm 2, less than 1 km distant.

In contrast to *H. arator* predominantly being captured in light traps during autumn, the greatest rate of captures in pitfall traps occurred in spring. Peak light trap captures were in April while pitfall traps caught most *H. arator* during October-November (fig. 1). Where pitfall traps were operated at two sites in the one season, a very similar

pattern in the time of activity occurred at both. In spring, there was a pattern of sharp increase in captures during September and rapid decline in December. The only deviation from this site/year congruence occurred in early spring 1995, with captures at site 4 increasing later than at site 2 (fig. 1). Notably, the area where the pitfall traps were located at site 4 was low-lying and partially waterlogged during winter and early spring. Captures in pitfall

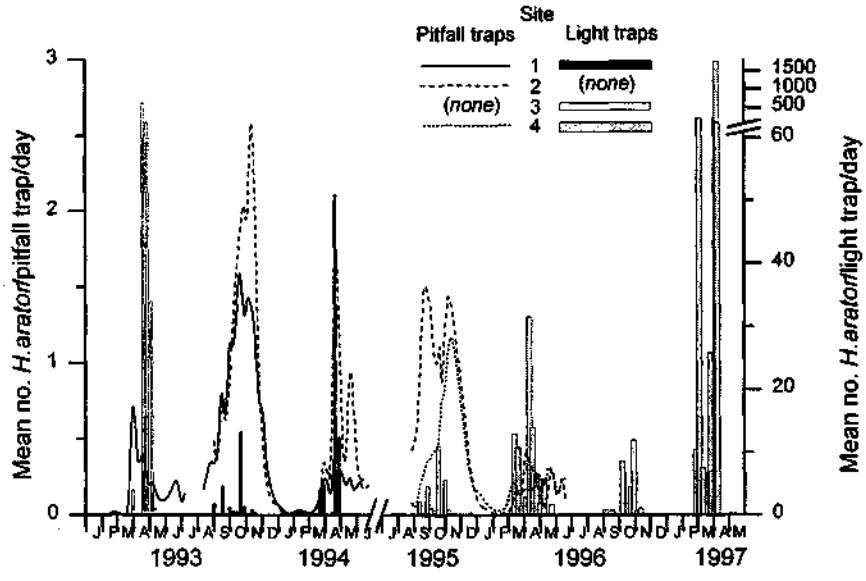

Figure 1. Mean numbers of *H. arator* caught in pitfall (left axis) and light traps (right axis) at several sites in the Busselton area during 1993-1996 (no traps were operated during July-June 1994-95). The pitfall traps at site 1 were the large type; all others were the small type (see text). A spline interpolation has been used for the pitfall trap data to enhance clarity.

traps occurred year-round, although at very low levels during January-February, the summer inter-generational interval (Matthiessen & Ridsdill-Smith, 1991). In contrast, light trap captures were confined to narrower periods in autumn and spring (fig. 1).

When data for all the traps used at the various sites and times were examined, two periods of opposite seasonal equivalence were common to all. These were periods of 78 days, from 6 September-23 November ('spring') and 6 March-23 May ('autumn'). Table 2 gives the total number of beetles caught in each type of trap in each 'season'. The data were analysed using a linear multinomial model with site and year as the dependent multinomial variables and season and position as classifying factors. Hypothesis tests were evaluated using the likelihood ratio (LR) criterion (Woodward *et al.*, 1990).

The tests showed that there were highly significant differences in capture probability for the light traps between spring and autumn (χ^2 too large to calculate, $p<0.001$, 2 df) and between the above ground and ground level positions ($\chi^2=26.8$, $p<0.001$, 2 df). For pitfall traps, more *H. arator* were captured in spring than in autumn ($\chi^2=10.1$, $p=0.017$, 3 df). There was a significant season x position interaction ($\chi^2=25.6$, $p<0.001$, 2 df), substantially higher numbers being caught in the above-ground light traps in autumn compared to spring. The window traps caught few *H. arator*. Although the ground-level trap caught more in both seasons, and both the ground-level and above-ground traps caught more in autumn than in spring, no significant differences could be

discerned between the capture probabilities.

Table 2. Mean total number of *H. arator* captured per pitfall trap and in light traps during two periods of 78 days, common to each site and year, and seasonally equivalent between spring (6 September-23 November) and autumn (6 March-23 May).

			Total no. *H. arator* per trap			
		Pitfall traps	Light traps		Window traps	
Site & years (as in fig. 1)	Season		Above ground	Ground level	Above ground	Ground level
1 (1993-94)	Spring	89	165	206	-	-
	Autumn	18	539	101	-	-
2 (1993-94)	Spring	121	-	-	-	-
	Autumn	49	-	-	-	-
2 (1995-96)	Spring	98	-	-	-	-
	Autumn	18	-	-	-	-
4 (1995-96)	Spring	39	197	420	6	27
	Autumn	15	659	365	21	54
4 (1996-97)	Spring	-	213	279	4	13
	Autumn	-	7025	4317	11	68

Figure 2 illustrates the relative captures of *H. arator* during each season in the above-ground and ground-level light traps, and in pitfall traps and window traps, on the same trapping occasions. During autumn captures in above-ground light traps were high relative to those in the pitfall traps compared to those in the ground-level light trap, and to both types of light traps in spring (fig. 2a). Light trap captures reflected rises in the number caught by similarly-placed window traps, although the relative increase of numbers in the ground-level light trap tended to be less than in its ground-level window trap counterpart in autumn (fig. 2b).

The numbers of *H. arator*, and their sex ratio, caught in pitfall traps and the two differently-elevated light traps is given in table 3 for each site and time traps were operated. Over all sites, males predominated in pitfall trap captures during spring, while females predominated at an approximately equivalent bias during autumn (table 3). Captures in light traps, irrespective of their elevation and season, were dominated around 2:1 by females (table 3).

Figure 3 shows pitfall trap captures in relation to the density of *H. arator* adults in the soil during spring and autumn, the data being combined from sites 2 and 4 in 1995/96. Captures in the pitfall traps were recorded on the day of core sampling. As that was the cumulative capture over the previous week, the capture on the next recording occasion one week later was also used for plotting against the core sample density estimate to give a balanced result either side of the core sample. Spring pitfall trap captures were substantially greater relative to density than those in autumn, and capture rates were generally similar in each of the two weeks either side of the coring occasion, indicated by the closeness of the two values at each density estimate (fig. 3).

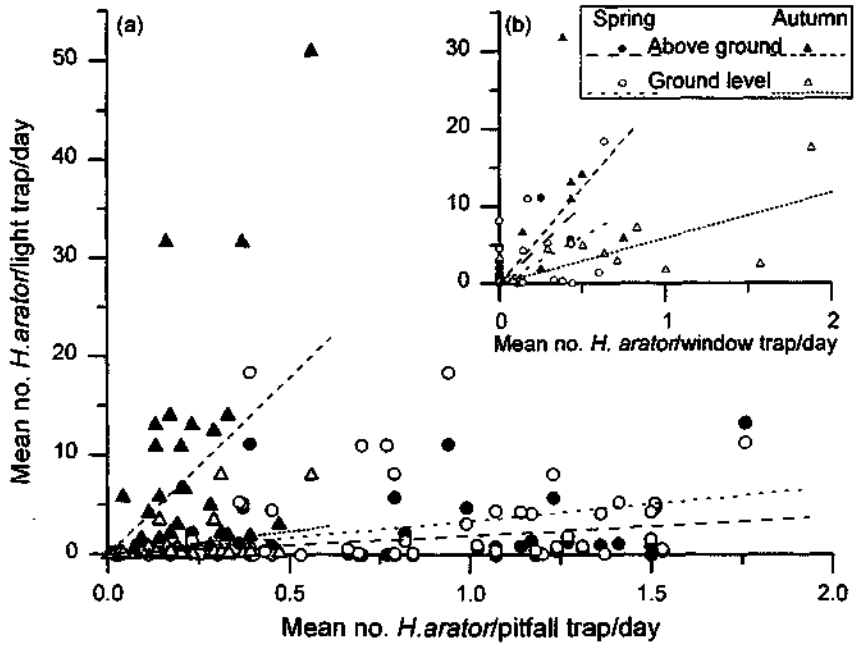

Figure 2. Captures of *H. arator* in ground-level and above-ground light traps relative to captures in pitfall traps (a) and window traps (b) on the same trapping occasion. Best-fit linear regression lines have been included to indicate trends.

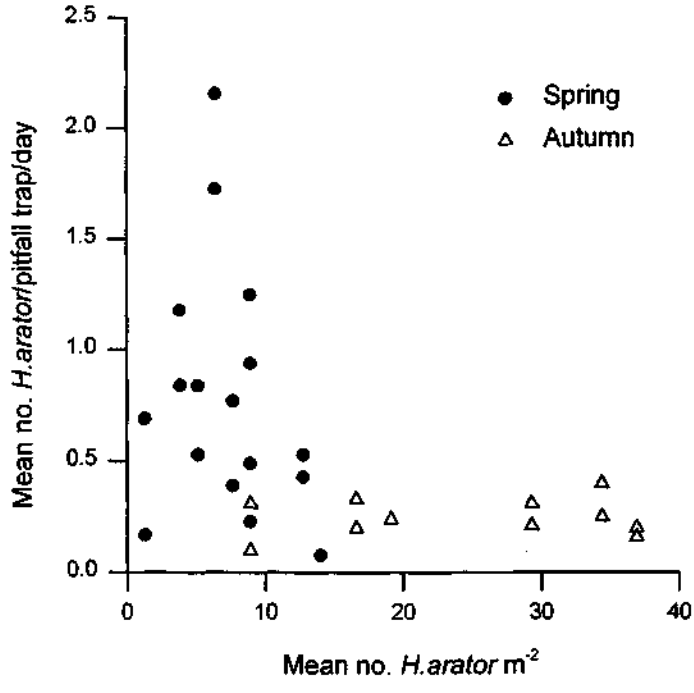

Figure 3. The rate of capture of *H. arator* in pitfall traps, relative to the species' estimated density in the adjacent soil, during spring and autumn. Average pitfall trap capture rate during the week preceding and the week following is shown for each estimate of density determined by core sampling.

Table 3. Total number and sex ratio of *H. arator* caught in pitfall traps and the two differently-placed light traps during spring and autumn.

Pitfall traps				
	Season			
Site & years (as in fig. 1)	Spring		Autumn	
	Total no.	Sex ratio (♂/♀)	Total no.	Sex ratio (♂/♀)
1 (1993-94)	2214	1.14	881	0.99
2 (1993-94)	1325	1.37	646	0.82
2 (1995-96)	1179	0.95	256	0.5
4 (1995-96)	646	1.45	243	0.86
Mean		1.23		0.79

Light traps								
	Above-ground				Ground-level			
	Spring		Autumn		Spring		Autumn	
	Total no.	Sex ratio (♂/♀)	Total no.	Sex ratio (♂/♀)	Total no.	Sex ratio (♂/♀)	Tota l no.	Sex ratio (♂/♀)
1 (1993-94)	178	0.34	542	0.63	218	0.31	321	0.34
4 (1995-96)	208	0.32	536	0.7	427	0.22	259	0.61
Mean		0.33		0.67		0.27		0.48

Oviposition and egg development

The 88 *H. arator* females held in the laboratory at ambient temperature in boxes of soil during spring, after being collected as reproductive immatures in autumn, laid a total of 1827 eggs, an average of 20.8 eggs/female. Oviposition commenced in late August and increased sharply during October (fig. 4). Half of all eggs had been laid by the end of the first week of October, and around 80% by the end of that month. Oviposition slowed during November and terminated with the death of the last females in early December (fig. 3). The rate of mortality of females was approximately constant during the oviposition period, with around 50% remaining in mid-October, while mortality of males was more rapid such that only 20% of the starting cohort remained at the same time (fig. 4).

The mean number of days for *H. arator* eggs to hatch under constant temperature in the laboratory ranged from about 80 days at 5°C to around 10 days at 26°C (fig. 5). Soil temperature in the field rose from around 13°C in July to 23°C in January (fig. 5). Greatest increase occurred during September-October, when average rainfall in the area diminishes rapidly and air temperatures begin to rise after winter (fig. 6). Combining the laboratory and field results produced an estimate of the time for *H. arator* eggs to hatch in the field of approximately 25 days in September, 18 days in October and 15 days in November (fig. 5).

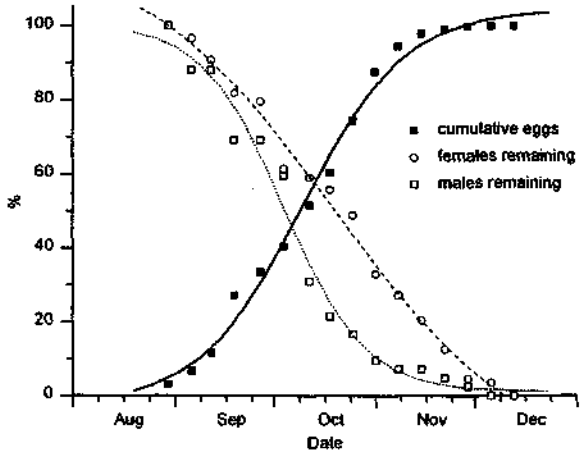

Figure 4. The timing of oviposition and cumulative percentage of eggs laid by a cohort of *H. arator* maintained in the laboratory at ambient temperature, and the survivorship of the male and female beetles in the cohort.

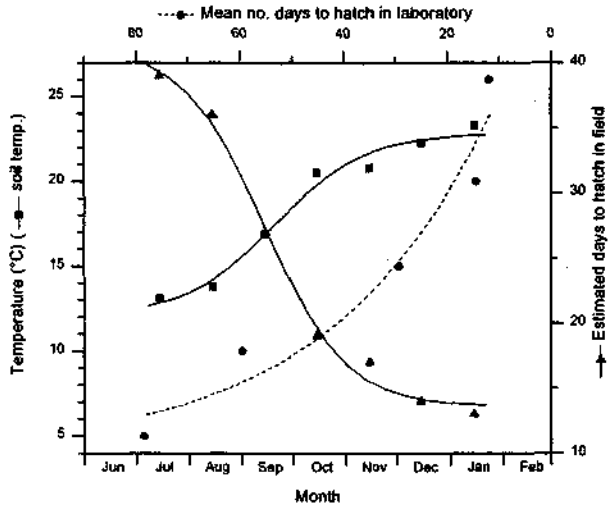

Figure 5. The mean number of days for *H. arator* eggs to hatch at different constant temperatures in the laboratory (top x-axis/left y-axis), the temperature of soil at 10 cm depth in the field from winter to summer (bottom x-axis/left y-axis), and from both of those an estimate of the time for eggs to hatch in the field at different times of the year (bottom x-axis/right y-axis). An exponential curve and sigmoidal curves are fitted to the egg development data, and the field temperature and time to hatch estimate, respectively.

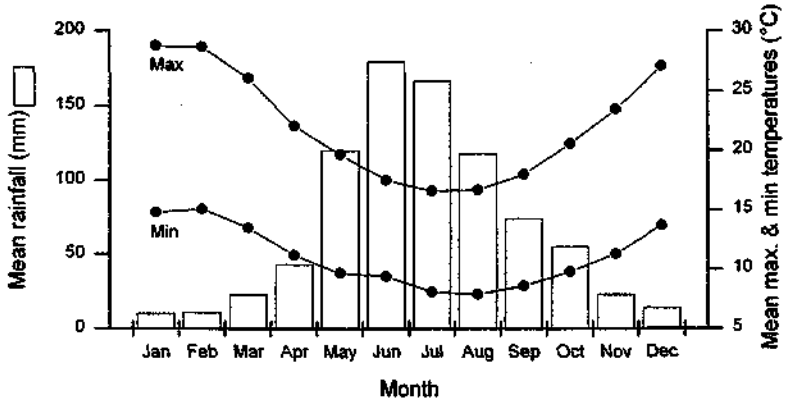

Figure 6. Long-term mean monthly rainfall and maximum and minimum temperatures at Busselton.

Densities in pasture and potato crops

The estimated density of *H. arator* in potato crops compared to adjacent undisturbed pasture during the late summer-early winter potato growing season at Busselton is shown in fig. 7(a). Temporal changes in the ratio of density in crops relative to the pasture over time are shown in fig. 7(b). With only one exception, the density of *H. arator* in potato crops was less than in the pasture, and generally substantially so (fig. 7(a)). In the exception, *H. arator* density was high at the beginning of the non insecticide-treated crop 2 of 1990 (fig. 7(a&b)). It subsequently fell (fig. 7(a)), reversing the ratio in favour of pasture at that site (fig. 7(b)).

The six crop/pasture combinations sampled in 1993 had very low densities of *H. arator* in the crops, ranging from 0 to only 1.4 *H. arator* $^{-2}$, despite densities in pasture ranging up to 38 beetles m^{-2} (fig. 7(a)). While the ratio of density between crop and pasture is subject to showing no change if the two densities simultaneously rise or fall, or could potentially increase because of decline in pasture rather than increase in the crop, there were no changes in the 1993 samples that caused the density of *H. arator* in the crops to approach that of the adjacent pasture (fig. 7). Although the six crops were all treated with insecticide prior to planting and during growth, four were not treated after the major flights of *H. arator* that occurred in mid-April (table 1).

More dynamic events were indicated in the 1990 season. The clear upward trend in the density ratio in untreated crop 1 (fig. 7(b)) primarily resulted from an increase in density in the crop (rising at each sample from 0.4 beetles m^{-2} in February to 6.6 beetles m^{-2} in May). The density ratio in insecticide-treated crop 1 indicated a generally similar upward trend over the season; the temporary fall in April resulting from a low collection of *H. arator* in the crop (fig. 7(b)).

At site 2 in 1990, the data were more variable in both crops and in the pasture. In the untreated crop the density of *H. arator* fell from 10 to 5.8 m^{-2} in the two weeks between the first and second samples, thereafter ranging narrowly between 3.7-4.4 beetles m^{-2} (fig. 7(b)). The density of the beetles in the treated crop varied little during the season, ranging between 0.9-2.5 *H. arator* m^{-2}, with no trend. In the pasture, however, the first and last samples of the season gave density estimates of 3.8 and 8.9 beetles m^{-2}, considerably less than the average of 20.4 m^{-2} for the three samples in the middle of the season. These low denominator values were therefore major contributors to the relatively high crop/pasture density ratios at the beginning and end of the season at site 2, although the major fall in density in the untreated crop between the first two samples also contributed substantially to the decline in the ratio (fig. 7(b)).

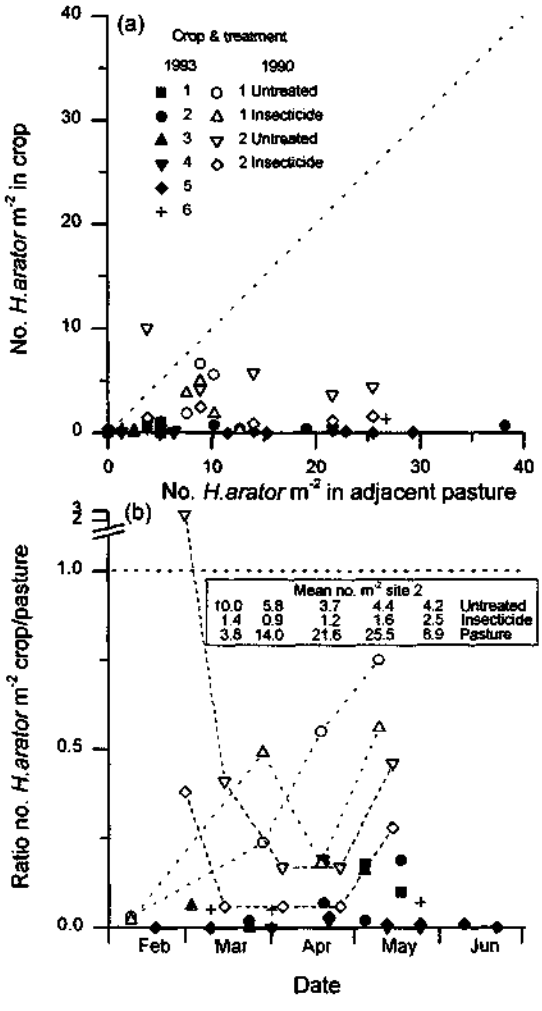

Figure 7. Density of *H. arator* in potato crops relative to density in adjacent undisturbed pasture, with 1:1 line for reference (a), and ratio of density in crop relative to pasture over time (b). The data for site 2 in 1990 are included to show the basis of swings in the crop/pasture ratio there.

Discussion

The seasonal contrast in flight and crawling activity by *H. arator* adults reveals the operation of two distinct behaviours. In autumn, when the beetles are most abundant and are newly-emerged and immature (Matthiessen & Ridsdill-Smith, 1991), high levels of capture in the above-ground light trap relative to the pitfall traps shows that the characteristic of beetles coming to the surface at that time of year was to either rapidly take flight or walk little during preparation for take-off. In contrast, high captures in pitfall traps during spring when beetles are present in low abundance and light trap catches were low reveal a high level of crawling activity on the surface of the ground, particularly by males, and relatively little flight.

It seems likely that the surface activity in spring is related to mate-seeking and oviposition. Moist soil and the

annual pasture plants being in growth then would provide generally favourable oviposition habitat throughout and little stimulus for the insects to disperse to other localities. Flight in spring is also likely to be inhibited by the reproductive maturity of *H. arator* at that time as generally more extensive flight in the Coleoptera is undertaken by immatures (Johnson, 1969). Crawling activity increased rapidly during September to peak in late October. By then, however, around 70-80% of the season's cohort of eggs had been laid and probably only around 50% of females and 20% of males remained, gauging from the cohort maintained under ambient conditions in the laboratory. Oviposition started in late August in the laboratory cohort, as occurs in field populations (Matthiessen & Ridsdill-Smith, 1991). By the time the median egg was laid in mid-October, time to hatch was estimated to be around 20 days, indicating that the majority of eggs would hatch during the first half of November, a considerable time after commencement of oviposition.

Lower captures during autumn in the low-level light trap compared to the elevated trap, and increased captures in the passive window traps at that time, give further indications that *H. arator* flight in autumn is highly active, with a tendency to exceed some minimum distance above the ground. Since the bottom of the high trap's 60 cm-long fluorescent light tube was only 60 cm higher, at 1.2 m above ground, than the top of the low trap's light, it appears that the beetle's activity once flying is to avoid being less than about one metre above the surface. In contrast to autumn, the close equivalence of captures in the different height light traps during spring and the lower numbers caught in both those and window traps suggests that spring flight activity is highly localized. It seems probable that light trap captures in spring are of beetles close to the traps exhibiting short bursts of flight, whereas those in autumn are of beetles that have fully taken to flight and are moving greater distances.

The predominance of captures of *H. arator* in light traps in autumn, and with the great majority occurring during the same short period in April, in south-western Australia fits with the general pattern of sporadic massed flights in autumn observed in New Zealand (Watson, 1979b). While the light traps in pasture caught very large numbers of beetles, those located away from pasture at the coast and in forest caught few. This suggests that the flight occurring in autumn, while at times highly active, is a comparatively small-scale dispersal. Simultaneous occurrence of the peak in captures in all habitats suggests that the most likely explanation for some catches in light traps located away from major areas of pasture is that they represent a wider dispersal of only a minor proportion of the beetles flying. Moreover, the very low captures in the forest trap, located only 200 m from pasture, indicate that forested areas are a habitat that is avoided by *H.arator*, or it greatly impedes the movement of beetles.

The coincidence of the highly active dispersive autumn flight activity with the decline in *H.arator* abundance in heavily-infested areas and increase in more lightly-infested areas (Learmonth & Matthiessen, 1993) indicates that dispersal has an influence on the regulation of populations on a localized scale. Whether dispersal in autumn is sufficient to solely account for the typical pattern of abundance in areas of inter-generational increase returning at that time to the level of the parental generation (Matthiessen & Ridsdill-Smith, 1991), and thereby filling a major mortality role, remains obscure without knowledge of survival in the late immature stages during that period. While Matthiessen (1993) found that the density of *H.arator* in field cages declined during the late immature stages in summer-autumn, few cadavers were found. For that reason, and because the decline in numbers coincided with adult emergence, it was not possible to discriminate with certainty *in situ* mortality from possible escape of new adults from the relatively shallow cages (Matthiessen, 1993). The most apparent characteristic of the autumn dispersal is that it contributes to restoring a more uniform abundance of *H. arator* across the landscape before the next spring breeding season. This appears to occur through a random redistribution of beetles from areas where breeding was successful to areas where it was poor, although in the process some individuals appear to become 'lost' to habitats that are likely to be unfavourable for breeding such as forest.

Light trap captures were consistently heavily biased in favour of females, with sex ratios around 0.3-0.6, a similar range to that reported by Watson (1979b) in New Zealand. In contrast, the sex ratio in pitfall traps was biased to males in spring, possibly reflecting mate-seeking behaviour, but equivalently biased to females in autumn. The greater occurrence of females in both pitfall and light traps in autumn indicate female *H. arator* may be more dispersive than males at that time of the year, although it is also possible that males are less attracted to light than females.

Despite the very large autumn captures of *H. arator* in light traps, two of which were located adjacent to potato crops, the abundance of beetles in crops was, with only a single exception, markedly less than in the surrounding pasture. The exception was at the beginning of a particularly heavily infested crop not treated with insecticide. While the six commercial crops were treated with insecticide, the fact that four were not treated after the major *H. arator* flight in the middle of April suggests that the observed pattern of consistently lower abundance in crops relative to pasture is not totally an artefact of insecticide application.

In terms of pest status of *H.arator* in high-value crops such as potatoes, the sampling of crops and pasture additional to the more limited earlier results noted by Matthiessen & Learmonth (1995) confirm that crops are not a disproportionate 'sink' for dispersing beetles. However, the extremely low threshold density for economic damage by *H.arator* in potatoes (Matthiessen & Learmonth, 1995) means that movement of any beetles into crops during their growth is highly undesirable. At present, the only means of countering such movement into crops is through application of insecticide, the targetting of which could probably be enhanced through use of light traps indicating the timing, and perhaps the magnitude, of major flights.

More positively, the new finding of high levels of surface crawling activity by *H.arator* in spring potentially opens an avenue for more efficient pre-cropping management of the species. It is now evident from the present study that the greater than expected success of spraying the ground surface with insecticide in late winter/early spring in reducing *H. arator* abundance (Learmonth & Matthiessen, 1993), with its advantages of being less costly and less destructive of soil structure, was most likely a consequence of beetles coming to the surface at that time to an extent not previously envisaged. The surface activity of *H. arator* is greatest in late October, suggesting that greatest mortality of adults may be achieved by applying insecticide then. However, most of the season's cohort of eggs were laid by that time and the aim of an adulticide spray should be to maximize the effect on the abundance of the subsequent larval population.

Since the immobile eggs are likely to be protected from a surface insecticide application, the information gained in this study suggests that an adulticide treatment would be best timed to precede as much of the season's oviposition as possible, while taking advantage of the likely greater vulnerability of the adults caused by their increasing surface activity, but which coincides with oviposition accelerating. Advantageously, the success of an application of insecticide to pasture in late August (Learmonth & Matthiessen, 1993), even before oviposition commences, indicates a high degree of vulnerability of adults well before their surface activity is maximal. It also implies that the beetles occur close to the soil surface in winter-spring. These factors together suggest that a potentially wide window of opportunity exists for the adulticide approach to management of *H. arator* in winter-spring, that it may be possible to use reduced quantities or 'softer' insecticides and that there could be a role for alternative treatments such as baits in reducing its populations.

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Surface activity of African black beetle in spring favours a pre-oviposition adulticide approach to its management.

John Matthiessen[A] & Stewart Learmonth[B]

[A]CSIRO Entomology, Private Bag PO, Wembley WA 6014.
[B]Agriculture Western Australia, Manjimup WA 6258.

Abstract

African black beetle, *Heteronychus arator*, is a widespread pest in pasture/horticulture rotations in non-arid regions of southern Australia. It is univoltine, with overwintering adults breeding during spring, and subterranean in all of its life-stages. Traditionally, control has been through use of soil-incorporated insecticides. In summer-planted horticultural crops the approach has been pre-planting treatment when larvae or newly-emerged adults occur. It has long been known that adults periodically fly actively during autumn. Recent studies have revealed that a high degree of surface crawling activity, but little flight, occurs during mating and ovipositing in spring. This activity pattern is reversed in autumn when newly-emerged adults occur. Peak surface activity occurs in late October, but by that time the great majority of the season's cohort of eggs has been laid. Insecticide applied to the surface of pasture as early as late winter, before oviposition commences, has the greatest impact and markedly reduces the subsequent larval population. Doses lower than incorporated by cultivation into pasture soils are effective and night spraying conferred no improvement in the level of population reduction. The effect is limited to a single season, as autumn dispersal results in re-colonisation of treated areas.

Introduction

African black beetle, *Heteronychus arator* (Fabricius), is a pest of horticulture, pastures and turf in Australia, New Zealand and South Africa. It is univoltine and subterranean in all of its life-stages, but the adults fly actively at times. Captures in light traps indicate that flight activity predominantly occurs in autumn as sporadic massed events, with spring captures making up only a very small proportion of the annual total (Watson, 1979a,b). Newly-emerged adults occur in autumn, while those in spring are mature (Watson 1979a; Matthiessen & Ridsdill-Smith, 1991).

The conventional approach to controlling African black beetle in potato production, for example, is to incorporate insecticide into the soil with a rotary hoe prior to planting the crop, in order to maximise the chances of the insecticide contacting the soil-dwelling insects. In turf, the approach has been to incorporate insecticide with irrigation. In all instances, control has been directed at the obligate soil-dwelling larval stage which occurs during spring and early summer (Matthiessen & Ridsdill-Smith, 1991).

Matthiessen & Ridsdill-Smith (1991) showed that oviposition occurred in spring, commencing in about the first week of September and ending in early December. Learmonth & Matthiessen (1993) tested to what extent insecticide applied only to the surface of pasture prior to the commencement of oviposition, and with no physical or irrigation incorporation, could reduce subsequent abundance of African black beetle, given that adults are subterranean. A single application of insecticide in late winter produced a very large reduction in the larval population that persisted during spring and summer (Learmonth & Matthiessen, 1993). It suggested that adults were more accessible to surface insecticide treatment than had been considered possible, and indicated that an adulticide approach to insecticidal management offered an effective alternative to treating larvae.

Adult activity patterns

To better understand the basis of the empirical effectiveness of the adulticide approach, the characteristics of African black beetle adult activity were further investigated with simultaneous light and pitfall trapping. It was found (Matthiessen & Learmonth, above) that a high degree of surface crawling activity, but little flight, occurs

during mating and ovipositing in spring. This relative activity pattern is reversed in autumn when newly-emerged adults occur. Peak surface activity occurs in October.

It appears that the surface crawling activity which dominates in spring is related to mating and oviposition that is occurring then as the resident populations are stimulated into activity by changing seasonal conditions. In autumn, it would seem that the more active flight activity is a dispersal mechanism. One purpose that dispersal activity serves is re-infestation of areas where breeding has been less successful because of higher than average immature mortality (Matthiessen, below). It also apparently contributes to re-infestation of insecticide-treated areas, meaning that the treatment effect of both the conventional and the spring adulticide approaches to management are lost the following autumn (Learmonth & Matthiessen, 1993).

Evaluation of the adulticide approach

Tests of insecticide treatments applied at various times in the spring to the surface of pasture in horticultural situations where potato cropping is rotated with pasture have been carried out to evaluate their effect on the abundance of the subsequent generation of African black beetle.

To determine the seasonal pattern of African black beetle oviposition, adults were collected in autumn and maintained in an outdoors laboratory at ambient temperature. Oviposition commenced in late August and continued until early December, with about 80% of eggs being laid by the end of October (Fig. 1).

The insecticide used was chlorpyrifos, as it is the currently-recommended agent for control of African black beetle in potato production in south-western Australia. The recommended quantity is 3 kg AI ha^{-1} incorporated with a rotary hoe prior to planting the summer potato crop in January. Application was made by boom spray to pasture that had been grazed short. Plot size was typically 100 m x 20 m.

Abundance of all stages of African black beetle was determined by soil core sampling prior to treatment and at times after treatment coinciding with the major population events of egg hatch, final instar larvae, newly-emerged adults and the stabilised population the following winter.

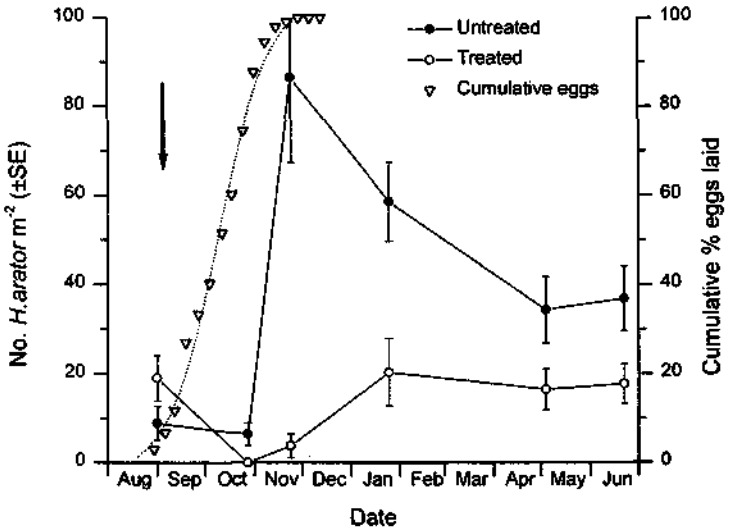

Figure 1. Seasonal pattern of African black beetle oviposition, and typical effect of a single application of chlorpyrifos at 3 kg AI ha^{-1} to the surface of pasture in early September on African black beetle abundance.

Figure 1 shows a typical example of the effect of a single application of insecticide to pasture in early September on the subsequent abundance of African black beetle. The pattern of oviposition has been included to illustrate the timing of the insecticide to precede it. There was a large reduction in abundance in the treated area, most apparent when first instars peaked in late November (Fig. 1). It is not possible to say whether the trend towards evenness in the abundances by late January represents survival of late-laid eggs or dispersal of newly-emerged adults into the treated area.

A summary of a range of adulticide tests carried out in pasture is given in Table 1. Mortality was estimated by comparing the abundance in the treated area with that in the untreated area on the occasion that peak numbers occurred. That was typically when first instars occurred in about late November (Fig. 1). The tests included halving the dose of chlorpyrifos and applying it at night. It was also applied twice, once in early September to precede oviposition and again in late October to coincide with peak crawling activity, at both the standard and half doses. Application was also made only in late October.

The data (Table 1) show a substantial impact of the pre-oviposition insecticide treatment at the normal dose of chlorpyrifos used for soil-incorporated control of African black beetle when large larvae are present in summer. They also suggest that half the quantity has a major effect, and that application at night does not confer a large advantage, nor does two applications. The major standout in the data was the much reduced effect of the insecticide applied in October when activity was greatest, but oviposition was almost complete (Table 1, Fig. 1). It would appear that chlorpyrifos applied to the surface has little effect on larvae.

Table 1. Estimated percentage mortality caused to African black beetle by various treatments of the surface of pasture with chlorpyrifos.

Treatment	No. tests	Mean %	Range
3 kg ha^{-1}, day	9	79	57-97
1.5 kg ha^{-1}, day	2	70	61-80
3 kg ha^{-1}, night	1	82	-
3 + 3 kg ha^{-1}, Sept. + Oct., day	2	88	87-89
1.5 + 1.5 kg ha^{-1}, Sept. + Oct., day	2	82	80-83
3 kg ha^{-1}, Oct., day	2	24	0-48

Conclusions

The alternative adulticide approach to suppression of African black beetle populations shows that advantage can be taken of the hitherto unsuspected high level of surface activity or near-surface presence of pre-reproductive African black beetle adults in late winter and early spring. It provides rational use of conventional chemical pesticides to reduce abundance of larvae in the next generation. Treatments applied much earlier in the year than is conventionally the case, at times when insect activity is not noticeable, were highly effective. It also appears that the adulticide approach offers prospects to reduce the quantities of pesticides used, and reduce the use of soil structure-destroying rotary hoes in horticulture. Overall, the adulticide method provides robust scope for management of African black beetle, the broad window of opportunity allowing it to fit flexibly with both production or management schedules and its capacity to take efficient advantage of the species' biology providing for use of alternatives in products or chemical groups.

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Soil factors influence reproductive success of African black beetle, *Heteronychus arator* (Coleoptera: Scarabaeidae).

John Matthiessen

CSIRO Entomology, Private Bag PO Wembley, Western Australia 6014

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Abstract

Field and laboratory studies showed that soil factors markedly influenced the survival and abundance of African black beetle, *Heteronychus arator* (Fabricius) in the Mediterranean-type climate region of south-western Australia. Survival was high in the early immature stages when soil is moist during spring. Mortality was greatest late in the immature stages, probably mainly in the pupal stage, which occur during the warm, dry summer. The mortality occurred in contrasting soil types despite not all drying equally during summer and supplementary watering in some plots. It is concluded that mortality in the late immature stages is the major factor limiting *H. arator* populations under a Mediterranean-type climate. Few soils could produce an inter-generation increase in their *H. arator* population. The variation in survival results in patchiness within pastures in their favourability to support *H. arator* breeding, which on average results in the consistent abundance typically observed between generations in south-western Australia.

Introduction

African black beetle, *Heteronychus arator* (Fabricius) originates in southern Africa, and is a soil-dwelling pest of horticulture, pastures and turf in Australia, New Zealand and South Africa. Studies of the biology and ecology of *H. arator* carried out in perennial pastures in New Zealand showed the major population-regulating factor to be mortality of young larvae in early summer, caused by their susceptibility to high soil moisture (King *et al.*, 1981b). If rainfall was lower and temperatures were warmer than usual in spring and early summer, breeding was more successful than average. A succession of such seasons amplified such success into periodic population outbreaks (King *et al.*, 1981c). Observational accounts in coastal regions of eastern Australia suggest that a similar pattern of periodic *H. arator* outbreaks is associated with drought, and that 'plagues' develop if a series of such seasons occurs (Wright, 1958). Like New Zealand, that region is characterised by summer rainfall and perennial pastures.

In contrast to New Zealand and east coastal Australia, south-western Australia has a Mediterranean-type climate characterised by a markedly defined dry summer and wet winter of great consistency. Pastures are composed of annual grasses and clovers that die in late spring. The region is considerably drier and warmer than New Zealand, particularly during the period of *H. arator* larval development in spring-summer. Population sampling has indicated that survival is high in the early immature stages (Matthiessen & Ridsdill-Smith, 1991; Matthiessen, 1993). Consequently, mortality in those stages did not appear to play the important regulatory role in *H. arator* population dynamics apparent in areas with a wetter spring or summer. Despite these apparently favourable conditions for high survival of early immatures, however, outbreaks of *H. arator* populations are neither a recorded nor anecdotal phenomenon in the region.

Contrary to New Zealand, the greatest losses from a cohort of *H. arator* in south-western Australia occurred late in the pre-adult development phase (Matthiessen & Ridsdill-Smith, 1991). The insects gradually disappeared between late final instar and adult, during the dry late summer and autumn, with abundance returning to a level similar to that in the previous spring (Matthiessen & Ridsdill-Smith, 1991; Matthiessen, 1993). Flight activity is high in autumn in the region (Matthiessen & Learmonth, 19xx) which suggested that the observed population decline could be related to dispersal of newly-emerged adults (Matthiessen, 1993).

King *et al.* (1981b) showed that high losses from populations could also occur in the late larval or early adult stages. They were primarily confined to perennial ryegrass (*Lolium perenne* L.)-based rather than paspalum (*Paspalum dilatatum* Poir)-based pastures. The losses were ascribed to ryegrass being a poorer adult host in autumn and winter, resulting in substantial dispersal and/or mortality which caused population decline then (King *et al.*, 1981b). It was not defined what component of the population decline in the autumn was true mortality *in situ* and what was the result of dispersal of adults. That study did, however, suggest the possibility that soil or plant factors could influence survival in the late immature stages.

Although dispersal of adults was also inferred to be a possible contributor to populations in localised areas returning to pre-breeding abundance by winter, there are no indications that dispersal results in high mortality of *H. arator*, as movement appears to be generally restricted to a relatively small scale within the pasture habitat (Matthiessen & Learmonth, 19xx). A possibility was that reproductive success was highly patchy and that high flight activity in autumn represented redistribution of *H. arator* from areas where breeding had been successful to where it had been poor. The large net loss from some areas, yet only a small gain in others, such as in areas of pasture treated with insecticide to reduce *H. arator* (Learmonth & Matthiessen, 1993), implied that the areas where breeding was successful were a relatively small part of the total area. A related possibility was that the reduction in abundance during the late immature stages was the result of high levels of mortality during those well-developed stages. There was no residual evidence of mortality in screen-covered field cages (Matthiessen, 1993), but it has been noticed that decomposition of cadavers is complete even for late instar larvae held individually in containers in the laboratory (D. Dall, CSIRO Entomology, Canberra pers. comm.).

The Mediterranean climate conditions of dryness and heat during summer would appear to be a potentially harsh environment for development of a soil insect at that time. In the field, the most obvious variable factor that seemed likely to impact on the success of breeding by *H. arator* was soil type, as pastures are generally uniform mixtures of annual grasses and legumes. The climate could be expected to affect drying and compaction of various soils differently, which may in turn alter the favourability of habitats for development and survival of *H. arator*. The aim of this study was to examine the reproductive success of *H. arator* under different soil conditions to better determine the levels of mortality in the immature stages and assess the impact of soil-related factors in the regulation of its populations.

Methods

Immature survival and development

Experimental studies aimed at determining the effect of soil type and moisture on survival and development of the immature stages of *H. arator* were carried out in the field and laboratory. Since the disturbance of soil removed from the field was likely to compromise the field applicability of laboratory experiments, but logistical difficulties prevented determining absolute *H. arator* survival values from placement of know numbers of larvae in the field, a complementary set of experiments was carried out in both the field and the laboratory. Field studies were undertaken in the Busselton (33° 39' S, 115° 21' E) district in south-western Australia, where *H. arator* is an agricultural pest. The *H. arator* for use in field cage experiments, and to produce hatchling larvae for use in laboratory survival experiments, were collected in autumn and maintained in boxes of soil in the laboratory at ambient temperature and fed on carrots.

Field experiments

Four areas of highly contrasting soil type (a fine sand, a sandy loam, a coarse sand and a clay loam) on the one farm were selected. The bulk density of each soil was measured in spring and again in mid summer from the dry weight of soil in a 10 cm diameter x 12 cm deep core. A hand-held penetrometer was used to measure the penetration resistance of the soil at the surface and at 15 cm depth at the same times. Organic matter content was determined in summer by ashing core samples of soil taken from the surface to 5 cm depth. Moisture content was measured every 2-4 weeks from spring-autumn at depths of 1-5, 10-15 and 20-25 cm by digging four trenches at each location and pushing a plastic vial into the wall of the trench at those depths. The vials were sealed and

returned to the laboratory for gravimetric determination of moisture content.

Following the commencement of the rainy season in autumn (May), twenty 30 cm diameter x 30 cm deep cylinders cut from heavy-duty PVC sewer pipe were hammered 25 cm into the ground at each soil type, using a mechanical vibrating plate compactor. After placement of these cylinders as field cages, the dead and newly-germinating pasture within each was removed and the soil checked for the presence of *H. arator*, which were removed. Disturbance of the soil was kept to a minimum by confining searching near to the surface. Each cage was sown with an equal quantity of annual ryegrass seed to standardise the pasture in each. The four areas were fenced to exclude livestock and left during winter for the grass to grow.

In late August, just prior to oviposition commencing in the laboratory-held *H. arator*, two pairs of those beetles were added to each field cage. Each cage was covered with a tall mesh cover to prevent escape of the beetles, while allowing the ryegrass to grow. Once the rain declined in late spring, half of the field cages at each soil type were watered weekly with approximately 30 mm water. The covers were removed in early December, when the cohort of *H. arator* remaining in the laboratory culture had completed oviposition and died.

Samples were taken twice, with half of the watered and unwatered cages at each soil type being removed at each time. At each sample the soil within the cages was sieved to extract the *H. arator*. The first sample was in early January, timed to coincide with the late larval stage. Each remaining cage was then covered with a mesh cover to prevent escape of emerging beetles. The second sample was in early April, coinciding with the adult stage. Recovered insects were separated to stage and counted. Third instar larvae in the first sample, and adults in the second sample, were oven-dried at 70°C to constant weight.

The characteristics of the naturally-occurring *H. arator* population adjacent to the field experiments in each of the four soil-type areas was assessed by digging the soil out of ten 45 cm square (0.2 m^2) areas to a depth of 30 cm. Each sample location was selected randomly by casting a quadrat. There were four sample occasions: early September to characterise the baseline population in each area at the time oviposition commences in the field, early January to coincide with the late larval stage, late March when newly-emerged adults occur but prior to the major flight activity period in April when re-distribution of local populations was likely to occur, and late July when populations have stabilised by virtue of low activity during winter (Matthiessen & Ridsdill-Smith, 1991). Insects recovered were assigned to stage, counted and their dry weight determined. Eight pitfall traps were also operated at each soil-type area, with captures being counted, sexed and dry weight determined.

Laboratory experiments

For the laboratory experiments, soil to approximately 20 cm depth was collected adjacent to each field experiment area in autumn. After removing plant matter and any *H. arator*, the soils were returned to the laboratory and each type mixed for uniformity before being placed in 35 cm diameter plastic plant pots . These had 1 mm brass mesh melted over the drain holes to prevent escape of *H. arator* that were to be added later. Each pot held approximately 12 L soil. Annual ryegrass seeds were then planted and the pots were placed in the open during winter.

Eggs collected from the laboratory culture of *H. arator* during spring were held individually on synthetic sponge moistened with 4% sodium hypochlorite solution as a fungistat in the wells of microtitre plates at 22°C until they hatched. As new larvae became available they were placed individually in a 10 mm deep x 6 mm diameter hole in the soil of each pot, which was gently squeezed closed, sequentially amongst all pots until each pot had 15 larvae. This occurred between late September and mid November. All pots were watered daily with an automated fine mist spray until mid December when half the pots in each soil type were removed from being watered.

In early January, coinciding with late-stage larvae, the soil in half the pots in each treatment was removed and sieved for *H. arator*. Mesh covers were placed over the remaining pots at that time to prevent escape of emerging adults. In early March, coinciding with the occurrence of adults, the remaining pots were emptied and the soil sieved to collect the *H. arator*. At each sampling occasion the insects were separated to stage, counted and their

dry weight was determined.

Results

Soil characteristics

Measurements of the characteristics of the soils at each location are given in table 1. The clay loam stood out as a low bulk density and high organic matter content soil, while the coarse sand was lowest in organic matter and generally of low compaction. Patterns of seasonal change in the physical characteristics of the soils were inconsistent.

Table 1. Characteristics of the soils at each location.

		Fine sand	Sandy loam	Coarse sand	Clay loam
Bulk density (g cc^{-1})	October	1.12	1.25	1.36	0.76
	February	1.32	1.23	1.19	0.76
Compaction - surface (kg cm^{-2})	October	15.5	15.1	11	14.5
	February	14.7	28.5	17.3	10.7
Compaction - 15 cm (kg cm^{-2})	October	17.8	12.3	6.8	10.6
	February	20.7	16	8.3	9.1
Organic matter (%)		10.6	8.7	5.1	26.2

The measurements of soil moisture content at three depths at each soil type location over the spring-autumn period of the field experiments is shown in figure 1, together with the monthly total rainfall at the site. There was considerable contrast in the moisture contents of the soils, with clay loam maintaining the highest moisture levels throughout the period while the coarse sand was consistently the driest. The fine sand and sandy loam had similar moisture levels about mid-way between the coarse sand and the clay loam (fig. 1).

Most soils showed a decline in moisture during November that tended to level out in December, despite the higher summer temperatures. This was probably a reflection of the low-lying nature of the landscape in the region. An abnormally high rainfall total in December was caused principally by one rainfall event, while the remainder of summer and early autumn was very dry (fig. 1).

Immature survival and development

Field and laboratory estimates of survival

The mean number of *H. arator* occurring in the field cages to which two pairs of beetles had been added in spring is shown in figure 2. In January mainly larvae were present, with 88% of all *H. arator* obtained being third instars, 10% being second instars and 2% pupae. There was large variation between the different soils in the number of *H. arator* obtained, ranging from about three per cage in the watered sandy loam to around 26 per cage in both dry and watered treatments of the fine sand (fig. 2a). Adding water produced an inconsistent effect, with decreased numbers in the sandy loam and increased numbers in the clay loam. The dry-treated coarse sand was the only situation in which second instars predominated (fig. 2b).

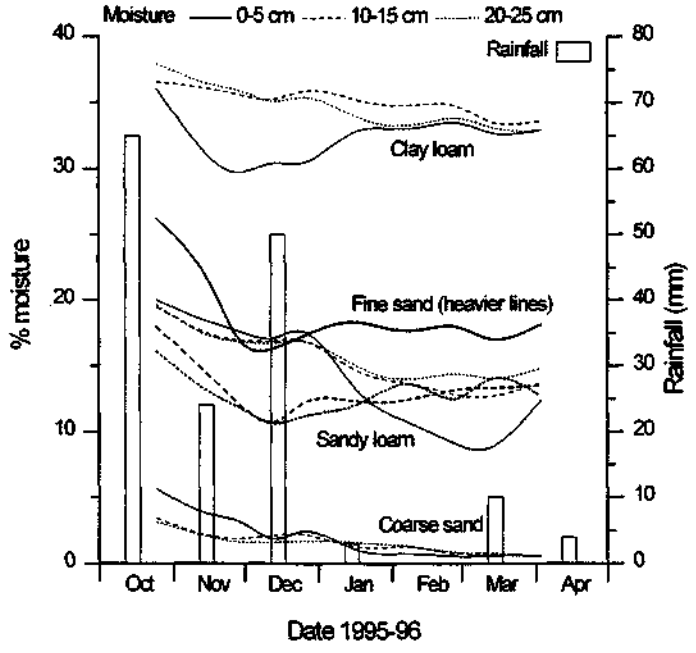

Figure 1. Changes in the moisture content of the different soil types at three different depths during the period of the field experiments, with the monthly total rainfall on the farm where the experiments were carried out.

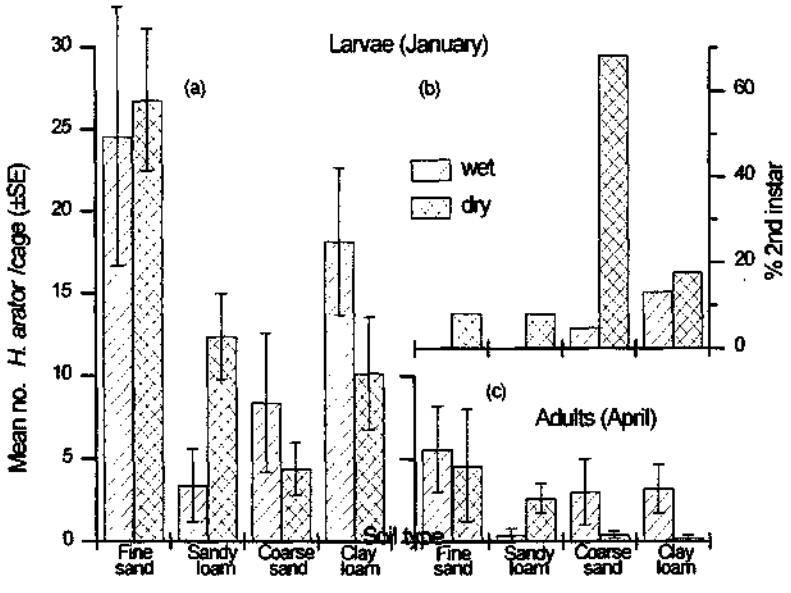

Figure 2. The mean number of *H. arator* obtained from field cages that were infested with two pairs of *H. arator* in September and allowed to dry normally or were watered weekly during summer, in the different soil type locations. Samples were taken in January when predominantly third instar larvae were present (a), except in the coarse sand which had mostly second instars (b), and in April when adults were present (c).

By April, the number of *H. arator* occurring in the field cages had declined (fig. 2c). When compared to the numbers present in January, the recovery in March represented mortality during that interval ranging from 31 to 100%, averaging 74% in the watered treatment and 94% in the dry treatment. In March, 98% of the *H. arator* in the mesh-covered cages were adults; the only exception was the only two individuals recovered from the five cages sampled in the dry-treatment coarse sand which were third instars. The effect of the watering treatment remained inconsistent (fig. 2c). No cadavers were found in the soil within any field cage.

Based on the fecundity of 20.8 eggs/female achieved by a cohort of *H. arator* held in the laboratory (Matthiessen & Learmonth, 19xx), and assuming a sex ratio of one, a survival of 9.6% from egg to adult would be required to maintain replacement levels between generations. At the January sampling of predominantly third instars, survival as a percentage of the laboratory potential ranged from 8-64%, with all treatments except the watered sandy loam being greater than 9.6%. By the April sampling, survival as a percentage of the laboratory potential ranged from 0.5-13%, with the only instances over 9.6% being both treatments of the fine sand.

In the laboratory experiment, the first set of pots was sampled in January when all *H. arator* were third instars. Survival of the 15 first instars added to each pot was generally low, at around 5-10% and reached a maximum of only a little over 40% in the watered coarse sand (fig. 3). Watering of the soil in the pots slightly enhanced survival or had no effect.

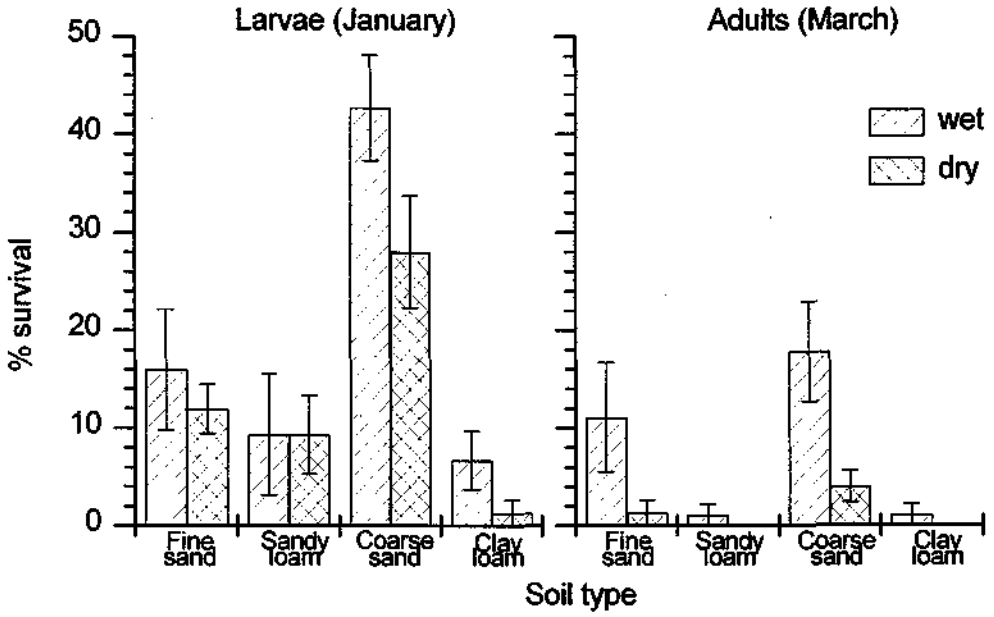

Figure 3. Survival of *H. arator* from known numbers of first instar larvae placed in pots of the contrasting soils in spring, with half being watered regularly. Samples were taken when third instar larvae were present in January (a) or when adults occurred in March (b).

When sampled in March, 91% of the *H. arator* recovered from the pots were adults, with 6% and 3% being third instars and pupae, respectively. Of those immatures, all occurred in the coarse sand and all the larvae were in the dry treatment. Survival diminished between the January and March samples, and ranged from zero in the dry sandy loam and clay loam treatments to approximately 18% in the watered coarse sand (fig. 2). When compared to the numbers present in January, the recovery in March represented mortality during the intervening period ranging from 33 to 100%, averaging 71% in the watered treatment and 94% in the dry treatment. These figures were very similar to the estimates of mortality during that period in the field experiments. As in the field cages, no cadavers were found in any pot.

Field population events

Changes in the density of *H. arator* in the soil at each soil type location, measured by quadrat sampling in spring, summer, autumn and winter are shown in figure 4. No *H. arator* were found in the coarse sand area. Density ranged around 1-10 m^{-2} in September at the other locations, and all had markedly increased by January when larvae were present. The increase ranged from just under 10-fold in the clay loam to around 30-fold in the fine sand and sandy loam (fig. 4). Between the occurrence of larvae in summer and new adults in autumn, abundance declined around 5-fold in each location. A further comparison of abundance between the summer peak and mid winter showed an overall decline of approximately 15-fold in the fine sand, 2-fold in the sandy loam and 8-fold in the clay loam (fig. 4).

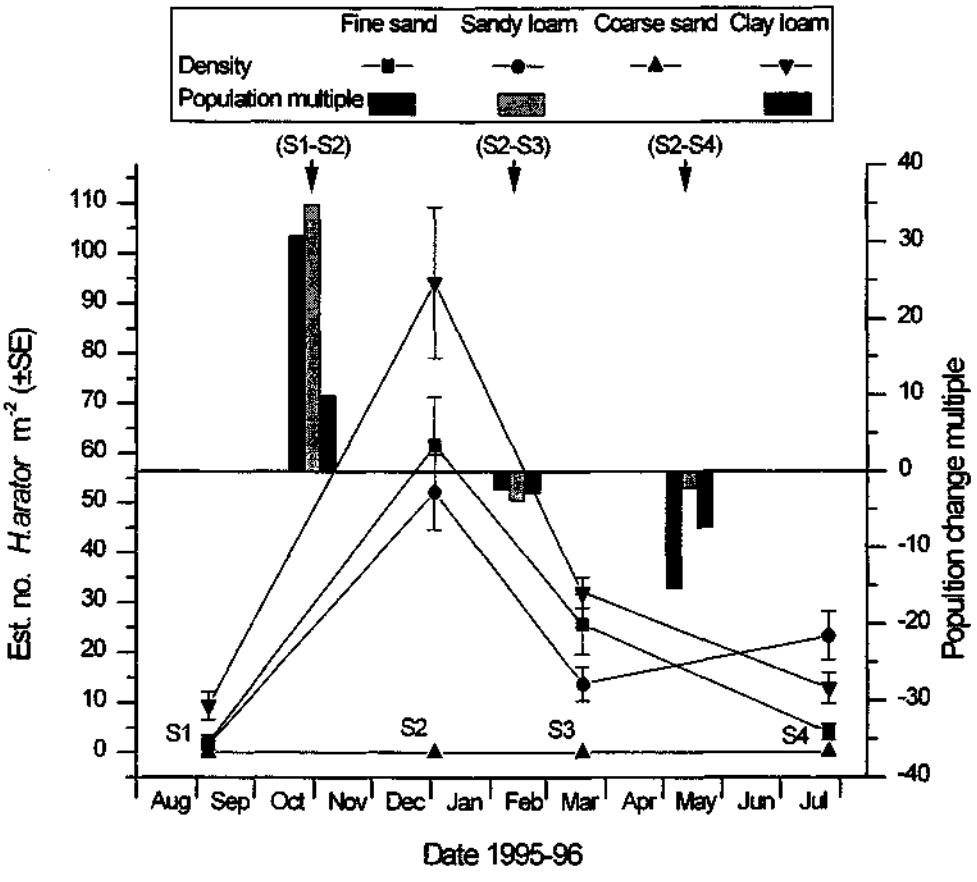

Figure 4. The estimated density of *H. arator* determined by quadrat sampling at four times in each soil type location, with the change in abundance being expressed as a population multiple to clarify the magnitude of the changes (note that the third set of bars is the change from sample 2 to 4).

Discussion

Rearing *H. arator* in soils of different characteristics caused substantial variation in the survival of immatures. However, differences in responses in the same soil type between the field and the laboratory experiments suggest that generalisations about the specific impact of particular soil factors or characteristics on the performance of *H. arator* populations in the field will be difficult to make, and probably subject to many confounding factors. Nevertheless, the information obtained in this study indicates that soil characteristics play a major role in the demographic performance of *H. arator* populations through effects on survival in the immature stages.

The laboratory studies suggested that mortality could be high in both the early and late immature stages. However, that indication tended not to be universally borne out in the sampling of the natural population in the field. In two of the soils, natural population increases between spring oviposition and summer occurrence of late instar larvae were estimated to reach approximately 30-fold. When compared to an estimated average fecundity in a bulk laboratory culture of 20.8 eggs/female (Matthiessen & Learmonth, 19xx), it indicated that very high levels of early immature survival occur in favourable situations, as had been inferred from sampling field populations (Matthiessen & Ridsdill-Smith, 1991).

Of greater relevance to the question of the stage at which most mortality occurs was the finding, in the three soil types where *H. arator* populations occurred, that the experimental results of consistently high mortality in the late immature stages were confirmed in the sampling of the field population. This occurred irrespective of instances of high mortality earlier in the laboratory experiments (probably the result of poor establishment of the first instar larvae, which appeared sensitive to handling), or low numbers of large larvae being present in the field experiment cages. In the case of the field experiments, where two pairs of *H. arator* were added to each cage, it is impossible to say whether low numbers of larvae resulted from low oviposition or high mortality early in the larval stage. It is highly unlikely that the large reduction in abundance of the field population resulted from dispersal of adults because the sample was taken in March, before the main flight period in April (Matthiessen & Learmonth, 19xx). Taken as a whole, the results strongly support the proposition that late immature stage mortality is the major factor limiting *H.arator* populations in the mediterranean-type environment in south-western Australia.

The factors responsible for such mortality can only be speculated about. Cadavers or chitinised body parts could never be found in the caged situations. This suggests highly active lysis by microbes was occurring, but whether such action was responsible for death, rather than physical factors such as desiccation is unknown. It does, however, seem unlikely that desiccation would cause high mortality of the well-developed final instar larvae that occurred in summer. The most evident effect of desiccation was in the very dry coarse sand, where development was slowed. Most larvae there were in the second instar in summer when third instars predominated in the other soils and in the watered coarse sand. Furthermore, the natural moisture content in the soils used in the present study extended across a wide range. This was particularly the case in the clay loam where high levels were maintained throughout the summer, but where late-stage mortality was also high.

The occurrence of highest survival of larvae in the laboratory pots in the coarse sand illustrates the difficulty of extrapolating such pot results to the field. It suggests that the better drainage likely to have occurred in that soil type when confined in pots was a critical factor affecting *H. arator*. In the field, survival was low and development markedly slowed in the coarse sand. Together with the absence of a field population in that area, it indicates that the coarse sand area was a very harsh environment for both the establishment and the persistence of an *H. arator* population.

Apart from particularly unfavourable soil type conditions such as the coarse sand, the data taken together show that mortality can be very high in the late immature stages. In the undisturbed field populations there was a clear indication that mortality reaches its highest level then. Only fecundity vastly greater than observed in laboratory culture would allow high levels of early-stage mortality to counter such a conclusion. The presence of third instars in high abundance, but adults in vastly lower abundance indicates that mortality was greatest after the final larval instar. It suggests that the pupa is the more vulnerable stage. The pupa appears less chitinised than the head capsule of a third instar larva, further suggesting that it could readily decompose without trace.

The occurrence of population-regulating mortality in the late immature stages in mediterranean-type environments means that *H. arator* populations have the capacity to reach their most damaging potential where the large larva is the main pest stage, such as in pastures and turf. Third instar larvae occur in about mid-December. By that time, however, the annual pastures characteristic of such regions have become senescent after reaching peak production in spring. While heavy infestations of larvae are often observed separating the shoots of pasture plants from their roots, there is no direct loss of pasture production from the mature plants, unlike in the perennial pastures of summer-rainfall areas such as New Zealand and eastern Australia. The only losses that can occur is if livestock dislodge or break up the mat of dry pasture to an extent that is destructive. The situation is different

in irrigated perennial turf, where mortality after the actively feeding third instar larval stage would have no value in minimising damage, at least within that season. *H. arator* is frequently the target of insecticidal control in turf, but not in pasture, in mediterranean climate regions.

In horticulture, such as potato production, the adult of *H. arator* is the most damaging stage (Matthiessen & Learmonth, 1993; Matthiessen & Learmonth, 1995). The comparatively low abundance of adults, however, offers no consolation to producers because the threshold density for economic damage is extremely low (Matthiessen & Learmonth, 1995), and prophylactic control using insecticide incorporated into the soil is commonplace.

The absence of reported or anecdotal outbreaks of *H. arator* in mediterranean climate regions, in contrast to summer-rainfall areas where climate-driven variation in the mortality of the early immature stages causes population fluctuations, indicates that the factors causing the late-stage mortality are highly consistent. The most characteristic attribute of the mediterranean-type climate is its consistency. While the amount of rainfall may vary annually, but usually not enormously, the pattern of receiving sufficient rainfall every winter is highly regular. This is no more the case than in south-western Australia, which has the most classically-defined mediterranean climate (highest proportion of annual rainfall in the winter months) on earth, especially in the higher-rainfall regions where *H. arator* predominates.

Ultimately, the regularity of the climate pattern may have a role in the consistent regulation of *H. arator* populations. It appears from the high mortality of late immature stages, which are not susceptible to climatic factors such as excessive wetness unlike hatchling larvae (King *et al.*, 1981b), that the key proximate factors may be biotic agents of remarkably consistent overall effect.

Acknowledgements

Mark Shackleton for technical assistance and Keith Rose for permission to carry out the field experiments on his farm.

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The origin of introduced whitefringed weevil populations.

John Matthiessen

CSIRO Entomology, Private Bag PO Wembley, Western Australia 6014

Background

Whitefringed weevil is an insect of South American origin that has been accidentally introduced into Australia, the USA and South Africa. In New Zealand, there has been considerable success in establishing parasitoids as natural enemies of Argentine stem weevil, also an accidental introduction from South America. During that program of research it was found that it was critical to match biotypes of the natural enemies to biotypes of the pest to achieve good establishment.

In a collaboration with New Zealand and United States researchers, we participated in studies aimed at establishing the genetic variation in whitefringed weevil populations in various regions with the aim of determining their likely regions of origin in South America, and similarity of the populations in different locations. The study was undertaken as a PhD study by Scott Hardwick at Ruakura research centre at Hamilton in New Zealand. He is currently writing up his thesis and this section aims to briefly summarise his results on the genetic variability of whitefringed weevil populations that he studied, with particular reference to the Australian populations that we and colleagues sampled.

Summary of results

Samples of whitefringed weevil adults were collected in several parts of South America, in a range of regions in Australia and New Zealand, and in North America and South Africa. The polymerase chain reaction-based randomly amplified polymorphic DNA (RAPD-PCR) technique was used to compare the genetic material from three species (*Naupactus* (formerly *Graphognathus*) *leucoloma*, *N. peregrinus*, *N. tucumanensis*) of whitefringed weevil, and 23 populations of *N. leucoloma*. Australian samples were collected from Manjimup in Western Australia, Mareeba in Queensland, Dervonport in Tasmania and Bannockburn in Victoria.

The three species were consistently distinguished from each other by the presence or absence of RAPD bands. It was possible to distinguish between six clones in the 23 populations sampled from Australia, North America, South Africa and South America. Several similarity groupings were evident. Queensland and Tasmanian populations were similar to those from New Zealand and South Africa, and most similar to Argentinian and Uraguayan populations in South America. The Western Australian population was similar to the North American and a local population at Ruakura in New Zealand, and most similar to the Chilean population in South America, while the Victorian population was least similar to the others, but the most similar to *N. peregrinus* and *N. tucumanensis*.

The results give a basis for any future investigations seeking potential biological control agents for whitefringed weevil to ensure that efforts are directed most appropriately in terms of obtaining well-adapted biotypes.

Thanks to Scott Hardwick for the summary of his results and to Harry Fay, Paul Horne and Lionel Hill for collecting whitefringed weevil in Queensland, Victoria and Tasmania, respectively.

Methods of application of metham sodium for whitefringed weevil management.

Stewart Learmonth

Agriculture Western Australia, Manjimup, Western Australia 6258

Aim

The use of metham sodium fumigant for the control of larvae of whitefringed weevil (WFW) in the Manjimup/Pemberton potato growing region of WA has increased over the past few seasons. This new approach to protection of potatoes has lead to the adoption of a range of methods for the incorporation of this fumigant into soil. Little information concerning the efficacy of these methods for this particular crop/pest combination was available.

The following series of experiments were undertaken to compare the relative efficiency of methods currently being used by potato farmers in the Manjimup/Pemberton region for applying metham sodium to protect potatoes from WFW attack.

Methods

Metham sodium is a soil fumigant with a broad spectrum of activity , having some effect on soil borne micro-organisms such as fungi, as well as nematodes, insects and plants. It has been applied using a range of methods - with irrigation water in light soil types; by dribble bar in front of some type of incorporation device such as a rotary hoe; by boom spray attached to the front side of a rotary hoe; at depth through nozzles either attached standard tines or to the tips of by duck-feet tines; by blade plough, whereby the chemical is pumped through nozzles placed evenly behind a v-shaped metal bar that is dragged through the soil at approximately 20 cm depth. As well as these methods of incorporation of metham sodium, soil may be rolled after treatment to slow the rate of escape of fumigant and so try to improve the efficacy of the treatment.

The most common methods of application in Western Australia are use of the rotary hoe, and injection through tines and the blade plough. These methods, some with and without rolling the soil after treatment, were compared for efficacy in controlling WFW larvae on four potato farms in the 1995/96 cropping season.

These farms were selected on the basis that some WFW larvae were present at the site prior to planting. Metham sodium was applied at least 2 weeks before planting to large unreplicated plots. The methods of application actually used on each farm varied and are shown on the graphs in the Results section below.

At crop maturity and before the crops were commercially harvested, ten plots measuring 10 m lengths of crop row were dug by fork and examined in a field shed for the presence of damage by soil insect pests. As well as damage by WFW larvae, some sites were infested by another soil insect that is a pest of potatoes in WA - African black beetle (ABB). While the damage caused by each pest is not always readily distinguishable, there are some differences that allow for a reasonable level of identification of the associated pest. Therefore, where ABB was observed to be present at the time of damage assessment, a record was made of tubers damaged by this pest also. Damage levels were based on weights of tubers and are a combination of shallow damage where tubers would be downgraded but still saleable at a lower grade, and where holes chewed by insects were so deep that the tubers would be rejected.

Results and Discussion

The percentage of tubers damaged by larvae of WFW and adults or larvae of ABB are shown in the figures below.

Over the four farms, the level of tuber damage by WFW or ABB in untreated plots ranged from just under 3% on Farm 2, through just over 3% on Farm 1, just over 6% on Farm 4, to around 14% on Farm 3. For the purpose of comparing treatments, only the last level is high enough to make meaningful comparisons. From experience, the patchiness of the distribution of whitefringed weevil in pasture in this potato growing region makes comparisons less meaningful unless high levels of tuber damage are encountered. Nevertheless, the results can be examined with this restriction.

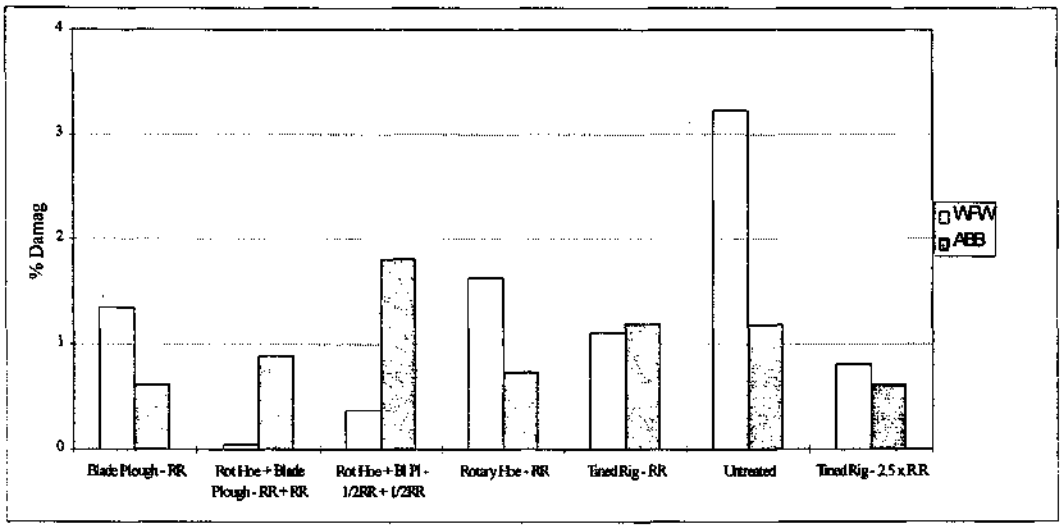


Figure 1. Metham sodium methods of application and percent tuber damage by WFW and ABB - farm 1. RR: recommended rate of metham sodium product per hectare; Rot. Hoe: rotary hoe.

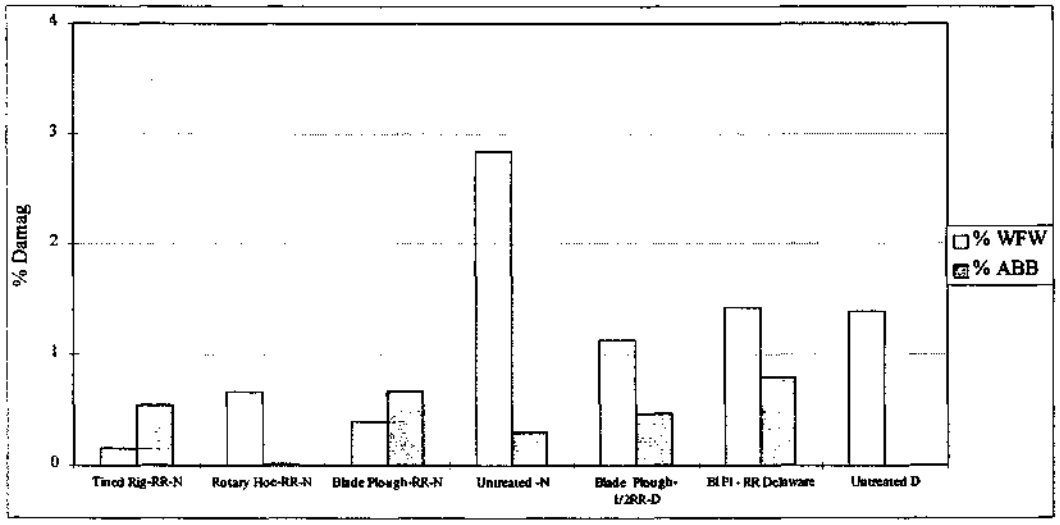


Figure 2. Metham sodium methods of application and percent tuber damage by WFW and ABB - farm 2. RR: recommended rate of 500l metham sodium product per hectare; N: potato variety Nadine; Untr: untreated; D: potato variety Delaware; Bl Pl: blade plough.

On all but one farm where use of the blade plough had no apparent effect on tuber damage, all methods of application methods for metham sodium reduced the level of tuber damage by the soil insects (see graphs and Table

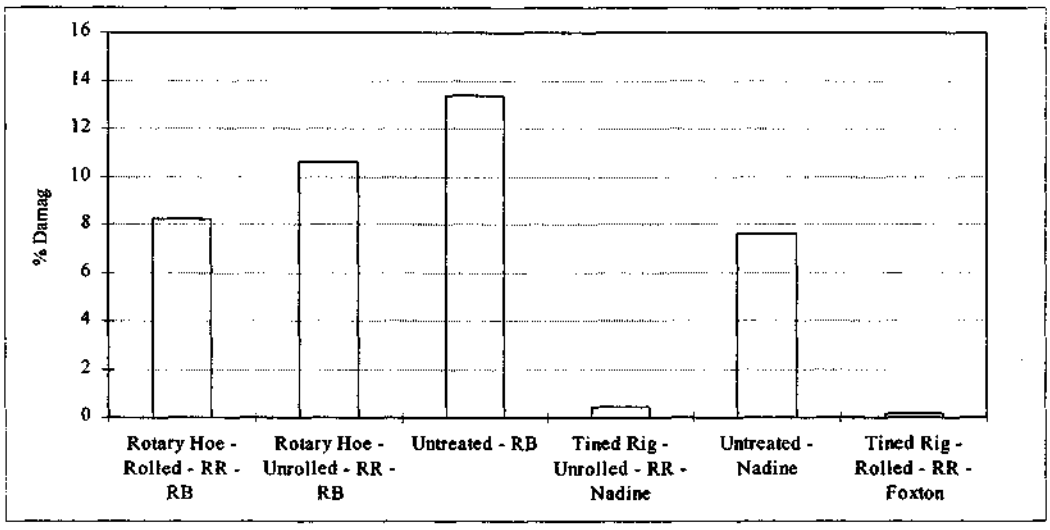


Figure 3. Metham sodium methods of application and percent tuber damage by WFW - farm 3. RR: recommended rte of 500l metham sodium product per hectare; RB; potato variety Russet Burbank.

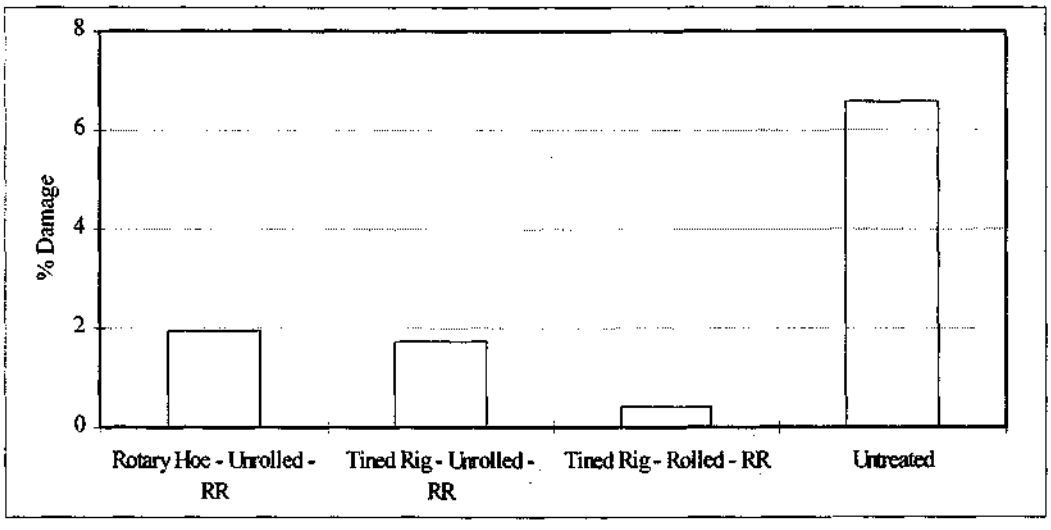


Figure 4. Metham sodium methods of application and percent tuber damage by WFW - farm 4. RR: recommended rate of 500l metham sodium per hectare.

1). On Farm 1, where metham sodium was applied as a combination of two methods of application, the level of control of WFW was improved. Rolling soil after the incorporation of the pesticide appeared to give only slightly improved control - see results for Farms 3 and 4.

Interpretation of the effect of the treatments on level of damage by ABB is not considered here because of the possibility of immigration of flying adults after the application of the metham sodium. This soil pesticide has a short duration of activity, probably of the order of 10 days. After this time, beetles that fly into the treated area would probably not be killed. The effect of the metham sodium is against resident pests.

This investigation was undertaken without replication of treated areas. This must be seen as a deficiency in conducting this type of research for these insect pests. The decision was made that there was a high risk of selecting a farm with an apparent heavy infestation of WFW and going to the expense of replicating the treatments, only to find that untreated plots were lightly infested - such is the vagaries of this soil pest.

Table 1. The level of tuber damage by WFW expressed as a percentage of damage to untreated plots on four farms with various methods of application of metham sodium.

Method of metham sodium application	Farm and WFW damage as % of untreated				
	1	2	2	3	4
Blade plough - RR	42	14	103	-	-
Rot hoe + blade plough - RR + RR	2	-	-	-	-
Rot hoe + Bl Pl - ½RR + ½RR	11	-	-	-	-
Rotary hoe - RR	50	23	-	79	30
Tined rig - RR	34	6	-	6	26
Tined rig - 2.5 x RR	25	-	-	-	-
Blade plough - ½ RR - D		81	-	-	-
Tined rig - rolled - RR	-	-	-	2	6
Rotary hoe - rolled - RR	-	-	-	61	-

This result where the level of damage to potatoes was really too variable to provide a more rigorous interpretation of the data, led to the experiment on assessing risk of WFW damage to potato crops (see below). There is a need to locate farms with heavy and uniform infestations of WFW in order to assess application methods where replication of treatments can be reliably made with confidence that levels of damage in untreated plots of the order of 10% or more will occur.

In the meantime, discussions with farmers applying metham sodium indicate that application by all methods examined here provide an acceptable level of weevil control. Other factors such as maintaining good soil structure by using application methods other than the potentially destructive method of rotary hoeing are important in deciding which method is selected. The effect of metham sodium on the operator is also taken into account - using tined implements and a blade plough which place the pesticide directly into the soil, minimising the level of gaseous by-products at the time of application, has lead to the selection of these methods by many potato growers.

Effect of cultivation on vertical distribution of whitefringed weevil larvae in soil.

Stewart Learmonth

Agriculture Western Australia, Manjimup, Western Australia 6258

Aim

Whitefringed weevil is an important pest of potatoes in the Manjimup/Pemberton potato growing region of WA. For control, the soil fumigant metham sodium is the main pesticide currently used. It is a more reliable pesticide than the registered insecticide chlorpyrifos, but even with metham sodium, some damage to potato tubers by larvae still occurs.

While metham sodium is a fumigant, current knowledge of it suggests that when the product is incorporated in soil, there is no downward movement of active constituent beyond the depth to which it is applied. Because of this activity, it is further suggested that one deficiency in its effectiveness is that at the time it is applied, some whitefringed weevil larvae may occur below the depth to which the metham sodium is incorporated.

If information were available on the depth to which whitefringed weevil larvae occur for different methods of land preparation prior to growing potatoes, the nature of the metham sodium application may be altered to enhance its effectiveness in controlling weevil larvae.

The aim of this investigation was to determine the effect of different methods of land preparation for potato cropping on the vertical distribution of whitefringed weevil larvae in soil.

Methods

On each of two commercial potato farms currently under pasture, the vertical distribution of whitefringed weevil larvae in soil was to be measured using a specially designed auger. The auger was attached to a mechanised tractor mounted post-hole digger and consisted of an internal PVC tube approx. 100 mm diameter and 350 mm long that had been cut into two equal halves lengthwise. The two halves of the PVC tube were taped together using thin paper tape to form a cylinder. This cylinder was held in the auger by a flange near the cutting tip of the auger.

As the auger was screwed into the soil to the depth of approx. 350 mm, the soil core was collected inside the PVC tube. After the auger was drilled to the required depth, it was removed from the soil and the PVC tube recovered. The tape holding the two halves of the PVC cylinder were cut, exposing the soil core. The soil core and one half of the PVC were laid in a cradle where different lengths (equating to different depths of the soil profile) were cut off from the core of soil and placed into plastic bags for subsequent extraction of larvae. The depths to which the soil core was divided were: the four top 50 mm sections and the rest - approximately 150 mm.

Larvae were extracted from the soil by a wash/float process, with subsequent microscopic examination for young larvae not easily seen with the naked eye.

Such measurements to determine the effect of cultivation on the depth distribution of WFW larvae were to be made in soil receiving the following cultivations:

1. no cultivation; 2. cultivated with a mouldboard plough; 3. Cultivated with an agro-plough or tined rig.

In Early July, the potential trial sites were examined using a spade to confirm a reasonable density of whitefringed weevil existed, based on presence of medium to large sized larvae as an indicator of the presence of the main

generation of the difficult to detect small larvae.

In each block, the following soil sampling regime was to be:
(a) mid July (before cultivation)
(b) late August
(c) late September
(d) late October
(e) early January - dry examination only, depending on stage of larvae in late October
(f) early March - dry examination only, depending on stage of larvae in early January

On each sampling occasion, except where indicated otherwise, the density of whitefringed weevil larvae was to be estimated by taking 30 soil cores using the auger described above, each core to be separated into five components as already described and each of these was sorted for whitefringed weevil larvae.

Results

Farms where this sampling was undertaken were infested only with very low densities of whitefringed weevil larvae. The activity was aborted because of time constraints in finding alternative more heavily infested trial sites soon enough such that the study would not be compromised by the target insect developing beyond the life stages of most relevance. The time consuming nature of the sample collection and processing was another problem in completing this investigation, given that other experiments had been planned.

The main achievement from this study was the development of a depth sampler capable of providing reasonably accurate estimates of the vertical distribution of insects down a soil profile. It was a very time consuming process to collect the soil and it would be an advantage to mechanise this aspect of the sampling before future efforts are made to complete this investigation. There are no plans to repeat this study in the near future.

Monitoring whitefringed weevil adults in pasture to indicate risk of infestation in following potatoes.

Stewart Learmonth

Agriculture Western Australia, Manjimup, Western Australia 6258

Aim

Whitefringed weevil is an important pest of potatoes in the Manjimup/Pemberton potato growing region of WA. For control, the soil fumigant metham sodium is the main pesticide currently used. It is a more reliable pesticide than the registered insecticide chlorpyrifos, but at a cost of around $700/ha, is approximately five times the cost of chlorpyrifos.

Despite this cost disadvantage, the reliability of control of whitefringed weevil with metham sodium and its broad spectrum activity in controlling other pests and diseases, have lead to the widespread use of it.

With respect to control of whitefringed weevil, there is a great deal of uncertainty as to whether a particular paddock to be used to grow potatoes will be infested with the pest at a level requiring the use of metham sodium. This has been demonstrated by Agriculture WA in trying to locate trial sites in paddocks with a history to suggest they would be heavily infested only to find they are virtually pest free. Also, at the other extreme, some growers have had crops destroyed by weevil larvae because they thought a particular paddock was not infested.

The reason for these inconsistencies is not readily apparent. Also, to undertake soil sampling to assess a risk level of a particular paddock would be prohibitively expensive using the current larva extraction method. However, one stage of the weevil that is detected relatively inexpensively is the adult stage.

Adult whitefringed weevil are large insects at approximately 20 mm long and can be reasonably easily detected. They feed on a range of broadleaved food plants, and, during the dry WA summer, are often found clustered at what are a few green plants in an otherwise inhospitable environment. If the abundance of such adults could be used as an indicator of the subsequent abundance of larvae, it would provide a reliable and relatively easy means to base decisions on whether to apply metham sodium.

The aim of this investigation was to determine whether the abundance of whitefringed weevil adults in pasture can be used to indicate whether a following potato crop requires soil pesticide treatment.

Methods

Eleven commercial properties used for potato production in the Manjimup / Pemberton region were selected in early summer of 1995 on the basis that they were to be planted to potatoes in the subsequent 1996/97 cropping season. On each of these farms, adult WFW were monitored to determine the abundance of whitefringed weevil adults.

The abundance of weevil adults in selected portions of the paddocks of approx. 0.5 to 0.75ha each, was assessed using three methods:

(a) **Time searches** were undertaken in each paddock of each farm for 30 minutes each fortnight starting in October and specifically checking green broadleaved plants during summer, which act as refuges for adults as the pasture dries off. Also, checks were made under objects for sheltering adults. Records were made of live and dead adults, the latter still being intact and obviously from the current season's emergence (presence of antennae was a good indicator of this). Some of the search time was spent checking the dry pasture as well. After the break of the season and with the germination of clover, the search was more random;

(b) **Pitfall traps**, which were plastic funnels 100 mm diameter sunk to be level with the soil surface and coated with Fluon, were installed along four transects, each transect with 5 traps and adjacent traps 15m apart. End traps were 20 m from the edge of the paddock and the edge of the plot used for the investigation. Transects were 20 to 40 m apart. Pitfall traps were 15 m apart along a transect. Traps were also checked fortnightly and live and adult weevils recorded;

(c) Using a **30 cm square quadrat**, weevil abundance was also assessed on an area basis, using a systematic sampling method of transects with adjacent quadrat positions being 8 m apart along a transect, with transects being approx. 25 m apart. Starting points for transects on each sampling occasion were selected randomly. Live and dead adults were also recorded using this method.

In order to determine the age and reproductive status of weevils being found, a maximum of 20 adults were dissected each fortnight. The weevils for this were collected from one farm where weevils were abundant, but these weevils not from within the plot area for testing so as to not affect the subsequent weevil abundance in that area. The presence of mature eggs in weevils was noted.

On only six of the farms initially sampled for weevil abundance, potatoes were subsequently planted. On these farms, the farmer was requested to leave certain areas untreated. Areas left untreated consisted of alternating treated and untreated plots to give approx. five replications of each. Each plot was approx. 5 m wide and approx. 80 m long.

At crop maturity for each of the six farms, 10 x 5 m of crop row sampling units were dug by fork from each of the treated and untreated plots and examined for both the presence of insect damage and surface diseases of tubers.

Results

The abundance of adult WFW on each of the eleven farms as recorded in the time searches, pitfall traps and quadrat sampling is shown in the following graphs (see Fig. 1). The greatest numbers of weevils were recorded in the time searches, with considerably fewer weevils being found in either the pitfall traps or the quadrats. The comparative abundance of weevils on the eleven farms varied considerably - from no weevils to the situation on Farm 1 where weevils were readily found on most sampling occasions. Emergence of WFW adults commenced in late December to early January and weevil adults were found up to late May - early June, when air temperature is declining and the winter rainy season has commenced. Both condition possibly result in an increase in mortality of weevil adults.

The proportion of WFW adults collected from Farm 1 with fully formed eggs is shown in Fig. 2. There were few occasions when weevils were not capable of oviposition during the monitoring period, with a high proportion of adults containing mature eggs. Similarly, the fecundity of adults was reasonably uniform over the monitoring period. The only exception to this situation was the fall in both fertility and fecundity of weevils in mid March, following a period of low rainfall.

The percentage of tubers damaged by WFW larvae and the incidence of surface tuber diseases from harvests in both untreated plots and those treated with metham sodium on Farms 1 to 6 are shown in Tables 1 and 2 respectively.

Table 1. Percentage damage to potato tubers by WFW larvae in untreated plots and plots treated with metham sodium on six farms monitored for the abundance of weevils in the preceding summer - autumn period.

Farm no.	Percent WFW damage to tubers	
	Untreated plots	Plots treated with metham sodium
1	13.3	2.6
2	3.3	1.2
3	0	0
4	1.0	0.4
5	2.6	0.6
6	0	0

Only one of the six farms was infested with WFW to the extent where damage was severe - Farm 1. This farm also had the greatest abundance of WFW adults when examined the previous summer -autumn period. Farms 2 and 5 where WFW adults had been observed in comparatively moderate numbers, had low but detectable levels of WFW damage to tubers.

The surface diseases of potato tubers most prevalent on the six farms were, black dot, silver scurf, and rhizoctonia, with a minor level of common scab. The application of metham sodium appeared to have no effect on the severity of black dot, silver scurf or common scab, but it seemed to reduce the severity of rhizoctonia.

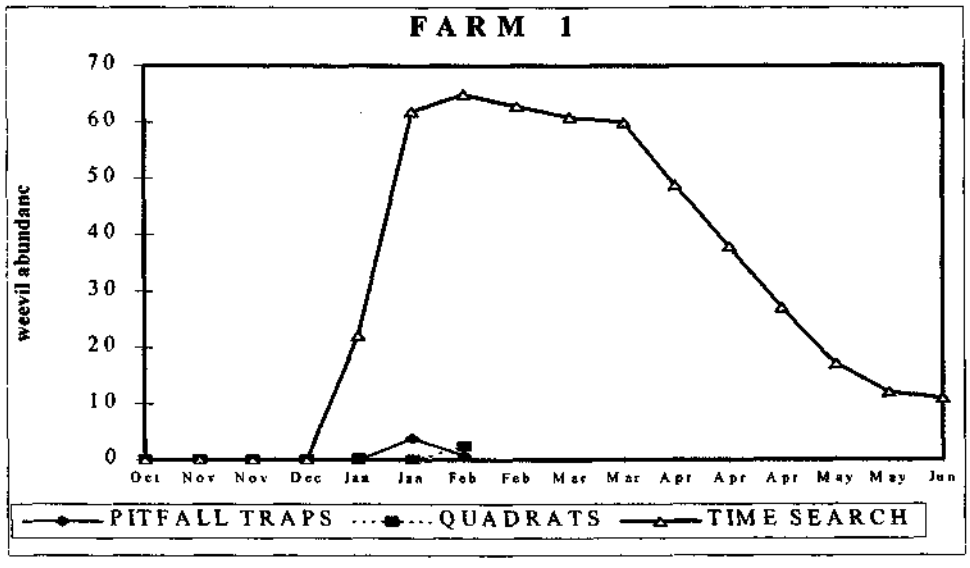
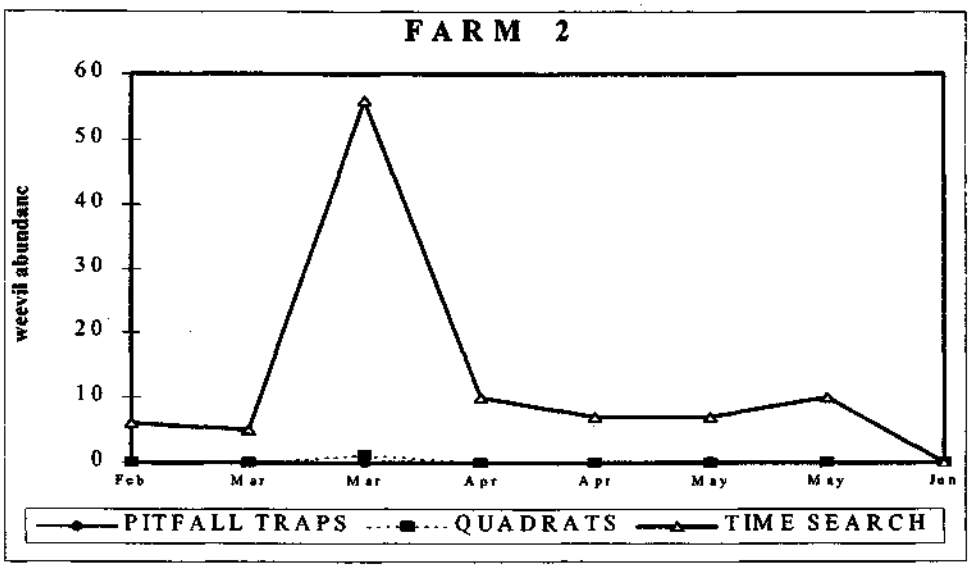


Fig. 1. Abundance of WFW adults on farms by three different search methods, for each of 11 farms.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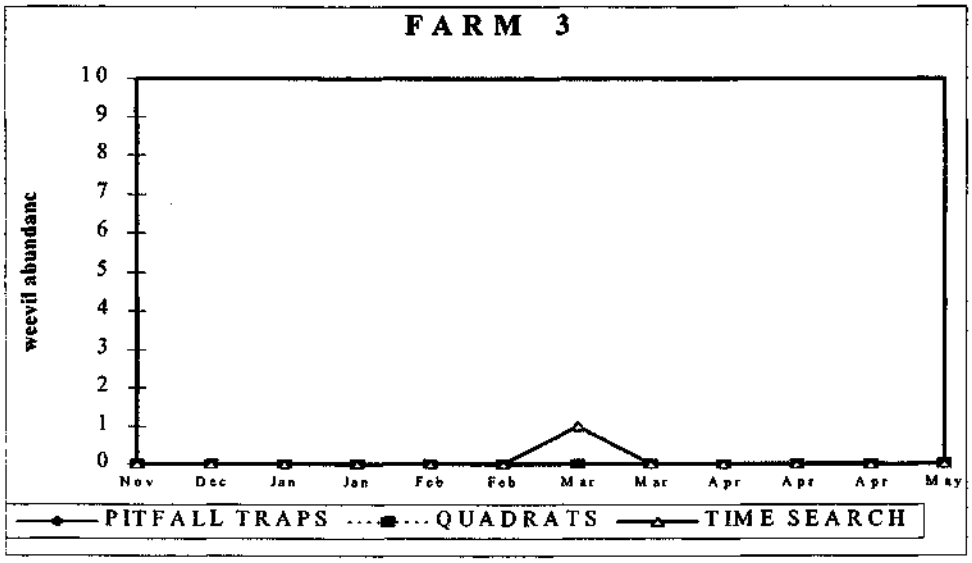
QUADRATS
TIME SEARCH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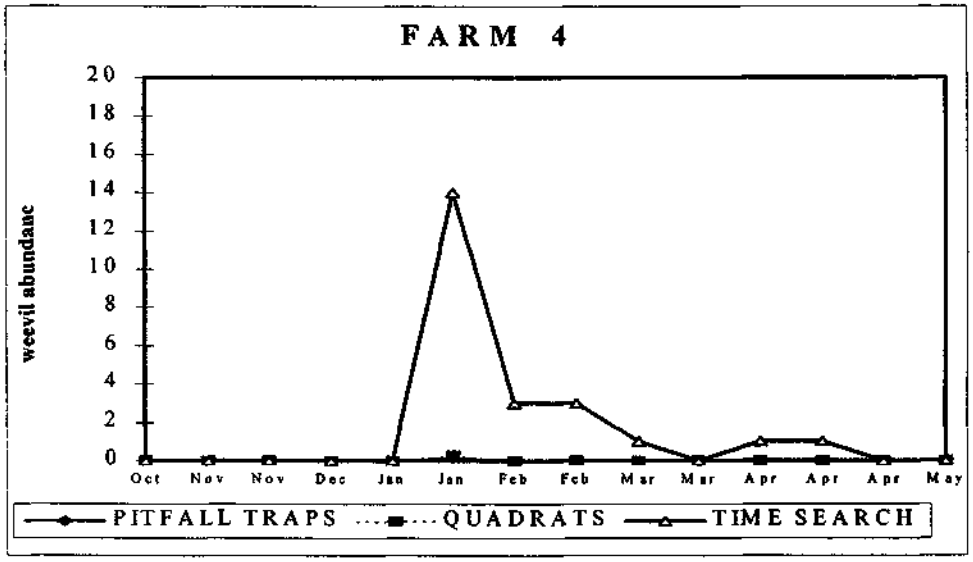
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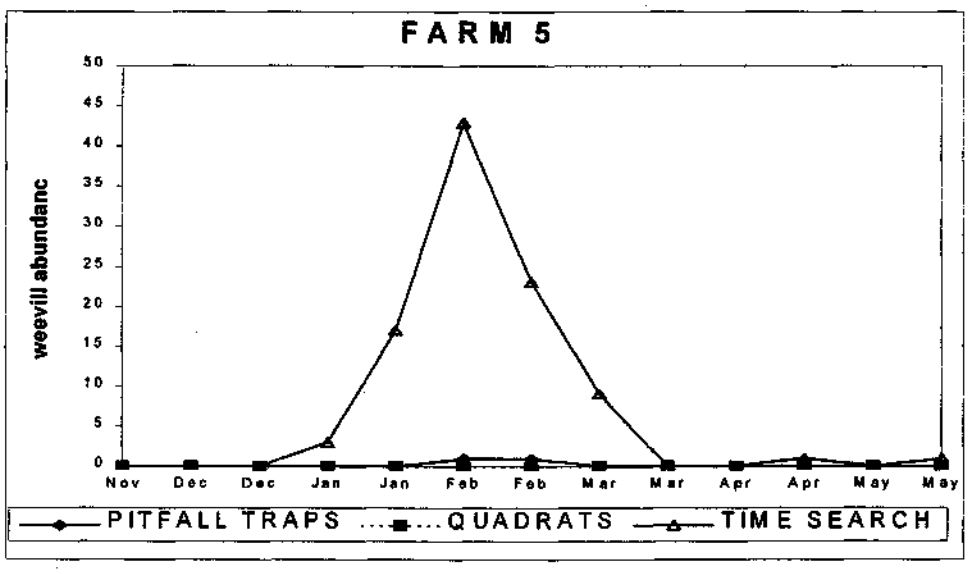
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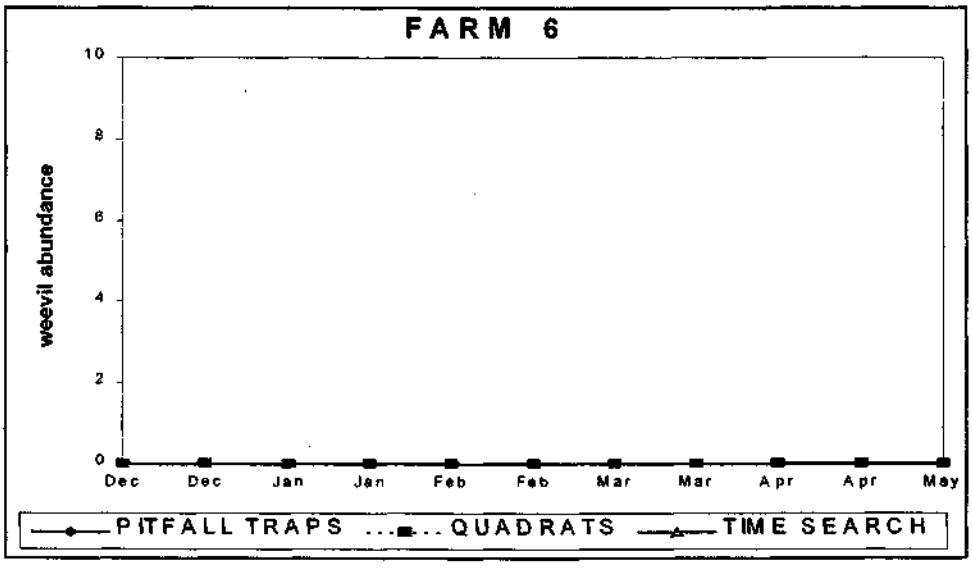
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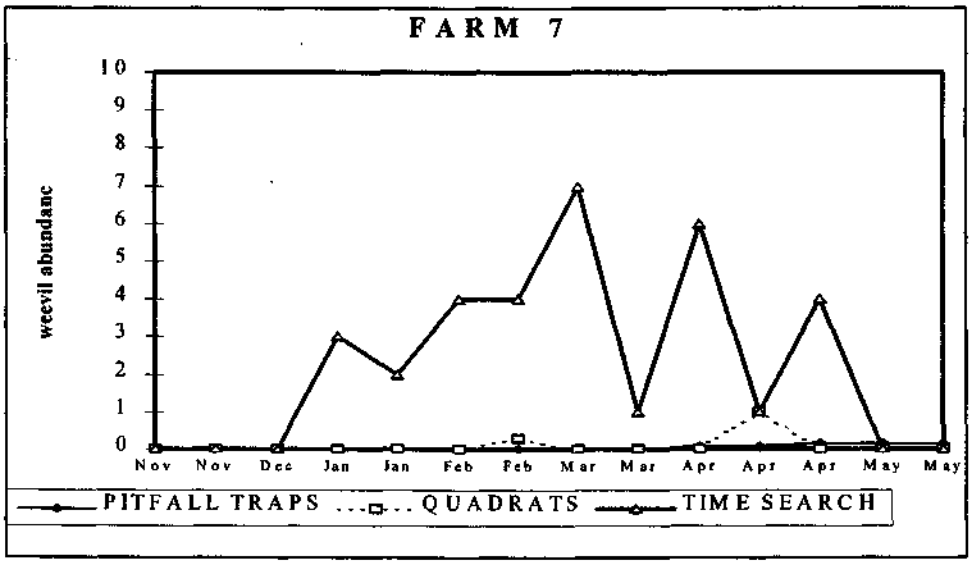
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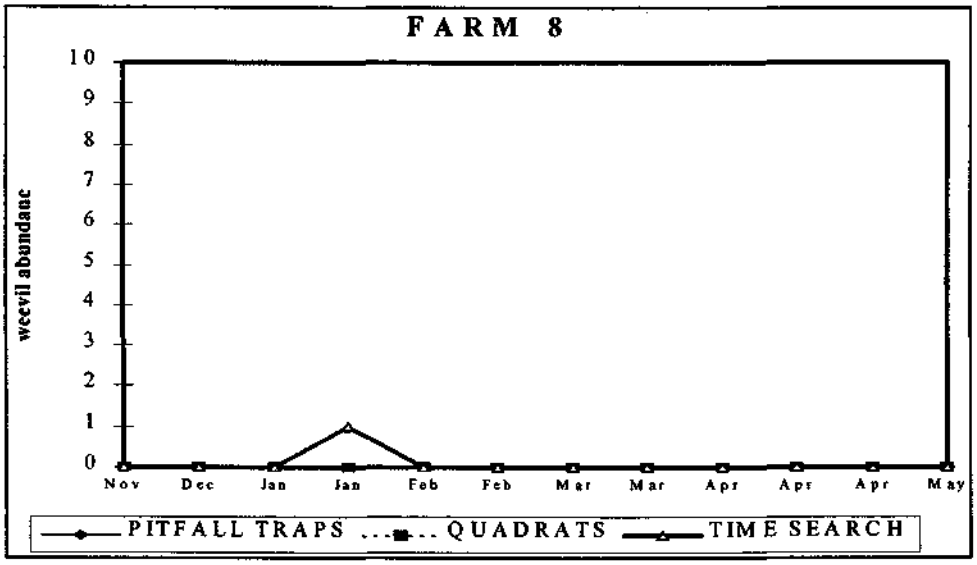
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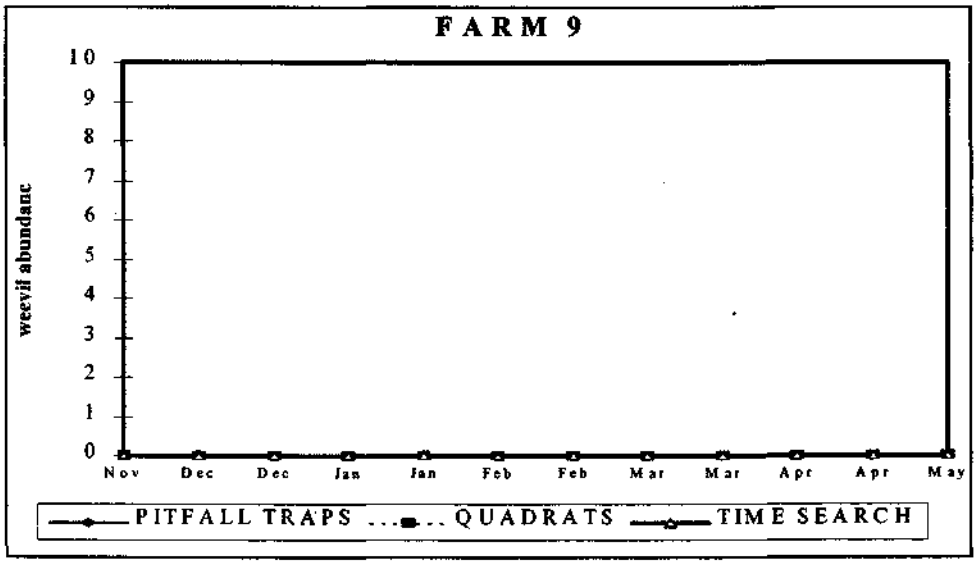
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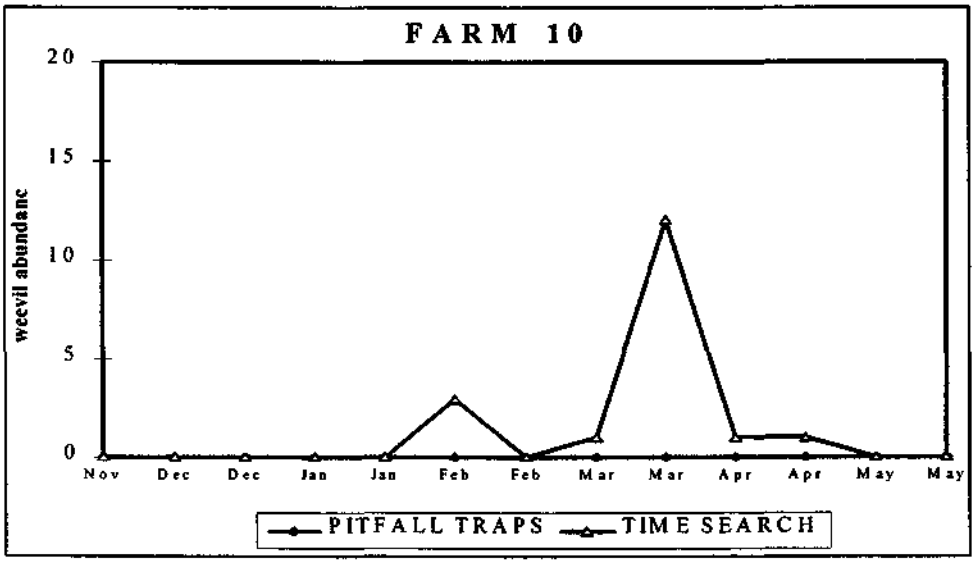
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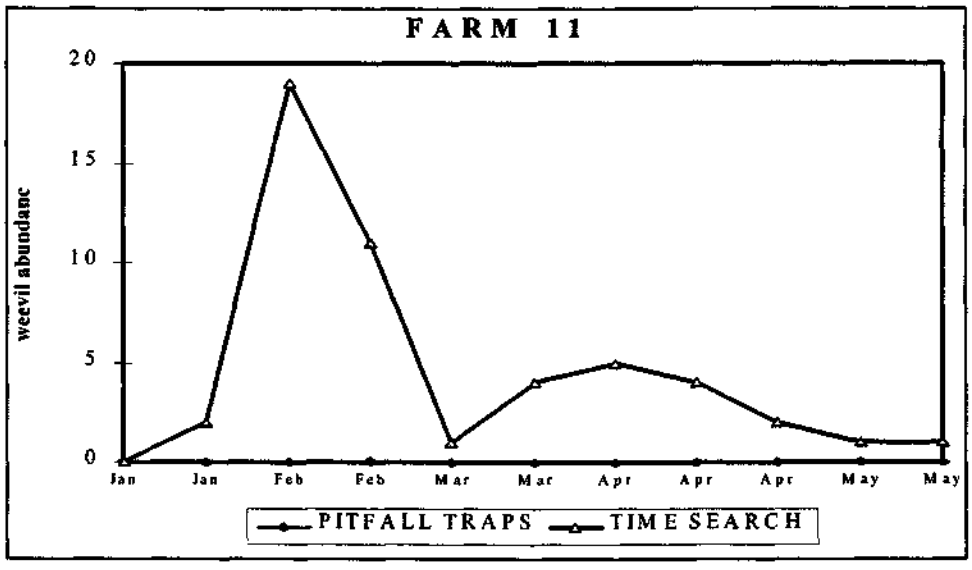


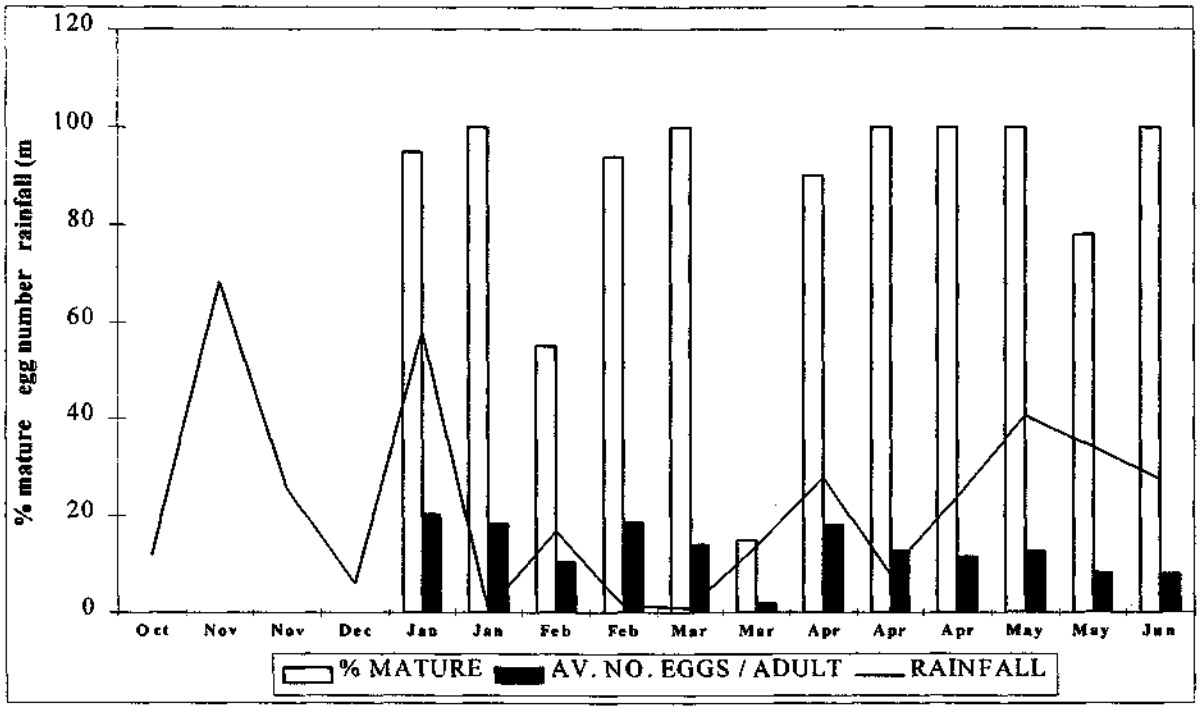


Figure. 2. Maturity status of WFW adults, average number of eggs per WFW adult and daily weekly rainfall on Farm 1 during the monitoring period.

Table 3. Percentage of potato tubers infected by the surface diseases black dot, silver scurf, rhizoctonia and common scab from untreated plots and plots treated with metham sodium on six farms monitored for the abundance of weevils in the preceding summer - autumn period.

FARM NO.	BLACK DOT						SILVER SCURF					
	1		2		3		1		2		3	
	UNT	MS	UNT	MS	UNT	MS	UNT	MS	UNT	MS	UNT	MS
1	3%	4%	4%	2%	0%	1%	5%	4%	2%	1%	0%	0%
2	11%	15%	10%	12%	17%	18%	14%	16%	16%	10%	7%	8%
3	22%	31%	14%	21%	19%	18%	0%	3%	0%	0%	0%	0%
4	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
5	15%	11%	16%	14%	7%	7%	1%	3%	1%	2%	0%	0%
6	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%

FARM NO.	RHIZOCTONIA						COMMON SCAB					
	1		2		3		1		2		3	
	UNT	MS	UNT	MS	UNT	MS	UNT	MS	UNT	MS	UNT	MS
1	18%	20%	11%	11%	2%	4%	0%	0%	0%	0%	0%	0%
2	27%	9%	4%	2%	4%	2%	0%	0%	0%	0%	0%	0%
3	25%	6%	13%	1%	10%	0%	0%	0%	0%	0%	0%	0%
4	47%	36%	11%	8%	3%	2%	0%	0%	0%	0%	0%	0%
5	18%	22%	15%	16%	19%	21%	1%	0%	0%	0%	1%	0%
6	10%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Discussion

Potato growers in the whitefringed weevil prone region of Manjimup/Pemberton of WA cannot afford to sustain damage to tubers that WFW larvae inflict. There is a tendency for growers who have suffered damage by this pest to treat subsequent crops on the assumption that WFW is present. The vagaries of the distribution of WFW in this region are that not all paddocks in the region are infested. This implies that some treatments for WFW are unnecessary, but the grower's worry about potential loss can outweigh the financial cost of applying pesticide. On the other hand, paddocks that have been used for potato cultivation in the past without damage by WFW larvae, are not necessarily uninfested when the paddock is cropped again after 4 or more years.

In late winter to mid spring when crops are planted in this region, WFW is in the early larval stages which are very small and difficult to detect. Also, the patchiness of the distribution of WFW larvae make intensive wash/float processing of soil samples somewhat inaccurate. The results of this investigation suggest that potato growers have a practical means of assessing risk of crop damage by WFW.

The large adult WFW are relatively easily seen in pasture.. If growers can scout those paddocks that will be planted to potatoes in spring, in the preceding summer - autumn period for adult WFW and find none over this period with around six checks of each of the paddocks, results here indicate that treatment for WFW is not necessary.

Potato growers will be made aware of the results of this study and be encouraged to undertake paddock scouting to try to assess risk of damage by WFW. With time and experience, it is hoped that growers will only be applying

pesticide on demand. Having decided that the pest is present and action is required to protect a crop, the challenge to find more environmentally acceptable means of managing WFW will then be the emphasis.

Another issue relevant to the use of metham sodium in potato crops is that of its potential to control other pests and diseases that impact on potato crops. The results in this study support the conclusion that metham sodium reduces the level of rhizoctonia, but has little impact on the disease black dot and silver scurf. Other fungicides are available for reducing the level of rhizoctonia and it is suggested that these more specific products are preferable to metham sodium with its more broad acting biocidal properties.

The activity of metham sodium against nematodes has been utilised by WA potato growers and could be argued as another reason for using this product. However, soil testing for the presence of nematodes is an option available to growers that can be utilised so that nematicides also are used on demand.

Alternative IPM-based strategies for management of African black beetle.

Stewart Learmonth[A] & John Matthiessen[B]

[A]Agriculture Western Australia, Manjimup WA 6258.
[B]CSIRO Entomology, Private Bag PO, Wembley WA 6014.

(1) Disinfesting pasture of African black beetle prior to planting potatoes.

Aim

African black beetle (ABB) is an important pest of potatoes in the Busselton potato growing region of Western Australia. For crops planted in summer, it is common practice to incorporate chlorpyrifos in soil prior to planting, to control the beetle. Best incorporation is achieved with the aid of rotary hoe. The beetles that damage such crops during their establishment phase are the progeny of beetles that occurred in the previous winter and completing their breeding cycle during the spring.

Previous research by Agriculture WA and CSIRO has demonstrated that surface spraying areas of pasture in late winter results in high levels of beetle mortality, but the reduction in the abundance of African black beetle has not been consistent nor of sufficient magnitude to provide reliable protection for a subsequent potato crop.

Recent research has indicated that a second application of insecticide around mid October when beetles are actively crawling and any minor invasion by flight would have occurred, could provide a more reliable and greater level of beetle mortality. This research also indicated that equivalent control of beetles might be achieved at half the rate that is usually applied.

As well as ease of pest control, pasture spraying obviates the need for rotary hoe incorporation of insecticide at planting and, by killing beetles prior to their breeding cycle in spring, is a more strategic time of application. Demonstration of success with this approach would also provide a basis for subsequent research on the use of baits for control of adult beetles.

The aim of this investigation was to determine whether two applications of insecticide in early- and mid - spring to pasture to control African black beetle provides superior and more reliable control of African black beetle compared to the previous use of a single application at the earlier time. Also to determine whether half rates of insecticide provide an equivalent level of control.

Methods

In early August, potential trial sites were sampled to confirm that ABB was present at a density considered to be potentially damaging to a subsequent crop of potatoes - 5-10 beetles/m^2. Two farms were found where the ABB density was near this level.

On unreplicated plots of pasture approx. 20 m by 100 m, on each of the two farms, the insecticide was applied by vehicle mounted boom spray at the times and rates shown in Table 1.

The effect of the insecticide treatments was assessed using a number of different sampling methods.

Soil core sampling was under taken throughout the experiment at different strategic times to measure the abundance of ABB on an area basis in each treatment block. Soil cores were 10cm deep x 10cm diameter. In each block, 100 soil cores were examined. All soil cores were examined in the field for beetles, except in spring when soil core were returned to the laboratory to extract small larvae. The times at which cores were collected were: (a) mid August (pre-treatment); (b) late September (post first spray); (c) mid - late November (post

second spray) - this sample was washed and floated to detect the very young immatures, which are the main stage of beetle present at this time; (d) late January; (e) late April; (f) July - near crop maturity.

Table 1. Details of rates and times of the insecticide treatments applied to pasture to determine whether disinfestation of a resident population of African black beetle can be achieved.

Treatment no.	Early September	Mid October
1	U	U
2	3	U
3	U	3
4	3	3
5	1.5	1.5

U: untreated; 1.5, 3: chlorpyrifos @ 1.5 & 3 kg AI/ha applied to pasture using a boom spray.

Pitfall traps, which consisted of a 100 mm diameter funnel attached to a 250 ml jar sunk into the soil to ground level, were used to measure the crawling activity of beetles. 10 pitfall traps were placed in each treatment block. Traps were arranged into two transects of 5 traps each, parallel and equidistant to the long side of each block. Pitfall traps were serviced weekly except during December and January when they were serviced fortnightly because adults usually are present only in very low numbers at this time. Beetles collected in pitfall traps were sexed and counted, then returned to the pasture near the trap. Also, whether beetles were live or dead was recorded.

Flight activity of African black beetle adults was recorded for the duration of the trial using **light traps.** These were set up at Vasse Research Station, which is approx. 15 km south east of the trial sites and where the daily catch was recorded, and on one of the farms used in this investigation, where the weekly catch was recorded.

Results

The density of all stages of ABB in the different treatment blocks on the two farms is shown in Fig. 1. Differences among the treatments were evident after the second soil sample, which was taken after the first application of insecticide. Untreated plots had greater numbers of ABB. The differences between the treated and untreated plots became larger by the time the third soil sample was taken in November/December. The greater abundance of insects at this sampling occasion is a reflection of the use of the more efficient extraction method of washing/floating, where small larvae can be recovered. The differences between the blocks that received no insecticide and those receiving only the late application was present for the duration of the trial on Farm 1, but with the differences declining by the conclusion of the trial. On Farm 2, the differences between treatments was not as great. The abundance of ABB was greatest in untreated plots and plots sprayed late only. By the conclusion of the trial, the abundance of ABB was roughly equivalent between treatment blocks.

The abundance of ABB adults in the pitfall traps for the two farms is shown in Figures 2 (a) and (b). The effect of the insecticide application is reflected in the pitfall trap catches, where the early spraying resulted in a drop in activity. The second application of insecticide in October resulted in a drop of pitfall trap catches in that treatment receiving the late application only. This second application of insecticide also resulted in a minor drop in catches in the treatment receiving the lower rate of insecticide, and to a less marked extent in the other insecticide treatments. The increase in trap catches in autumn with the emergence of the new generation of adult beetles, was greatest in the late spray blocks. By the conclusion of the trial in early winter, trap catches had dropped to uniform and low levels for all treatment blocks.

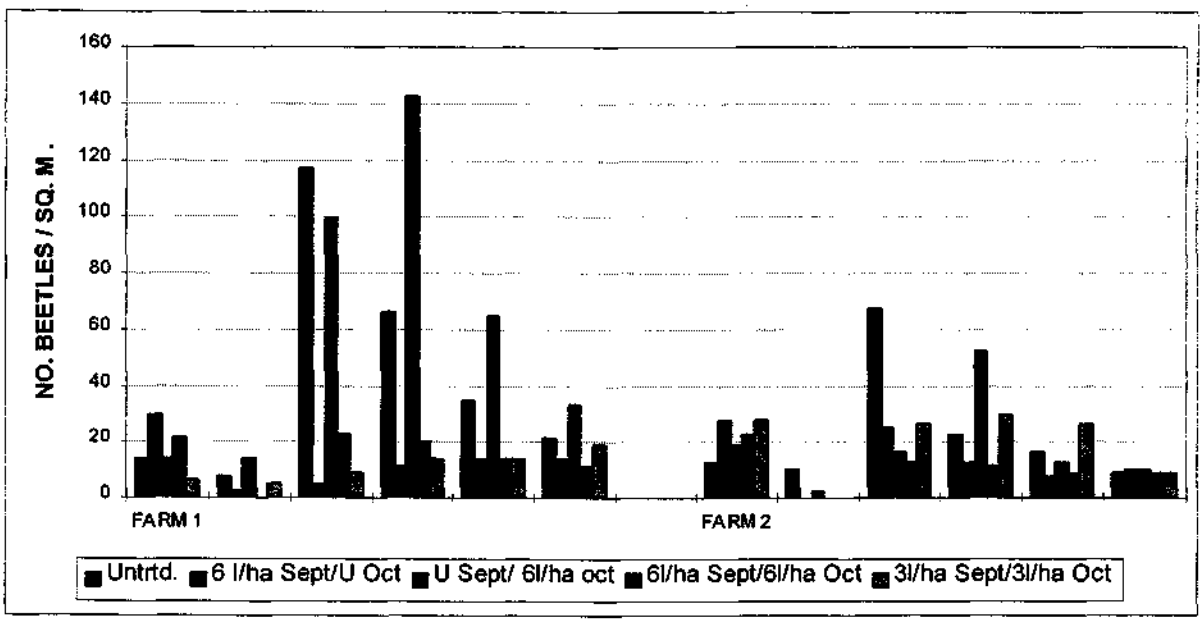


Figure 1. The average number of all stages of African black beetle per square metre from soil coring on two farms in blocks treated with chlorpyifos 50% EC at different times and different rates.

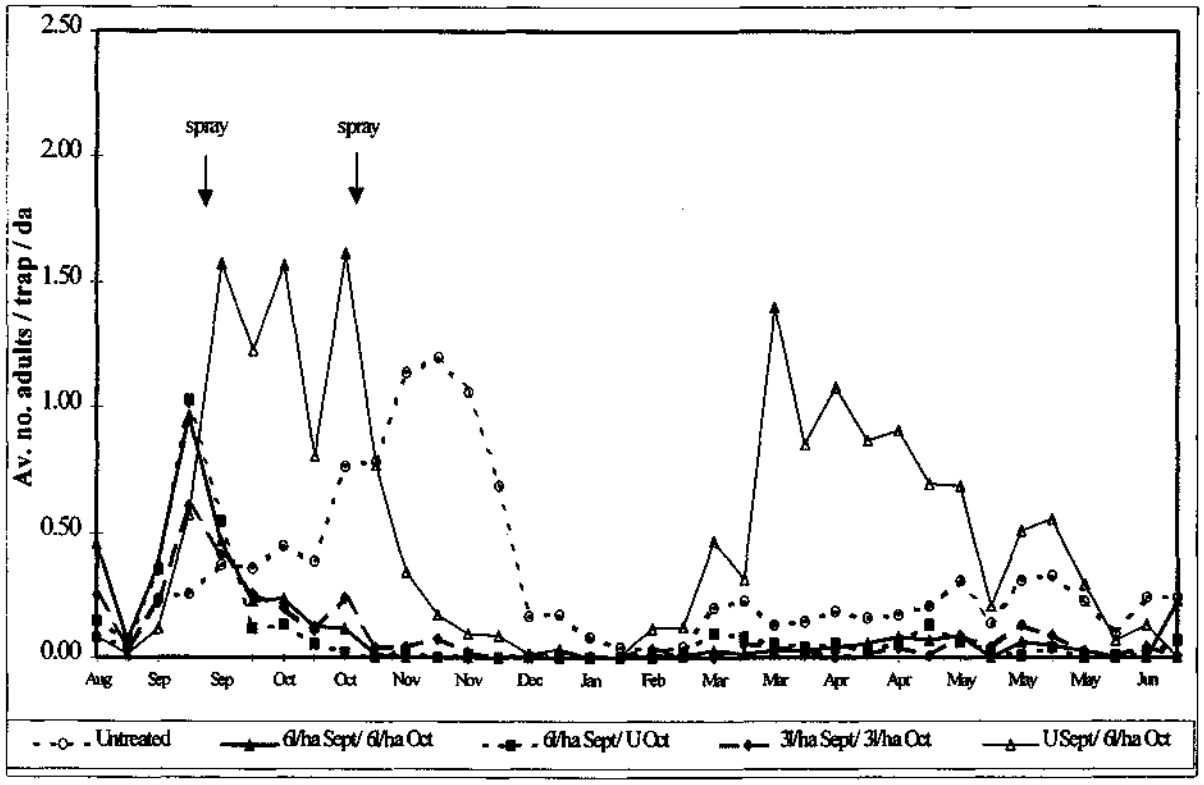


Figure 2a. The abundance of African black beetle adults in pitfall traps on farm 1 in blocks treated with chlorpyrifos 50% EC at different times and different rates.

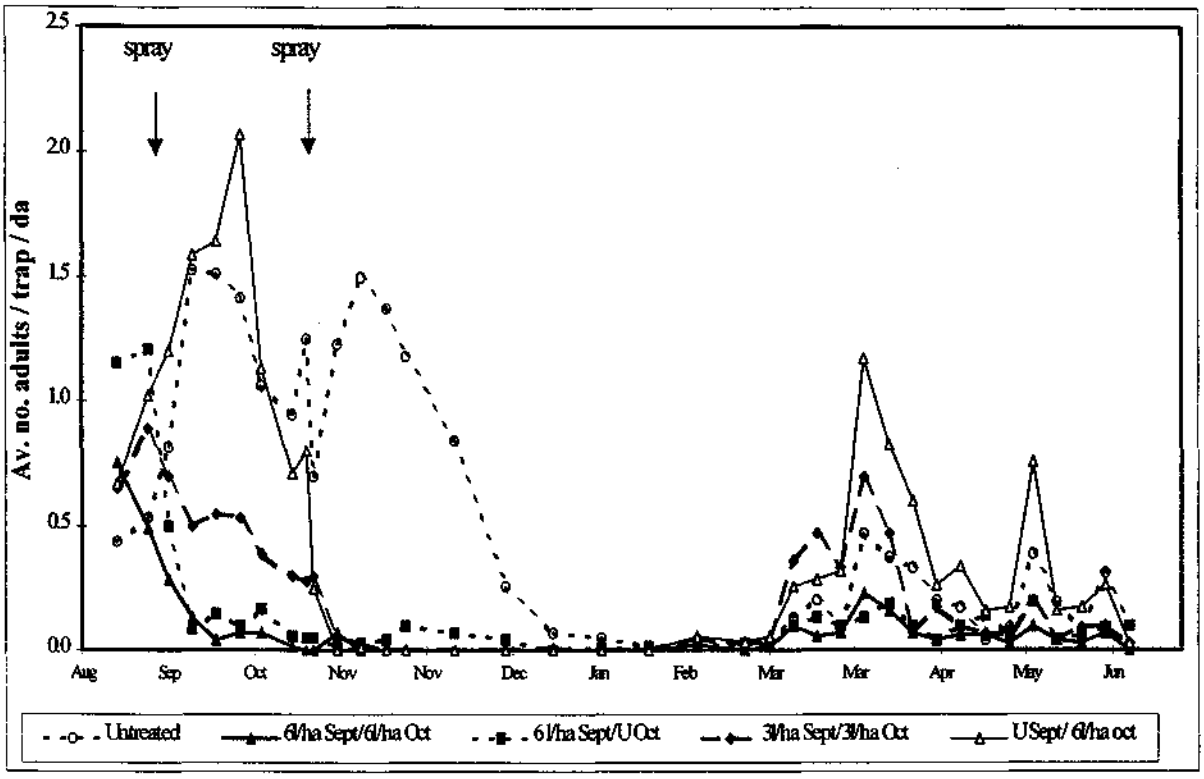


Figure 2b. The abundance of African black beetle adults in pitfall traps on farm 1 in blocks treated with chlorpyrifos 50% EC at different times and different rates.

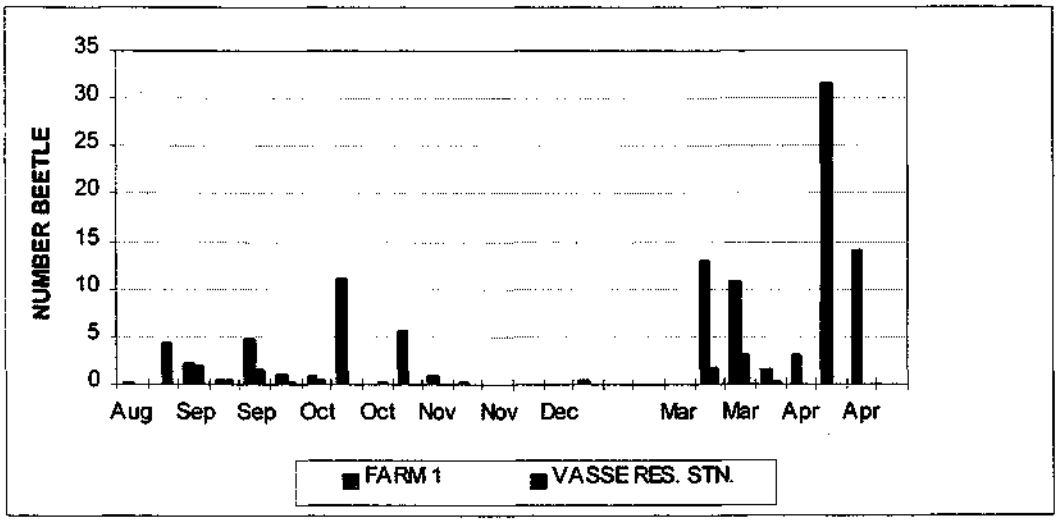


Figure 3. The average daily light trap catch of African black beetle adults in light traps operated at farm 1 and Vasse Research Station during the pasture spray trial.

The light trap catch data for the two light traps in operation during the trial, are shown in Fig. 3. Activity of beetles was evident during spring and again in autumn, with greater activity in the autumn.

Discussion

Reduction in beetle density and activity was recorded as a result of the pasture spraying on the two farms. The greatest reduction in ABB abundance, as indicated by activity levels in the pitfall traps and the soil coring, was achieved by the higher rates of chlorpyrifos and in particular when it was applied in September.

The application of chlorpyrifos in October alone and at the higher rate, although resulting in a reduction in ABB activity as recorded in the pitfall traps, must have been applied after a oviposition of beetles had commenced.

On Farm 1, the apparent poor activity of the late application of chlorpyrifos alone, was confounded by the lower abundance of ABB in the untreated block. This situation would be rectified by redesigning the trial to incorporate replication of treatments.

The return to approximately equal density of ABB across the treatment blocks in winter appears to be a combination of two factors. The flight activity of ABB would ensure some immigration into pasture areas. Also, as the data indicates here, there is an apparent mortality of ABB in late autumn. This could also be interpreted as an emigration, but whatever the cause, the effect is to remove the effect of the earlier differences in ABB density created artificially by the use of insecticide.

Nevertheless, the results obtained here indicate that a single application of the high rate of chlorpyrifos around late September has the potential to locally disinfest pasture. The results also indicate that the effect lasts at least until the following autumn when the flight activity of ABB and an apparent mortality of resident beetles, tends to return the pasture to a situation of more uniform abundance. This result is in agreement with earlier studies on the same treatments also involving pasture.

The next phase of this adulticide approach to managing ABB in potato crops is to examine whether the more efficient use of insecticide demonstrated here can be used where a crop of potatoes is planted in the following summer.

(2) Protecting summer sown potatoes from resident African black beetle by prior disinfestation of pasture.

Aim

African black beetle is an important pest of potatoes in the Busselton potato growing region of WA. To protect crops planted in summer, it is common practice to incorporate chlorpyrifos insecticide in soil just prior to planting. These beetles are the newly emerging adults that are the progeny of overwintering adult beetles that breed in the preceding spring in pasture.

Previous research by Agriculture WA and CSIRO, such as described in the previous section in this report, has demonstrated that surface spraying areas of pasture with insecticide in late winter results in high levels of beetle mortality that is sustained at least until subsequent immigrant flights in autumn. One application of chlorpyrifos EC insecticide at 3 kg AI per hectare in late September resulted in a reduction in beetle abundance equivalent to the best of any other treatment.

Therefore, it would appear that the incorporation of insecticide at planting may be able to be replaced by surface spraying of pasture with insecticide in the previous spring. This is potentially more efficient, and less deleterious on soil structure compared to use of a rotary hoe for incorporation of insecticide at planting.

Indications from this previous research on disinfestation by pasture spraying are that the reduction in the abundance of ABB has not been of sufficient magnitude to provide reliable protection for a subsequent potato crop. However,

possible additional mortality of beetles as a result of land preparation for potato production has not been assessed because the trials to date have been conducted only in undisturbed pasture, with the sole objective of measuring effects of the surface spray on insect abundance.

The aim of this trial was to determine whether application of insecticide to pasture in early- spring, together with possible additional mortality from cultivation involved in preparing the ground for potato production, provides sufficient mortality of African black beetle to obviate the need for a pre-plant application of insecticide to protect potatoes from a resident population of beetles.

Methods

Pasture paddocks in the Busselton region that were to planted to potatoes in the following summer, were assessed for the abundance of ABB adults in spring to determine whether they would be suitable as trial sites.

On each of two locations (farms) assessed as being infested with ABB adults at a density suitable for the trial, six plots approx. 25m by 50m were marked out. Two of the plots remained under pasture for the duration of the trial, with the other four plots being prepared in the usual manner and planted to potatoes in January/February.

Insecticide was applied to pasture plots by vehicle mounted boom spray in late August, and/or incorporated in soil just prior to planting for the following potato crop using a rotary hoe or disc plough, or the pasture and /or the subsequent potato crop were left untreated. The treatment details are shown in Table 2.

All plots of potatoes were protected from invasions of African black beetle during crop growth by foliar application of chlorpyrifos EC as required.

Operations on the land where potatoes were sown included stock grazing up to early September; plough in late September; fallow to end December; in January, pre-irrigate, cultivate, incorporate insecticide, where necessary, and plant; apply further insecticide 4 weeks after plant; apply further insecticide after March for immigrant flying beetles; harvest late May.

Table 2. Details of the timing of the insecticide treatments applied to pasture and incorporated in soil just prior to planting a potato crop, to determine whether disinfestation of a resident population of African black beetle can be achieved in the pasture phase to protect a subsequent potato crop.

Treatment no.	Timing of insecticide application	
	Late August (pasture phase)	January/February (crop phase)
1 Pasture (untreated)	U	U (pasture)
2 Pasture (sprayed)	spray	U (pasture)
3 Crop (untreated)	U	U
4 Crop (spray at planting)	U	spray
5 Crop (spray pasture only)	spray	U
6 Crop (spray pasture & crop)	spray	spray

Measurements were made on insect abundance and damage to potato plant parts as described below.

Soil Sampling: To estimate beetle density, 100 soil cores (15 cm deep x 10 cm diameter) were examined in each of the six plots initially and, after cultivation for preparation for potatoes, in the pasture plots only for the duration of the trial.

Pitfall traps: Ten pitfall traps were installed per treatment block in mid August. The trap consisted of a 100 mm diameter funnel attached to a 250 ml jar sunk into the soil to ground level. Traps were placed in two transects of 5 traps each, parallel and equidistant to the long side of each block. They were serviced weekly except during December and January when they are serviced fortnightly because adults are only present in very low numbers at this time. Beetles collected in pitfall traps were sexed, assessed as live or dead and counted. Beetles were then returned to the pasture near the trap. Traps placed in treatment blocks 3 to 6 were removed for cultivation in October and replaced later. These traps were removed from the blocks in late December as land preparation for potatoes began in earnest. For the two pasture treatment blocks, pitfall traps were serviced for the duration of the trial.

Abundance of ABB in potato cops and level of damage to potato plant parts: For Treatment 3 - 6, to estimate beetle density and damage to the potato crop, excavation sampling was conducted. This consisted of examining 40 x 0.5 m crop row sections, recording numbers and stages of insects and damage to potato stems and tubers, in late February, late March, late April and late May.

Yield assessment: At crop maturity, yield and beetle damage to tubers in 12 x 5 m crop row were assessed by hand harvests from each of the four crop treatment plots.

Light Traps: The weekly catch of ABB adults was recorded in two light traps - one on Farm 2 and the other within 2 kilometres of Farm 1, for the duration of the trial.

Results

The results of the soil coring for ABB are shown in Fig. 4. Both the effects of the pasture spraying and the abundance of ABB in the trial plots were cause for concern. On both farms, the effect of the pasture spraying in spring did not cause the desired effect of a reduction in ABB abundance - this was evident by November on Farm 2 and by January on Farm 1. The very low abundance of ABB through the season on Farm 1 was a problem in being able to record treatment effects.

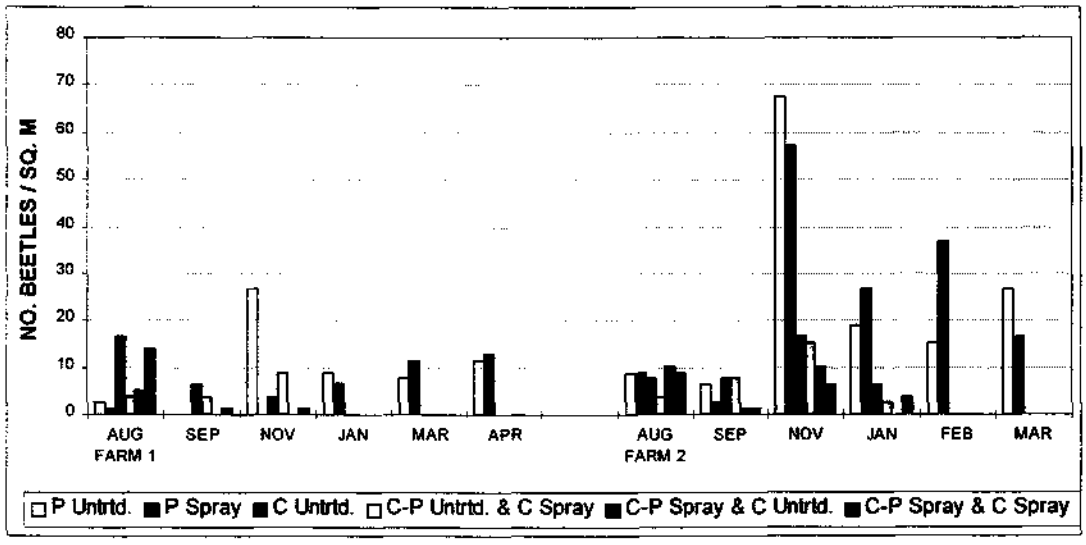


Figure 4. The average number of all stages of African black beetle per square meter from soil coring on two farms in untreated blocks and blocks treated with chlorpyrifos 50% EC insecticide at different times as shown on the graph, in either a pasture or a potato crop phase. The potato crops were planted in late January and early February. (P = pasture phase; C = potato crop phase; Untrtd. = untreated).

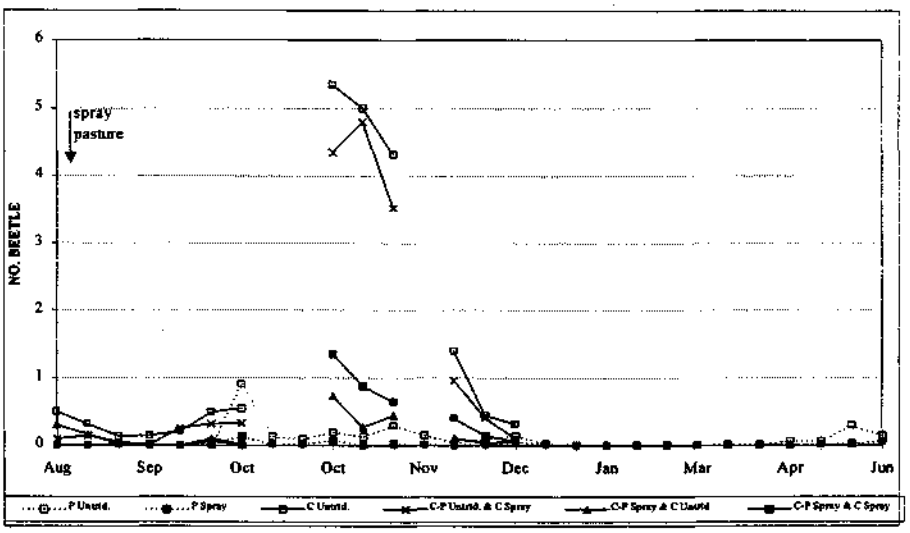


Figure 5(a) The abundance of African black beetle adults in pitfall traps on Farm 1 in untreated blocks and blocks treated with chlorpyrifos 50% EC insecticide at different times as shown on the graph, in either a pasture or a potato crop phase. The potato crops were planted in late January and early February. (P = pasture phase; C = potato crop phase; Untrtd. = untreated). The discontinuous portion of the graph are where traps were removed for cultivation or cropping.

The pitfall trap catches are shown in Figs. 5 (a) and (b). The generally low abundance of ABB is indicated in the catch results. The effect of the pasture spraying is evident in the pasture plots to be planted to potatoes, where more beetles were recorded in plots that had not been sprayed. The low abundance of ABB recorded from soil coring in the plots remaining under pasture for the duration of the trial, is reflected in the lower trap catches in those plots compared to pasture plots subsequently planted to potatoes.

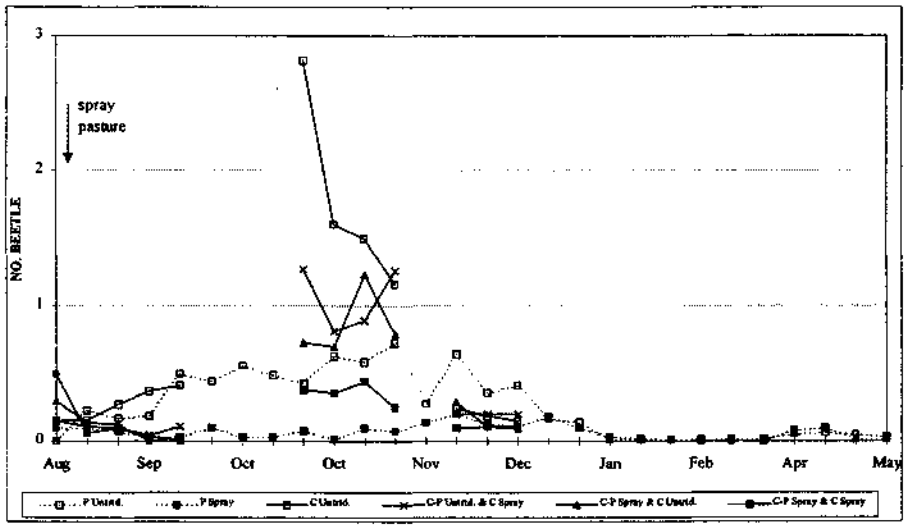
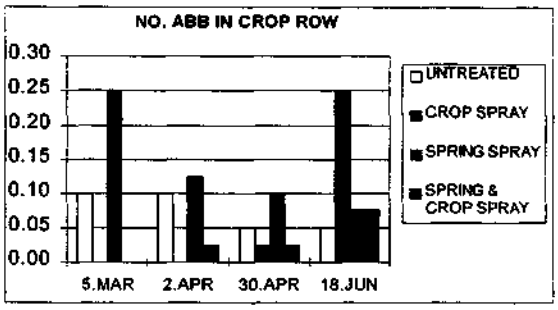


Fig. 5(b) The abundance of African black beetle adults in pitfall traps on Farm 2 in untreated blocks and blocks treated with chlorpyrifos 50% EC insecticide at different times as shown on the graph, in either a pasture or a potato crop phase. The potato crops were planted in late January and early February. (P = pasture phase; C = potato crop phase; Untrtd. = untreated). The discontinuous portion of the graph are where traps were removed for cultivation or cropping.



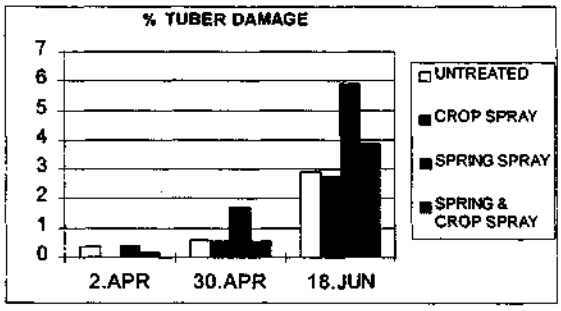
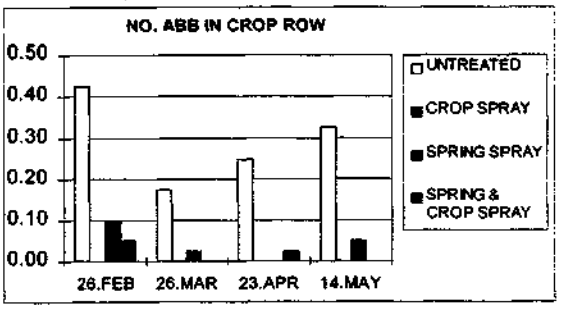


Figure 6(a). The average number of ABB per 50 cm crop row and percent potato tubers damaged by ABB in plots on Farm 1 that had been left untreated or treated with chlorpyrifos insecticide either in the previous pasture phase or just prior to planting potatoes as indicated.



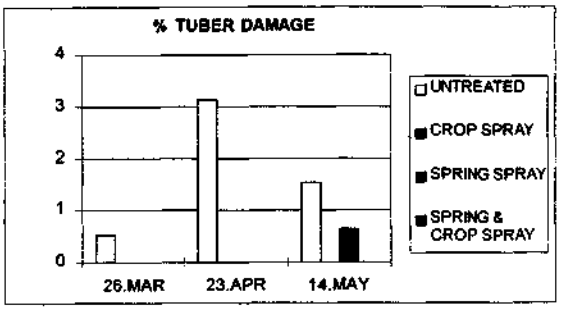


Figure 6(b). The average number of ABB per 50 cm crop row and percent potato tubers damaged by ABB in plots on Farm 2 that had been left untreated or treated with chlorpyrifos insecticide either in the previous pasture phase or just prior to planting potatoes as indicated.

The abundance of ABB and damage to potato tubers as recorded from excavation sampling within crops on the two farms, are shown in Figures 6 (a) and (b). The damage to tubers in these blocks and yield estimates at crop maturity are shown in Figures 7 (a) and (b). The generally low level of tuber damage and low level of abundance of ABB is apparent in these figures. On Farm 1, it appeared that the spring spray was ineffective, but it is more likely that the variation in beetle abundance masked any possible treatment effect - given that ABB abundance was lower in the untreated area. Likewise, on Farm 1, the spring pasture spray appeared to be effective, but given the low abundance of ABB at that trial site, the effect is not convincing. Similar comments can be made in relation to the marketable tuber yield and ABB damage to tubers as recorded at crop maturity in the hand harvests.

The light trap catch data is given in Fig. 8. Even though reasonably large flights of ABB were recorded in the study area, these appeared to have only minor influence on the abundance of ABB adults in either the pasture or potato crops during the trial. There was an increase in abundance of ABB recorded in the crop spray potato plot on Farm 1, but this was not a universal occurrence.

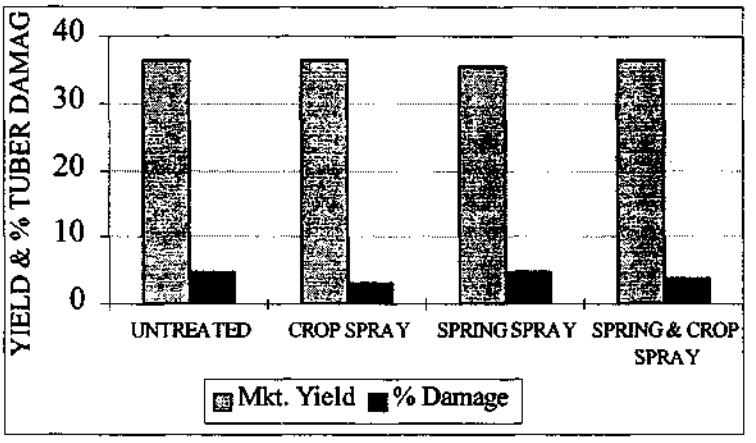


Figure 7(a). Average marketable yield (t/ha) and percent damage to tubers by ABB in plots on Farm 1 that had been left untreated or treated with chlorpyrifos insecticide either in the previous pasture phase or just prior to planting potatoes as indicated.

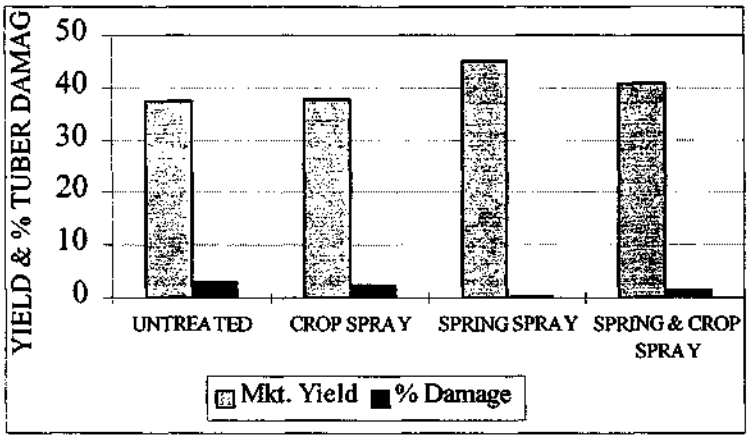


Figure 7(b). Average marketable yield (t/ha) and percent damage to tubers by ABB in plots on Farm 2 that had been left untreated or treated with chlorpyrifos insecticide either in the previous pasture phase or just prior to planting potatoes as indicated.

Discussion

After a long series of experiments to test the principle of an adulticide approach to protecting summer planted potato crops from attack by ABB, it as disappointing that this experiment where a potato crop was included for the first time, produced such inconclusive results.

A number of considerations arise as a result of this series of experiments. The first and most apparent is that trial areas need to have a high initial population of ABB to give the best chance of making valid comparisons between treatments. The best assessment of success in this regard is the level of stem damage to potato crops during crop establishment, when the effects of a dense resident population of ABB is most apparent and has the greatest effect on gross yield. It is the stem loss from ABB early in the life of a crop that has the greatest impact on gross yield in potatoes.

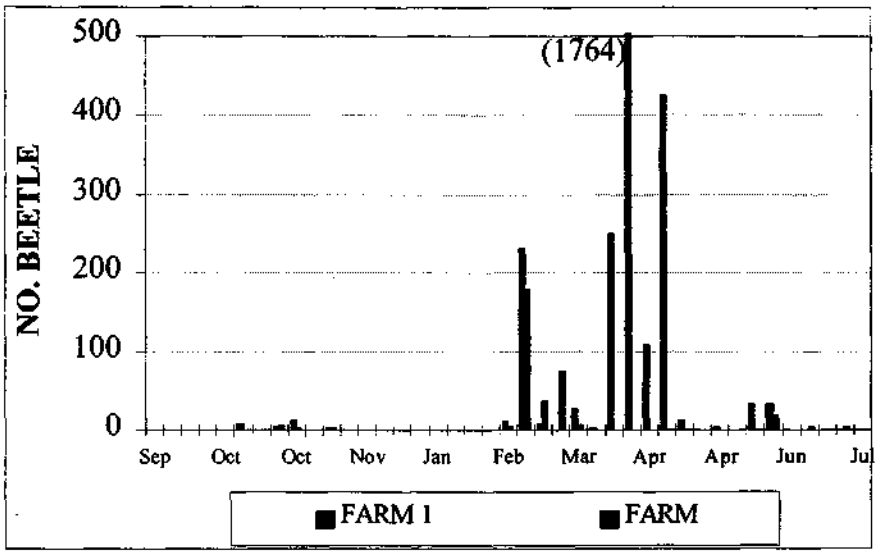


Figure 8. The average daily light trap catch of African black beetle adults in traps operated on two farms in 1996/97. The second trap (Farm) was on another potato farm approx. 2 kilometres from Farm 2.

The second aspect that needs consideration is the incorporation of replicated plots. This would have helped to overcome to some extent, the occurrence of what might be termed an intermediate density of ABB across the trial sites. If replication were included, a review of the number of sampling units would need to be made, or greater resources allocated to the experiment in view of the labour intensive nature of the sampling involved.

A third aspect is the detail of the spring application of insecticide. Even though this trial has not given conclusive results, it was apparent that the spring application of insecticide was not as successful as has been demonstrated in previous trials. The mechanism of efficacy of the application is undoubtedly related to the strong crawling activity of adult ABB at this time, probably related to this being the time of mating and oviposition. More detailed studies to gain a better understanding of the mechanism of the adulticide approach could be beneficial in achieving a more reliable result from the pasture spraying, for example, the effect of heavy grazing, rainfall, air temperature and the timing of the application or use of multiple applications.

The principle of the adulticide approach has advantages over the conventional at-planting use of insecticide. It offers an easier method of insecticide delivery, which is less destructive of soil structure than the at-planting use of a rotary hoe. The possibility also exists to experiment with the use of baits which are selective against the adult stage. Like the adulticide approach with the topical application of insecticide to the pasture, the use of a bait would be timed to occur prior to the spring breeding of ABB. If this were shown to be effective, it would offer an even more environmentally friendly method of disinfesting pasture prior to the summer potato crop.

Defining and consolidating further research into management of soil pests and diseases of WA potatoes - Pest and disease survey of WA potato industry - 1996/97

Stewart Learmonth

Agriculture Western Australia, Manjimup WA 6258

Aim

A considerable amount of intensive research has been undertaken on the management of soil insect pests of potatoes over the last 8 years. The objective was primarily to reduce the pest status of the two most damaging soil insect pests, whitefringed weevil and African black beetle.

As a result of this research, metham sodium is now widely used in the Manjimup/Pemberton area for control of whitefringed weevil.

Also, the research on African black beetle has provided improved knowledge of the insect's biology and pest status, early indications that pre-crop pasture spraying leads to a meaningful level of disinfestation of land to be cropped to potatoes and confirmation that this pest has not yet developed resistance to chlorpyrifos, the main insecticide used for its control in potatoes. Such advances have lead to a perceived overall improvement in crop protection from attack by African black beetle in WA potato crops.

Even though there are major concerns and reservations about the reliance on single pesticides for both insect pests - metham sodium for whitefringed weevil and chlorpyrifos for African black beetle - there are indications that the soil insect pest problems have been considerably reduced. To what extent is this reflected in the level of insect damage in commercial crops?

Suppression of diseases and nematodes is an added benefit of using metham sodium; however this fumigant can cause changes in the soil biota in treated land that may be deleterious to crop production in different ways than currently experienced.

The importance of diseases in W.A. potato crops is less well understood, compared to the situation for insects. In an investigation on cosmetic quality of ware potatoes in the Manjimup/Pemberton area by Andrew Harvey (1993), silver scurf/black dot, scabs and rhizoctonia were the most important diseases found. The relative importance of these various diseases has not been quantified across the W.A. potato growing regions.

The HRDC project PT447 on "Integrated management with biofumigation to control soil pests and diseases of potatoes", has the objective of maintaining or improving the quality of potatoes with reduced reliance on synthetic pesticides. Work prior to this current activity had considered only insect pests, but, with the inclusion of biofumigation as an alternative to fumigation with heavy doses of synthetic fumigant, the project more broadly encompasses pests and diseases of potatoes.

The aim of this activity was to identify the causal agents and levels of insect damage and disease across Western Australia's potato growing districts. A potential limitation of this survey is that, in using commercial crops, reliance will be placed on including sufficient crops that have been treated with metham and chlorpyrifos, and ones that have not. Pest levels will be compared to assess both the level of damage still occurring in W.A. crops as well as obtaining an indication of the efficacy of these pesticides.

The identification and quantification of the soil pests and diseases of potatoes will clearly indicate whether further research is justified and if so, the targets of the research.

Methods

This activity was a survey of commercial potato properties in Gingin, Perth Metro, Harvey, Busselton, Margaret River, Scott River, Donnybrook, Manjimup/Pemberton and Albany districts to identify and quantify the levels of insect and disease losses.

Only ware (fresh table) and Simplot processing potatoes were included in this survey. Other processing potatoes represent less of the W.A. industry and damage in these crops should be reflected in the results from the survey.

The survey consisted of three components - interview with growers selected at random from each of WA's potato growing regions to complete a questionnaire on a crop about to be harvested; field sampling of that potato crop to assess for insect damage; individual tuber assessment of a sample collected during the in-field assessment of insect damage.

Interview with growers. For each crop and variety surveyed, information was collected on cultural history of the site and crop cultural methods. See Appendix 1 for Cultural History Information Form.

Insect damage was assessed by hand digging 10 x 5m crop row samples. The samples were collected evenly over the paddock, commencing 5 rows from the edge and five metres from the end, then continuing in a 'v' shape over the cropped paddock. Insect losses were assessed on-site by hand sorting. In this category, damage was categorised to one the following pests: whitefringed weevil, African black beetle, potato moth, cutworm and slugs. The percentage loss by weight of potatoes to insects and disease for each crop and variety surveyed was calculated. Tubers were graded according to Western Australia's Potato Marketing Corporation ratings for ware tubers and Simplot Australia for processing tubers - See Appendix 2 for details.

Disease level, in terms of surface diseases of tubers only, to be assessed from individual examination of 25 tubers collected at each of the sampling stations for assessing insect damage, the tubers to be collected adjacent to and in the same row used for the insect damage assessment. Disease levels were assessed off-site on washed tubers, with follow-up laboratory diagnosis where required. All tubers were scored for disease after approx 21 days shelf storage in calico bags.

Disease prevalence was based on the proportion of surface affected by disease and, for Simplot potatoes, the weight of the portion of tuber removed that is affected by non-surface diseases was recorded.

The following diseases were assessed as they were considered to be the most likely to be encountered: black dot, silver scurf, rhizoctonia, common scab, powdery scab, soft rots, early blight and nematodes.

Results

No results or conclusions are presented here. The last of the sampling was completed after 30 June 1997 and the finalisation of this work was included in HRDC project VG97050 which followed on from this project. The collation and analysis of the data is yet to be completed and will form part of the final report of HRDC Project VG97050.

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TECHNOLOGY TRANSFER

Publications

The following is a list of publications by members of this team during the course of this project. The publications cover issues both directly and indirectly related to the project and related issues of soil pest and disease management in horticulture.

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Kirkegaard, J. A., Matthiessen, J. N. & Desmarchelier, J. M. (1995). Biofumigation - using *Brassica* species to control pests and diseases in Australian agriculture and horticulture. Pp. 1225-1227 *in: Proceedings of the 9th International Rapeseed Congress - Rapeseed Today and Tomorrow*. Cambridge.

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Kirkegaard J.A. and Sarwar M. (1998) Biofumigation potential of brassicas. 1. Variation in glucosinolate profiles of diverse field-grown brassicas. *Plant and Soil* (in press).

Kirkegaard, J.A., Wong, P.T.W., Desmarchelier, J.M. and Sarwar, M. (1996) Suppression of soil-borne cereal pathogens and inhibition of wheat germination by mustard seed meal In: Agronomy - Science With Its Sleeves Rolled Up, M. Asghar (ed), Proceedings 8th Australian Agronomy Conference (Toowoomba, Qld: January 30-February 2, 1996) pp. 353-356.

Matthiessen, J. N. (1995). Soil pest and disease management - overseas trends and developments. *Potato Grower*: November: 7.

Matthiessen, J. N. (1995). Metham sodium - just how good is it for whitefringed weevil control? *Potato Grower*: May: 2.

Matthiessen, J. N. (1995). Challenges in implementing IPM for low economic threshold soil insect pests. *International Plant Protection Congress*. The Hague. (*Abstract*).

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Articles:

Brassica break crops gain popularity *Farming Ahead*, January 1994 No.25, pp 20-21.

Benefits of canola in the rotation. *Good Oil*, Feb/March 1994, pp4.

Brassicas - natural soil fumigants. *Rural Research* **163**, Winter 1994 pp 4-8. Compiled by Alan Dick.

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Canola crops boost soil fertility. *Australian Farm Journal*. June 1995 pp 36-37.
Canola found to lift later wheat yields. *The Land*. April 20 1995. pp23.
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Meetings:

John Matthiessen - Grower meetings at Busselton and Manjimup, May 1996. Comprehensive outline of project and findings from overseas travel in 1995.
Diana Fisher - Two talks to industry (Perth and Manjimup), September 1996 on project progress.
Stewart Learmonth - Agriculture WA field day at Manjimup Horticultural Research Centre, February 1997.

Biofumigation Update newsletter

At the commencement of this project, contact was made with researchers in a variety of disciplines related to the management of soil-borne pests and diseases in order to establish a network to facilitate the interchange of information and further develop an understanding of various industries and regions where there would be interest in the pursuit of bioactive rotation crops as a management tool. This group, especially the GRDC-funded research aimed at elucidating the chemical profile of various brassicas and their potential for biofumigation, and in turn the study of the mechanisms by which biofumigant effects were achieved against pathogens and pests, formed a core resource underpinning the concept. It was considered that a network of interested researchers, growers and consultants would allow broader evaluation of the concept and help implement broader applications in a systematic way.

To further this networking idea, a small newssheet, named the 'Biofumigation Update' was produced. An issue was produced approximately every six months, with six being produced during the project. Each issue of the 'Biofumigation Update' is produced in two versions: Horticulture and Cereals. For each issue we have made it so that the front page is identical for both versions, covering issues of general relevance that relate principally to the core underpinning research. The back page is designed to cover aspects related more particularly to the relevant sector.

Interest in the newsletter grew rapidly, through word-of-mouth and by informing people at grower meetings. By the end of the project the Horticulture version was being sent to over 250 people, and the circulation list was still growing. The circulation list ranged broadly across researchers, growers, consultants and industry representatives across Australia, and there were also some international recipients.

A copy of each issue of the 'Biofumigation Update' forms Appendix 1.

Industry liaison

In addition to numerous articles written by the team members and published in industry magazines during the course of this project (see publications list), and regular publication of the 'Biofumigation Update', there have been several presentations at grower meetings, articles produced by journalists on the project and a television story on the major rural program 'Cross Country'.

Overseas visits

John Matthiessen travelled to Europe and the USA in 1995 to attend the International Plant Protection conference and hold discussions with researchers on soil pest and disease management issues in potato production. John

Kirkegaard also attended the International Plant Protection conference and the International Rapeseed conference in Europe in 1995. Stewart Learmonth visited South Africa, during which time he investigated current research on African black beetle. Both John Matthiessen and Stewart Learmonth attended the International Entomology Congress in 1996, with Stewart travelling through Europe and to the USA to study, amongst other things, potato pest management. John Matthiessen attended the European Union ADSAP (Accelerated Degradation of Soil-Applied Pesticides) workshop in Europe in May 1997.

PROJECT MANAGEMENT

Investigators

This project had three investigators:

John Matthiessen, Principal Research Scientist, CSIRO Entomology, Perth, Western Australia
John Kirkegaard, Senior Research Scientist, CSIRO Plant Industry, Canberra, ACT
Stewart Learmonth, Entomologist, Agriculture Western Australia, Manjimup, Western Australia

Scientific Staff

A Post-Doctoral Fellow (Muhammad Sarwar) appointed to CSIRO Plant Industry, Canberra on funds from the Grains Research & Development Corporation to carry out the specialised chemical analysis aspects of the biofumigation work. This HRDC project provided operating and part-time technical support to allow more extensive analyses relevant to horticulture.

Support staff

Two technicians were directly employed on this project:

Mark Shackleton (CSIRO) and Diana Fisher (*née* Doyle) (Agriculture WA).
Additional technical support was provided by staff of the Agriculture Western Australia Manjimup Horticultural Research Centre, CSIRO Plant Industry, Canberra and staff employed casually.

Collaborators

Scientific collaborators

The project received excellent support from colleagues within CSIRO, for such things as chemical analysis advice and facilities and collaboration on individual studies. There was also broad-ranging collaboration with colleagues in other organisations around Australia, much of which was facilitated by and through the 'Biofumigation Update' newsletter. This collaboration and association covered such aspects as obtaining seed of various brassicas and the analysis of effects of biofumigation on pathogens and nematodes. Associations were developed with various collaborators overseas who provided such things as chemical standards, seed and information on key aspects of the chemistry and toxicity of isothiocyanates.

Farmer collaborators

Almost all of the field work associated with this project was carried out on potato growers' properties. Many farmers, too numerous to mention individually, in the Busselton, Manjimup-Pemberton and Albany areas provided access to their crops and land for sampling, experimental and trial work.

CONCLUSIONS

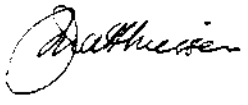
The research carried out by the multi-disciplinary team during this project has provided a high level of basic scientific information on the biology and ecology of soil pests, the chemical profile and biofumigation potential of brassicas, methodology for studying the effects of and mechanisms of biofumigation and other alternative integrated pest management-based strategies for suppression of soil-borne pests and diseases.

This information will soundly underpin continued development of alternative, solidly biologically-based strategies for the sustainable management of soil-borne pests and diseases. The fundamental information obtained on the biofumigation potential of various brassicas is already prompting significant interest amongst seed companies with interests in such lines as fodder brassicas to consider selecting for biofumigant traits. Most *Brassica* breeding has been directed at enhancing the palatability of oilseed and fodder lines through reduction in the concentration of isothiocyanate-precursor glucosinolates. The presence of some lines with very high levels of biofumigation capacity indicate that there is clearly significant potential to move along a pathway of development of specialised lines for biofumigation.

Metham sodium is clearly a highly useful soil-borne pest and disease management agent for potato producers, especially for control of whitefringed weevil which is otherwise intractable. There are, however, concerns about the long-term sustainability of metham sodium use as it is a very broad-spectrum biocide with a likely high capacity for disruption of soil biota and associated ecological functioning in soil. It is also subject to the phenomenon of enhanced, or accelerated, biodegradation which means that repeat usage should be prudently handled. Biofumigation with rotation green manure *Brassica* crops offers an alternative for suppression of soil-borne pests and diseases, perhaps especially in a preventative capacity in areas where pest or disease problems are less severe, leaving the use of chemical fumigation for situations of more chronic infestation.

There is clearly scope for use of chemical insecticides better targetted to the biology of the pest insects. Scouting for whitefringed weevil adults during summer is a useful guide to larval infestations in the following spring and thus a guide to whether application of metham sodium, as the best available means of control, is necessary. The adulticide approach to African black beetle management, by spraying chemical insecticide onto the surface of pasture in early spring to precede oviposition and take advantage of surface crawling activity that has been discovered to occur then, is the apotheosis of the concept of integrated pest management through highly efficiently-directed and minimal use of chemical insecticides.

It is clear that management of soil-borne pests and diseases in potato production needs to rely on a prophylactic approach. Options for implementation of control strategies during crop growth are very limited because of the potential for disruption of the crop's development. This means that a 'think ahead' approach is required, as many critical biological events in the pests' life system occur long before the short-term potato crop is planted. Such an approach is necessary both for more appropriate use of conventional chemical pesticides and for the concept of growing biologically active *Brassica* green manures for biofumigation.



John Matthiessen

Chief investigator

December, 1997

APPENDICES

Appendix 1. Copies of each of the six issues of the 'Biofumigation Update' newsletter produced during the course of this project.

Appendix 2. Copy of letter (unsolicited) received from a potato farmer recipient of the 'Biofumigation Update' expressing positive feedback about the research.

Appendix 3. Pest and disease cultural history information form.

Appendix 4. Tuber grading ratings for pest and disease survey.

HORTICULTURE
Biofumigation Update

No.1 September 1994

Biofumigation Research Project Gets Under Way

Biofumigation is a term that has been coined by **John Kirkegaard** of the CSIRO Division of Plant Industry in Canberra to describe a concept of using *Brassica* plants to control soil-borne pests and diseases in other crops.

The concept is based on the knowledge that, depending on the species or variety, brassicas contain a large array of glucosinolates in a range of concentrations in different plant parts. Glucosinolates are precursors of isothiocyanates (ITC's), which are inimical to many soil-borne organisms. The ITC's are released when plants are physically broken up, and during the breakdown of residues in the soil.

The best known synthetic ITC is metham sodium, which releases methyl isothiocyanate when applied to moist soil. This very expensive fumigant is being used increasingly to control soil-borne pests and diseases in horticulture.

In Australia, the potential benefits of *Brassica* crops was initially recognised when it was observed that wheat crops grew better following canola and Indian mustard compared with other 'break' crops such as linseed or oats.

Laboratory experiments showed that pieces of canola and mustard root emitted volatiles that inhibited the growth of take-all fungus. Chemical analysis by **Jim Desmarchelier** of the CSIRO Division of Entomology in Canberra identified various ITC's being released from the roots and other plant parts.

A joint CSIRO Plant Industry & Entomology research proposal to **Grains Research & Development Corporation (GRDC)** has been supported to carry out basic studies of the types and distribution of ITC's in brassicas, and their biological activity. A post-doctoral fellow to carry out these studies will be appointed on this GRDC funding early in 1995. Applications are currently being considered.

At the same time, a proposal submitted to **Horticultural Research & Development Corporation (HRDC)** by **John Matthiessen** of CSIRO Division of Entomology in Perth, **Stewart Learmonth** of the Western Australian Department of Agriculture at Manjimup, and John Kirkegaard sought support for further developing the biofumigation concept for horticultural applications. Specifically, it was aimed to incorporate it into an integrated pest management program for soil insect pests of potatoes in WA.

Biofumigation network

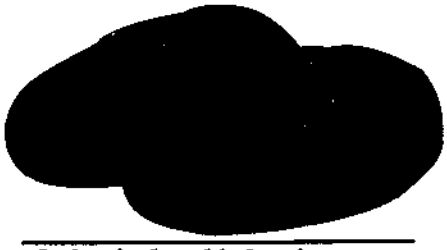
Part of the proposal was also to develop a network to explore other potential horticultural applications. HRDC was keen to speed investigations into disease control applications and John Matthiessen and John Kirkegaard recently visited pathologists in SE Australia to develop associations and potential future collaborations in that field.

CSIRO, with GRDC and HRDC support, is facilitating investigation of the potential of biofumigation for managing soil pests and diseases. The objective is to take it beyond empirical studies to systematically determine the most potent plants and the mechanisms of their impact on specific organisms. Collaboration will then take it to particular production systems.

The News Sheet

This news sheet is aimed at informing researchers who are involved in pest and disease control issues of current developments, specifically in the investigation and application of the biofumigation concept, and rotation or other amendment systems that can play a role in reducing reliance on chemical control agents.

The purpose is to increase awareness of the biofumigation concept and provide a means for information exchange within the biofumigation network.



In horticultural industries, HRDC is supporting research on the biofumigation concept, with the initial emphasis in potato production.

Most emphasis will be in horticulture, and initially this will be focussed on the potato industry. However, broader linkages are encouraged.

The news sheet will be produced on an occasional basis - determined by feedback and interest. Contributions of information, in 'snippets' form (eg. something like the inset on p2), are welcomed.

The co-ordinator is:

John Matthiessen
CSIRO Division of Entomology
Private Bag
PO Wembley WA 6014
Ph: 09 387 0641
Fax: 09 387 0646
E-mail: johnm@ccmar.csiro.au

The Potato Pest and Disease Biofumigation Network

The visit by John Matthiessen and John Kirkegaard to researchers in SE Australia in August 1994 was aimed at establishing personal contact principally with pathologists working in potatoes, and allied areas.

We sought to determine the main disease problems, inform other workers of the biofumigation concept and discuss potential applications for the approach in the future. This background information will help in the choice of the most appropriate post-doctoral fellow for the *Brassica* characterisation studies and ensure testing can be carried out against key organisms.

New South Wales

At NSW Agriculture at Rydalmere, vegetable pathologist **Len Tesoriero** indicated that ***potato early dying*** was becoming a significant problem in the Riverina area. This syndrome appears to result from a synergism between a root-lesion nematode and a fungal disease, principally *Verticillium dahliae*. Len, in collaboration with **Rod McLeod**, a nematologist at Rydalmere has identified *Pratylenchus coffeae* as the nematode associate. This is unusual; overseas *P. penetrans* is associated with early dying.

Rhizoctonia and **Colletotrichum** (black dot) are also a problem in NSW. All of these diseases and nematodes are potential targets for the biofumigation approach.

Rod is already assessing the potential of various brassicas, particularly readily available **fodder and green manure rapes** as inter-row covercrops, for their impact on nematodes in vineyards and is obtaining positive results. As it is likely that these plants have been selected to be comparatively low in glucosinolates, the potential exists to use more potent selections for nematode control.

Such identifications will be carried out by the post-doctoral fellow working with John Kirkegaard in Canberra.

Victoria

Powdery scab is the main disease problem in Victoria. Control strategies are being investigated by **Dolph de Boer** and **Megan Theodore** at the Department of Agriculture Horticultural Research Institute, Knoxfield, who are testing brassicas as hosts of the disease.

It was pointed out by **Paul Horne**, an entomologist at HRI that Victoria has many potato-growing systems. Chemical fumigation is mostly uneconomic. The soil insects whitefringed weevil and wireworm are also a problem to potatoes in Victoria. The economics and array of diseases and pests suggest potential for break crops with biofumigation activity in Victoria.

Also in Victoria, **Jillian Hinch**, a nematologist in Plant Sciences and Biotechnology of the Department of Agriculture at La Trobe University has carried out studies on the use of organic amendments for nematode control, and has some previous experience with brassicas.

South Australia

Rhizoctonia is the major disease of potatoes in SA and **Trevor Wicks** at Primary Industries SA, Northfield sees potential for the biofumigation approach as a means of control in SA.

The capability now exists through the work of **Dara Whisson** in SA Research & Development Institute to use molecular probes to identify and quantify Rhizoctonia groups in soil. Considerable work is also being carried out on Rhizoctonia in cereals in SA at CSIRO Division of Soils.

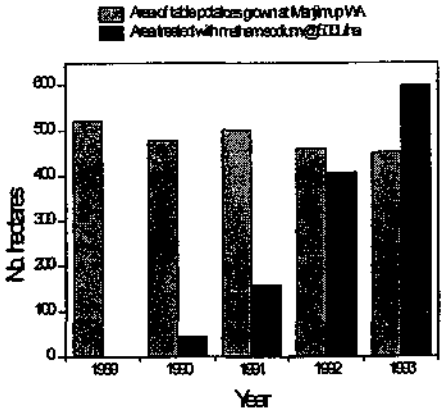
The nematology group of **Maria Scurrah** at SARDI also showed interest in the concept for nematode control.

Pam Strange, from consultants Scholefield Robinson Pty. Ltd., reports whitefringed weevil is becoming a serious pest on some potato-growing properties near Adelaide and metham sodium use is dramatically rising.

Tasmania

Most of the well-known soil-borne potato diseases such as **Rhizoctonia, common and powdery scab and black dot** are present in Tasmania.

Roland Laurence of the Department of Primary Industry and Fisheries at Devonport and **Calum Wilson** at DPIF in Hobart see a potential role for rotation and break crops in ameliorating diseases in potatoes and other horticultural crops. Work is being carried out in association with **Ian Macleod** of Serve-Ag Pty. Ltd. at Devonport in northern Tasmania.



Use of chemical control agents for soil-borne pests and diseases continues to be higher than the potato industry desires.

Although soil insect control has been forcefully moved away from use of residual cyclodiene insecticides, a clear trend in WA has been the adoption of metham sodium for control of whitefringed weevil.

The effective control offered by synthetic methyl isothiocyanate suggests whitefringed weevil as a potential target for biofumigation with naturally-occurring ITC's. Control of an insect in this way is novel and will require good understanding of its ecology and the characteristics and effects of biofumigant plants.

However, success against a large organism like an insect should bode well for success against microorganisms.

John Matthiessen & Stewart Learmonth.

HORTICULTURE
❖Biofumigation Update❖

No. 2 | *Compiled by John Matthiessen & John Kirkegaard* | May 1995

Postdoctoral fellow appointed

Dr **Muhammad Sarwar** took up his position as Postdoctoral Fellow on the Biofumigation Project at CSIRO Division of Plant Industry on 24 April. Dr Sarwar's position is funded by GRDC and HRDC is contributing to technical and operational support.

Dr Sarwar received his Bachelor and Masters degrees from the University of Agriculture, Faisalabad, Pakistan where his research interest was the efficiency of phosphorus uptake from soil. He received his PhD from the University of California, Riverside in 1993 where he investigated the evolution of auxins following microbial degradation of their precursors in soil, and the subsequent stimulation of crop growth.

During his PhD studies Sarwar identified the most appropriate concentration of auxin precursors, determined the micro-organisms responsible, measured the environmental conditions in which the evolution of auxins was maximised and assessed the effect on the growth of maize. Following graduation he took up a Postdoctoral fellowship at the University of Missouri, Columbia, where he was involved in a number of projects including further studies on differential effects of microbially-derived auxins on crop and weed species, overproduction of auxins in orchard soils following pesticide application and identification of allelopathic phenolics from vegetative parts of sorghum.

Sarwar's background combines extensive analytical skills including HPLC and GC-MS for analysis of complex molecules in plants and soil with wide experience in soil microbiology. These will be valuable skills in the screening of brassicas for glucosinolate content and subseqent release of ITC's in soil.

We take this opportunity to welcome Sarwar, his wife Saira and his daughters Zain (6) and Nayob (1).

***

The grains and horticultural (potato sector) industries, are supporting research on the biofumigation concept through GRDC & HRDC.

GRDC

GC - MS facility prepared

During his first weeks Sarwar has spent several days with **Jim Desmarchelier** of CSIRO Division of Entomology discussing and preparing analytical equipment for analysis of glucosinolates and ITCs in soil and plant material. They have purchased a sulphur column and will have a dedicated GC for the project.

Brassica collection

John Kirkegaard travelled to VIDA, Horsham recently to meet with **Phil Salisbury** (Breeder) and **Allan McIntyre** (Curator, Temperate Field Crops Collection) to discuss and assemble an appropriate range of *Brassica* types for inclusion in the screening for glucosinolate content and ITC release during 1995/96.

The day was very productive and together with seeds supplied by **Rex Oram** (CSIRO Plant Industry), **Percy Wong** and **Rod McLeod** (NSWA), a range (80) of brassicas representing all of the major species has been assembled. They will be screened in field and glasshouse studies for the type, concentration and distribution of glucosinolates in their tissues, and evolution of ITC's will be determined.

The principal compiler of the Horticulture Biofumigation Update is:

John Matthiessen
CSIRO Division of Entomology
Private Bag
PO Wembley WA 6014
Ph: 09 387 0641
Fax: 09 387 0646
E-mail: johnm@ccmar.csiro.au

Contributions are welcome and will be appropriately acknowledged.

Potato Pest and Diseases

Relative toxicity of fumigants to whitefringed weevil

As reported in the first Biofumigation Update, the current favoured method for controlling the soil insect whitefringed weevil is the fumigant metham sodium, and use for this purpose has risen sharply in the last few years.

Despite the general success of metham sodium as a control agent for whitefringed weevil, nothing is known about the relative toxicity of its active product methyl isothiocyanate (MIT), nor that of other known or putative fumigants, to the insect.

Field trials have shown that the usual quantity of metham sodium required to most reliably prevent damage in potatoes is 500l/ha, at a cost of around $700/ha.

Recently, the groups led by **John Matthiessen** and **Jim Desmarchelier** at CSIRO Division of Entomology in Perth and Canberra, respectively, jointly carried out *in vitro* tests of four fumigant chemicals against just-hatched whitefringed weevil larvae.

The spring-planted potato crop is most susceptible to whitefringed weevil damage because the larvae are active and growing during late spring and early summer. Pre-planting treatments are applied in late winter when whitefringed weevil populations are predominantly in the first larval stage, making the tests relevant to the field situation.

The fumigants tested were pure MIT, methyl bromide (MeBr), carbon bisulphide (CS2) and carbonyl sulphide (COS). The first three are fumigants of long standing, while CSIRO has recently received a usage patent for the fourth as an environmentally-friendly alternative to methyl bromide.

Figure 1 shows the extremely high toxicity of MIT to whitefringed weevil larvae compared to the other fumigants. The LD95 is around 1.5 mg/l/h.

However, assuming uniform application through the top 25cm of soil, the theoretical concentration of the active MIT produced by 500l/ha metham sodium is almost 48 mg/l of soil.

The large discrepancy results from the particularly high rate and degree of sorption of MIT by soil. From 500l/ha metham sodium, only about 1% of the MIT remains free within about 2h of its formation.

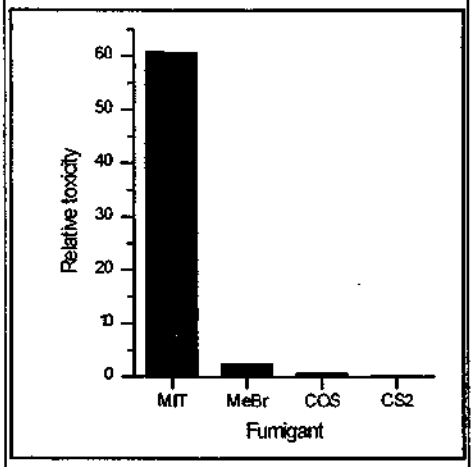


Figure 1. Relative toxicity of fumigants to whitefringed weevil larvae.

Figure 2 shows the relative extent of sorption of the fumigants by three contrasting soils from WA potato-growing areas. Soil with high organic matter content was most sorptive.

These data provide benchmarks against which to assess the efficacy of *Brassica*-derived isothiocyanates.

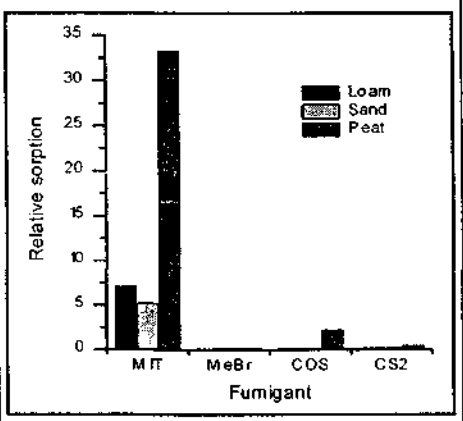


Figure 2. Relative sorption of fumigants by three soils.

Indian mustard kills potato Rhizoctonia

Recently, **Trevor Wicks** and his team at the South Australian Research & Development Institute in Adelaide have tested the effect of Indian mustard, *Brassica juncea*, against potato *Rhizoctonia*.

Indian mustard is noted as a high producer of isothiocyanates, and seed meal has given the best inhibition of *Rhizoctonia*.

In *in vitro* tests, the vapours arising from as little as 0.1g seed meal per plate gave 100% inhibition of growth in fungal colonies. Freeze-dried plant material was also tested but at 2g of plant material only 50% inhibition of growth was obtained.

A small field test assessed the effect of Indian mustard as a green manure. Plants were ploughed into the ground, some with seed meal added, and tubers with sclerotes were planted.

After four weeks the tubers were removed and the sclerotes assessed for viability. The plants with meal added to the soil had the greatest effect on sclerotial viability, decreasing it from 76% to 20%.

Field tests using seed meal and Indian mustard as green manure are continuing.

US group finds big variation in isothiocyanate potencies

A research team in Idaho has recently found that the toxicity of aromatic isothiocyanates to eggs of the black vine weevil is around 50x greater than those containing an aliphatic moiety. Methyl isothiocyanate from metham sodium is an aliphatic isothiocyanate.

Both types occur in brassicas and the profiling work being undertaken by Sarwar will identify species or cultivars containing the most potent forms.

HORTICULTURE
❖Biofumigation Update❖

No. 3 *Compiled by John Matthiessen & John Kirkegaard* December 1995

UK *Brassica* specialist visits Australia

Dr **Richard Mithen**, Project Leader in the *Brassica* and Oilseeds Research Department, John Innes Centre, Norwich, UK recently spent two weeks in Australia on a visit organised by **John Kirkegaard**. While here, he visited research groups involved in *Brassica* research in Canberra, NSW, Victoria and South Australia.

Richard's group has spent several years investigating the genetic control of glucosinolate biosynthesis in *Brassica* species and the effect of glucosinolates on pest and pathogen interactions. They have been able to successfully identify and map the genes in *Brassica napus* (canola) which regulate the aliphatic glucosinolate profiles in the leaves. They are now attempting to clone the same genes from *Arabidopsis* (a cruciferous plant used in genetic studies) in order to manipulate the glucosinolate profiles of other *Brassica* species.

Richard's team has been primarily interested in manipulating the glucosinolate levels in leaves of canola to reduce attack by pigeons, slugs and hares. They have developed a canola variety with a four times greater level of glucosinolates in the leaves than commercial canola, without altering seed levels. The types of glucosinolates are also more similar to those in mustard leaves. This has reduced bird and slug damage significantly and these varieties are currently being multiplied for commercial use.

The implications of Richard's group's work to biofumigation studies are significant. Genetic manipulation may provide opportunities to increase the levels of the most potent glucosinolates in root or shoot tissues to enhance the biofumigation effect while not altering seed levels. **Mark Potter** (Waite Institute) will work with Richard's group in Norwich in 1996 investigating the potential to make similar changes to Australian spring varieties.

Richard has made available seed of these high glucosinolate varieties to determine if biofumigation potential is enhanced. CSIRO will be including them in field trials next year and Richard hopes to return to continue collaboration. Thank you to all those who provided accommodation for Richard, which made the visit possible. It has set the basis for many fruitful collaborations.

The principal compiler of the Horticulture Biofumigation Update is:
John Matthiessen
CSIRO Division of Entomology
Private Bag
PO Wembley WA 6014
Ph: 09 387 0641 Fax: 09 387 0646
E-mail: johnm@ccmar.csiro.au
Contributions are welcome and will be appropriately acknowledged.

Evidence points to in-crop biofumigation

Recent findings from different research projects suggests that a significant biofumigation effect may result from root exudation during the growth of *Brassica* crops, rather than following incorporation of mature residues.

John Kirkegaard (CSIRO Plant Industry, Canberra) has shown that suppression of fungal pathogens in vitro by mature residues was minor, consistent with the low ITC release by such tissue, while **David Roget** (CSIRO Soils, Adelaide) found significant reduction in take-all inoculum in soil by living, non-incorporated, mustard.

Recently, **Jim Gardiner** (University of Idaho, Moscow, Idaho) has shown that isothiocyanates (rather than glucosinolates) occur in soil where *Brassica* plants are growing. One suggestion is that glucosinolates are exuded by the roots and quickly hydrolysed by myrosinase (perhaps microbial) to release the ITC's.

Glucosinolate analyses under way

Screening of 84 different *Brassica* lines for glucosinolate content of the root, shoot and seed tissues is underway at CSIRO Plant Industry, Canberra.

The autumn-sown field experiment is approaching maturity and the spring sowing is in flower. In addition to field grown plants, a subset of 11 species has been grown under different conditions in the glasshouse to determine the effect of different environments on glucosinolate content.

Muhammad Sarwar has calibrated an HPLC for analysis of the glucosinolates and anticipates having all 700 samples from the current screening analysed by February 1996. The information on glucosinolate profiles of the various species, particularly the root tissue, will be some of the first done on field-grown plants and will provide a sound basis for selecting *Brassica* types with greatest potential for biofumigation.

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

GRDC

Horticulture Pests and Diseases

Mustard green manure reduces bacterial wilt

There is some evidence that decaying residue of *Brassica* plants may reduce the level of inoculum, and the subsequent incidence and severity, of bacterial wilt.

Bacterial wilt (*Pseudomonas solanacearum* E.F. Smith) is a serious soil-borne disease of many crops including tobacco, potato, tomato, banana and groundnuts. Representatives of more than 35 families of plants are affected by the disease, especially under tropical and sub-tropical conditions.

Steve Akiew and **Peter Trevorrow**, Queensland Department of Primary Industries, Mareeba, have recently studied the effect of organic amendments on disease incidence.

Brassica and tobacco plants were chopped into pieces before mixing with soil containing approximately 107 colony forming units per gram of soil of a 'tagged' strain of *P. solanacearum*. An agar medium selective to this strain was used to monitor the survival of the bacterium over a period of six weeks, and tomato seedlings were transplanted to assess disease development.

The survival of the bacterium in the soil was reduced with either mustard or canola amendment. Among the *Brassica spp.* tested, *B. juncea, B. rapa and B. nigra* reduced the population of the bacterium to a non-detectable level after four to five weeks of incubation. *B. napus* suppressed the population to 40% of the control.

The tomato plants in the glasshouse wilted after two weeks without amendment or with tobacco residue. In contrast, survival was 100% and 53 % following mustard and canola, respectively. In the field, mustard and canola reduced disease incidence by 59% and 28%, respectively.

So far, the studies show incorporation of mustard, particularly *B. juncea*, to significantly reduce the level of inoculum and the severity of bacterial wilt at 5% to 10% rates of incorporation. The low rate (1.0%) used in the field , which is equivalent to 4 tons of plant residue probably accounted for the higher disease incidence. Steve and Peter are now aiming their research to integrate biofumigation with approaches involving biological control, crop rotation and the use of disease resistant plants.

Steve Akiew can be contacted on 070 928555.

*An interesting corollary to this work in tropical areas is that high glucosinolate Brassica juncea lines bred by **Rex Oram** at CSIRO Plant Industry were effective, but tended to flower early without producing large amounts of vegetative material, and were also host to the disease. Lines that are do not flower in long days or have a vernalization requirement may be appropriate for tropical situations as they would tend to remain vegetative and potentially produce more glucosinolates.*

*- **John Kirkegaard**, CSIRO Plant Industry, Canberra.*

Brassica effects on nematodes

Postgraduate student **Mark Potter** (Waite campus, University of Adelaide), in collaboration with **Trevor Wicks, Robin Harding** and **Robin Thrum** (SARDI) have recently measured positive effects of *Brassica napus* Rangi rape green manure in reducing *Pratylenchus* nematodes in potato-growing land in the Adelaide Hills.

Figure 1 shows the early results where green manuring of Rangi rape was compared with that of oats. The site was sandy and well-infested with nematodes. An adjacent potato crop the previous year had suffered heavily from the symptoms of early dying, in which nematode infestation is involved.

Mark Potter can be contacted on 08 3037216.

Overseas trends in use of synthetic fumigants

Figures obtained by **John Matthiessen**, CSIRO Entomology, Perth, on a recent visit to the Netherlands, show synthetic fumigant use there is in sharp decline.

The Dutch have a legislated program to halve 1984-88 levels of overall pesticide use, and reducing emissions to the environment beyond the target site by 90%, by the year 2000.

Of the fumigants. methyl bromide is banned, leaving metham sodium and 1,3 dichloropropene (1,3-D), (Telone II) as the most common agents. By law, fumigation of a site is allowed only once in four years. This will become once in five years in 2000.

To date, total pesticide use has declined by 50%, with most of the reduction resulting from a sharp decline in the use of fumigants, down 75% against a target of 45% (Fig. 2).

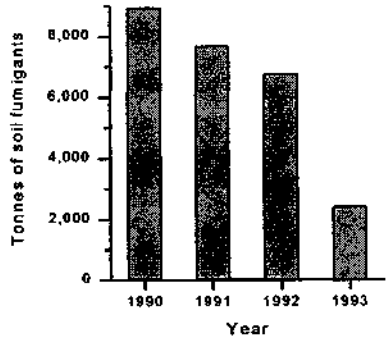


Figure 2. Recent trend in soil fumigant use in the Netherlands.

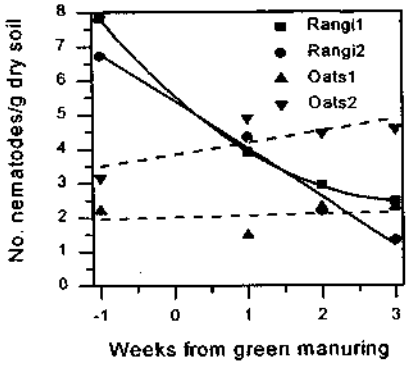


Figure 1. Effect of Rangi rape and oats green manures on *Pratylenchus*.

HORTICULTURE
◆Biofumigation Update◆

No. 4 *Compiled by John Matthiessen & John Kirkegaard* June 1996

Glucosinolate analysis of *Brassica* collection completed

Measurement of glucosinolates, the precursors of the bioactive isothiocyanates, in the roots and shoots of over 100 different brassicas has been completed by **Muhammad Sarwar** and **John Kirkegaard**, CSIRO Plant Industry, Canberra.

The brassicas assayed included oilseed, fodder, weed and vegetable types relevant to the total Biofumigation Project (cereals and horticulture).

The main collection was sown in the field at CSIRO's Ginninderra Experiment station in autumn 1995, while a subset of 11 diverse species was sown there in spring, and under three different controlled-environment glasshouse conditions.

Shoot and root material was freeze dried and analysed by HPLC to detect levels of 20 different glucosinolates.

Plants were sampled at buds visible, flowering and maturity, and seed was also collected for analysis.

Preliminary analysis of the data allows some general conclusions:

(1) Concentration of all glucosinolates declined as the plants matured and was very low in dry mature tissue.

(2) The overall glucosinolate profiles of field-grown plants were similar to those of plants grown under controlled conditions, with the exception of some minor glucosinolates found only in the field-grown plants. The actual concentrations found in the tissue varied in different environments.

(3) Glucosinolate concentrations varied between species and also within species. Table 1 shows the range of major ITC-releasing glucosinolates in the roots and shoots of different species from the autumn field sowing.

The ten-fold range within species indicates that there is great potential to select or develop brassicas with high levels of glucosinolates for biofumigation.

(4) Generally winter *B. napus* (canola) varieties had higher glucosinolate concentrations than spring varieties. Both Oscar and Dunkeld were notable for the low level of shoot and root glucosinolates compared to European winter canola types although variation within Australian spring varieties was evident.

Sarwar is currently collating the large data set for publication. Meanwhile he will concentrate on testing pure isothiocyanates for toxicity to several fungal pathogens.

He is currently working with **Jim Desmarchelier**, CSIRO Division of Entomology calibrating a GC for detection and measurement of the ITC's and preparing for the *in-vitro* fungal tests that will commence in July.

Table 1. Range of glucosinolate types and concentrations (μmol/g) in *Brassica* species at flowering (autumn-sown field crop).

	Shoot			Root	
	Propenyl	Butenyl	Pentenyl	Phenylethyl	OH benzyl
B. napus (oilseed)	0	0-3	0-8	2-19	0
B. napus (fodder)	0	1-9	1-5	7-20	0
B. juncea (oilseed)	0-19	0-8	0	3-13	0
B. nigra	11-26	0	0	1-3	0
S. alba	0	0	0	0-4	3-4
Wild turnip	0	35	1	26	0

IAMA group visits CSIRO, Perth

A 50-strong group of IAMA consultants visited the CSIRO laboratories at Floreat, WA during their national conference in Perth in March, 1996. **John Matthiessen** CSIRO Entomology, outlined the biofumigation work and added many names to the Biofumigation Update mailing lists.

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

GRDC

The principal compiler of the Horticulture Biofumigation Update is:

John Matthiessen
CSIRO Division of Entomology
Private Bag
PO Wembley WA 6014
Ph: 09 387 0641 Fax: 09 387 0646
E-mail: johnm@ccmar.csiro.au

Contributions are welcome and will be appropriately acknowledged.

Horticulture Pests and Diseases

Glucosinolate degradation products in soil

Studies carried out by **Jim Gardiner**, University of Idaho, Moscow, Idaho and **Charlotte Eberlein**, U. of I. Experiment Station, Aberdeen, Idaho have identified glucosinolate degradation products in soil after *Brassica* breakdown.

Rape (*B. napus*) was sown in autumn and ploughed under in spring. Soil samples were taken at intervals over the next three weeks. The soil samples were extracted with methylene chloride and analysed by GC-MS.

Plant samples were also taken at plough-down and these too were analysed by GC-MS.

Nine glucosinolate degradation products were identified in the soil samples (Table 2).

Products of the degradation of 2-phenylethyl glucosinolate accounted for 87% of the total, by molarity. Products of the degradation of the glucosinolates found principally in the roots accounted for 93% of the total.

It is interesting that root glucosinolates should play so preponderant a part in the degradation products in the soil, virtually to the exclusion of the above-ground parts of the plant. This is something to be kept in mind by breeders, who tend to rely mostly on assays of leaf and seed tissue in their selection programs.

Why the shoot glucosinolates left so little trace remains a question. They may have degraded to simpler forms - sulfur and nitrogen gases - which were not being looked for, or maybe they never escaped into the soil in the first place. A third possibility is that they degraded to compounds that bind to the soil in forms inaccessible to the extractant.

Mark Potter, University of Adelaide, suggests isothiocyanates with short chain alkane R-groups may be more prone to bind to soil organic matter. (**John Matthiessen** and **Jim Desmarchelier** have shown methyl isothiocyanate to sorb heavily to soil - see *Biofumigation Update* No. 2).

From the vantage of the plant it would make sense that the roots should be dominated by a glucosinolate (2-phenylethyl) that produces volatiles that do not bind, and thus remain free to permeate the soil to the detriment of its other inhabitants, some of which are potentially antagonistic to the plant.

- contributed by ***Jim Gardiner***, University of Idaho, Moscow, Idaho.

Table 2. Glucosinolate degradation products identified in soil.

Compound	Parent glucosinolate	Where it mainly occurs
Isothiocyanates:		
2-phenylethyl	2-phenylethyl	roots
3-butenyl	3-butenyl	shoots & roots
4-pentenyl	4-pentenyl	shoots & roots
4-methylthiobutyl	4-methylthiobutyl	roots
5-methylthiopentyl	5-methylthiopentyl	roots
Nitriles:		
benzenepropanitrile	2-phenylethyl	roots
5-methylthiopentanitrile	4-methylthiobutyl	roots
6-methylthiohexanitrile	5-methylthiopentyl	roots
Oxazolidinethione:		
goitrin	2-hydroxy-3-butenyl	shoots & roots

Further basic studies on fumigant effects against soil pests under way

Recent studies by **John Matthiessen** and **Jim Desmarchelier**, assisted by **Mark Shackleton** and **Le Trang Vu** established baseline toxicity of several fumigants, including methyl isothiocyanate, against whitefringed weevil larvae *in vitro*, and their sorption characteristics in contrasting soils (see *Biofumigation Update* No. 2).

Studies planned to commence in late June will be aimed at determining temperature effects on toxicity of the fumigants *in vitro*, and testing methods for assessing their toxicity *in vivo*, to gain a meaningful evaluation of likely impacts in the field.

A significant logistical problem in conducting *in vivo* studies of the small (<1mm long) early-stage larvae is extracting them from the soil in a non-injurious way to accurately determine mortality. Future tests of biofumigants against insects in both the laboratory and field mean that it is essential to overcome this constraint.

These studies will test a fine mesh caging system developed by John and Mark which it is hoped will allow realistic soil insect mortality tests.

Update circulation

The circulation list for the ***Horticulture Biofumigation Update*** has grown to about 140 names, and includes researchers, extension workers, farmers and consultants.

We are keen to include snippets of practical-experience information from farmers and consultants, to broaden the content of the *Update*. Also, additions to the mailing list are welcome.

Please phone, fax or mail any contributions, no matter how small, or requests to be on the mailing list, to John Matthiessen.

HORTICULTURE
Biofumigation Update

No. 5 *Compiled by John Matthiessen & John Kirkegaard* December 1996

Biofumigation potential of Brassicas

The biofumigation potential of 75 entries from 13 different *Brassica* and related weed species sown at Canberra in autumn 1995 has been assessed by **Muhammad Sarwar** and **John Kirkegaard**, CSIRO Plant Industry, Canberra. Glucosinolate (GS) concentration in root and shoot tissue was measured at flowering. Fig. 1 shows the large variation both within and between the species for the total (root + shoot) glucosinolate production on a ground area basis.

Some had high GS production because of high tissue concentration, others from high biomass. Root and shoot GS levels were not correlated with seed levels. Selection for higher levels in root and shoot to enhance biofumigation should be possible without compromising seed quality.

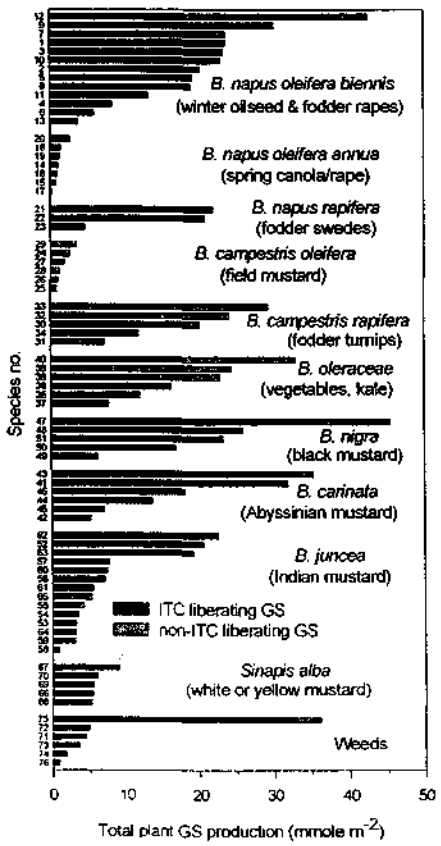


Fig. 1. Glucosinolate variation within and between species.

Shoots contained mainly aliphatic GS's. Aromatic GS's, particularly 2-phenylethyl, dominated in roots. Root GS's averaged 26% of total plant GS's and may be more important for biofumigation than previously thought (see **Mark Potter's** article). Roots would be the main source of GS's for biofumigation in rotation or companion crops where shoot material is not incorporated into the soil.

The type of GS's present was similar within, but varied between, species. *B. napus, campestris & oleracea* were high in hydroxylated GS's which do not release isothiocyanates (ITC's). *B. nigra, carinata, juncea* and weeds had mainly ITC-liberating GS's, particularly 2-propenyl, 3-butenyl and hydroxybenzyl.

The variation in the biomass, GS profiles and concentrations in both root and shoot tissues provides scope to select or develop Brassicas with enhanced biofumigation potential.
- ***John Kirkegaard***, CSIRO, Canberra (06 246 5080).

Caution on *Pythium*

Laboratory experiments by **Peter Stephens** and **Bill Davoren**, CRC for Soil & Land Management, Adelaide showed Indian mustard inhibited vine-infecting pathogens such as *Pythium* and *Phytophthora*.

However, field trials of mustard and canola incorporated into soil at 50% flowering in two vine nurseries showed increased *Pythium* propagules and reduced strike of cuttings. It took about 60 days for propagule abundance to decline to near starting levels.

Recent studies by **John Kirkegaard** and **Percy Wong** showed *Pythium* was the least sensitive fungus to volatiles from mustard *in vitro*.
- ***Peter Stephens*** (08 8303 6703).

Non-glucosinolate pest suppression by Brassicas?

The volatile aliphatic ITC's that occur in the leaves of *Brassica* species are known to suppress pests and diseases in laboratory assays.

Recent studies amending soil with leaf tissues suggest that leaf glucosinolates are not the only factor imparting a suppressive quality to *Brassica* leaves for root lesion nematodes, as illustrated in Fig. 2.
- ***Mark Potter***, Waite Campus, University of Adelaide (08 8303 6737).

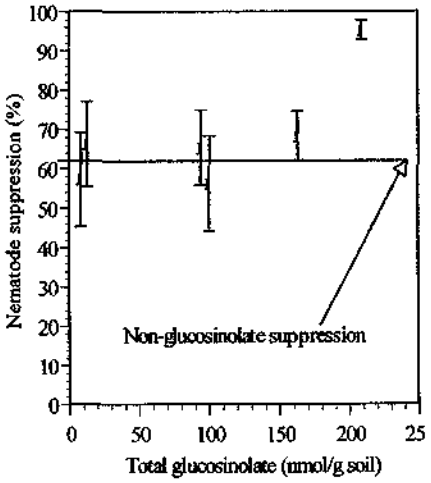


Fig. 2. Nematode suppression by *Brassica* leaf tissue.

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GRDC

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CSIRO Division of Entomology
Private Bag
PO Wembley WA 6014
Ph: 09 387 0641 Fax: 09 387 0646
E-mail: johnm@ccmar.csiro.au
Contributions are sought and will be appropriately acknowledged.

Horticulture Pests and Diseases

Mustard meal volatiles kill soil insects

Recent assays by **John Matthiessen**, assisted by **Mark Shackleton**, CSIRO Entomology, Perth show that volatiles from mustard seed meal in contact with water are highly toxic to whitefringed weevil (WFW) larvae. In contrast, canola meal was very weak.

Unlike some soil-dwelling weevils, such as black vine weevil, newly-hatched larvae of whitefringed weevil (1mm long) are hardy, and survive without food or shelter, thus making *in vitro* experimentation possible.

Median longevity of the larvae is 35 days at 10°C, falling to 25 days at 5°C and 15 days at 15°C. Soil temperatures in Western Australian potato-growing regions are around 10°C during winter when the first-stage larvae dominate the population for many weeks, and around 15°C in early spring when metham sodium fumigation control treatments are being applied in readiness for cropping.

The assays were carried out at 15°C, by exposing larvae in sealed glass jars to only the volatiles being emitted by hydrolysing seed meal for 24h. The larvae were then transferred to clean containers and checked for mortality at 24h intervals for up to 7 days.

Mortality amongst the larvae rose with time, generally plateauing at around 96h, so this was adopted as the end-point of the assay. Results are shown in Fig. 3.

The next step will be *in vivo* assays where the insects are caged in soils of various sorptive capacity.
- ***John Matthiessen***, CSIRO, Perth (09 387 0641).

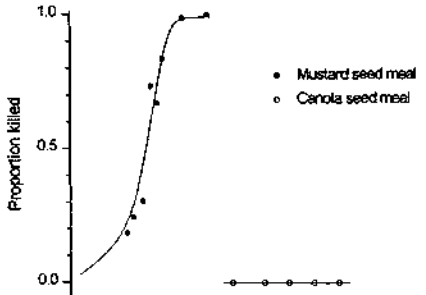

Fig. 3. Toxicity of mustard and canola seed meal to whitefringed weevil larvae. Note log scale on x-axis.

Recent papers

Kirkegaard, J A, Wong, P T W & Desmarchelier, J M (1996). *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathology* **45**: 593-603.

Matthiessen, J N, Desmarchelier, J M, Vu, L T & Shackleton, M A. (1996). Comparative efficacy of fumigants against hatchling whitefringed beetle larvae, and their sorption by soil. *Journal of Economic Entomology* **89**: 1372-1378.

Contact John Kirkegaard or John Matthiessen for reprints.

Test plots growing in WA

Small test plots of about 20 different brassicas, selected by **John Kirkegaard**, and aimed at gaining broad information on development and growth in mediterranean potato regions, are growing this year at the AgWA Manjimup Horticultural Research Centre, and at the Busselton farm of **Keith & Paula Taylor**.

Plantings were made in May and September. Growth of many, especially in the deep sandy loam potato soils at Busselton, and without irrigation, has been spectacular. Growth data will be linked to **John Kirkegaard** and **Sarwar**'s glucosinolate analyses to gauge biofumigation potential. - ***John Matthiessen***.

Seminars update potato industry in Western Australia

In early September, the Potato Growing Industry Trust Fund of WA and HRDC sponsored two seminars for potato growers. Organised by **Dennis Phillips** of Agriculture Western Australia (AgWA), they covered a wide range of potato production issues by many speakers.

With John Matthiessen (CSIRO) and Stewart Learmonth (AgWA) absent at the International Congress of Entomology, Stewart's assistant **Diana Doyle** filled the breach admirably. Growers were updated on insect pest management, including the biofumigation research.

Brassica rotations popular in NW Victoria

Tony Kourmouzis **of T & T Kourmouzis Irrigation & Crop Monitoring Service, Swan Hill, Victoria reports:**

Within the groups of farmers we are working with, it would appear that brassicas certainly have a place.

In particular, a few of the larger vegetable producers have taken to growing canola and forage rape as green manure crops over the colder months in a big way. One grower grew approximately 200 acres in 1996 for the purpose of turning it back in.

They are happy with the growth of canola and forage rape, bulk matter appears to be sufficient and they are happy to utilise them as green manure crops in rotation with the commercial crops. They are also used as inter-row green manure in grapevines.

The opportunity of growing varieties that are more active has been discussed, and would certainly be tried. However, a number of factors would need to be considered, such as the effects on subsequent crops, seed germination and emergence of commercial crops, plant-back periods, etc. Having more information regarding these factors would be beneficial.

Could brassicas suppress soldier fly in sugarcane?

Sugarcane soldier fly *Inopus rubriceps* is a pest in Queensland and northern NSW. Larvae suck the roots, causing poor growth and ratoon failure. The last effective insecticide was the highly residual dieldrin. Prolonged fallow, which is difficult and expensive to maintain, is the only currently available management tool.

Brassicas are being investigated for their activity against soldier fly, as a possible bioactive alternative to fallow for disinfesting fields before planting cane, by **Peter Samson**, BSES, Bundaberg (071 593228). The work forms part of a Sugar Research & Development Corporation-funded project investigating management methods for soldier fly.

Horticulture
Biofumigation Update

CSIRO AUSTRALIA

No. 6 *Compiled by John Matthiessen & John Kirkegaard* May 1997

Biofumigation potential of brassicas

During 1995 and 1996, a range of different *Brassica* species was grown in small plots at Canberra, by **John Kirkegaard**, and at two sites in the horticultural regions of south-western Australia - coastal Busselton and inland Manjimup, by **John Matthiessen**.

Glusosinolate (GS) analyses were carried out by **Muhammad Sarwar** on the shoots and roots of Canberra plants, and shoot and root biomass at flowering was measured at all sites. The GS concentration values obtained in Canberra were applied to the plants grown in WA.

Figure 1 shows the estimated concentration of isothiocyanate (ITC)-producing GS's that could ***potentially*** occur in soil, assuming a uniform distribution and total breakdown in the top 25cm.

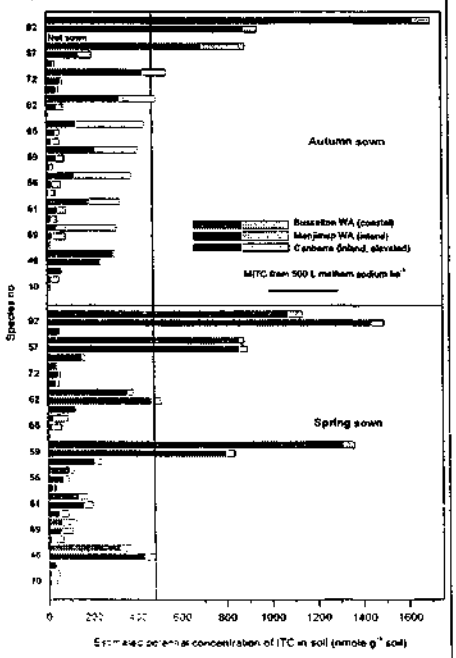

Fig.1. Estimated potential production of ITC's by several brassicas grown in two seasons at three locations, relative to metham sodium fumigant.

To obtain some indication of what this potential means, the concentration of methyl ITC from 500 L metham sodium/ha, also assuming full breakdown and uniform dispersal through the top 25 cm is shown for comparison. A soil bulk density of 1.35 g/cc was assumed.

It can be seen from Fig. 1 that some species grew much better in different locations. The climatically milder WA sites favoured vigorous growth of most species, especially the mild coastal site at Busselton. There were also differences in the plants' growth between autumn and spring sowings.

At this stage, the species are numbered for commercial confidentiality reasons for some. Not all are commercially available. Number 92, the apparent peak performer, is a white mustard originating from Germany. Of the other ten species shown, eight are fodder rapes, kales or swedes, one is an Indian mustard and one is a canola.

Of interest (and some frustration for potential biofumigation applications) is that at least two of the better-performing fodder rapes are no longer on the market. They have been replaced by improved varieties - assumed to be *lower* in GS's, explaining their better fodder attributes.

It must be remembered that these figures are derived from multiplying concentration x biomass, for the total ITC-producing GS's. The analysis takes no account of the different ITC profile in each species, and the likely different toxicities of those different ITC's to pests and diseases.

- ***John Matthiessen***, CSIRO, Perth (08 9333 6641 (note new number) & ***John Kirkegaard***, CSIRO, Canberra (06 246 5080).

Senior GRDC fellowship for John Kirkegaard

John Kirkegaard, CSIRO Plant Industry, Canberra has been awarded a GRDC Senior Fellowship to work with **Richard Mithen** at the John Innes Centre, Norwich UK from July-December 1997.

John departs in late May, visiting the group at Moscow, Idaho on the way.

At Norwich, John will focus on measuring the volatile ITCs released by growing and decaying canola and mustard roots. The levels of ITC's exuded will be compared to GS concentrations in intact tissue.

An attempt will also be made to determine the relationship between ITC exudation and pathogen suppression by inoculating pots.

The *Brassica* populations used will be carefully selected to assess potential to genetically enhance ITC levels - Richard's main research interest.

John's address will be: Brassica & Oilseeds Department, John Innes Centre, Norwich NR4 7UH, UK. Fax: +44 1603 259882.

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HRDC GRDC

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John Matthiessen
CSIRO Entomology, Private Bag
PO Wembley, WA 6014
Ph: 08 9333 6641 Fax: 08 9333 6646
E-mail: johnm@ccmar.csiro.au
Contributions are sought and will be appropriately acknowledged.

Horticulture Pests and Diseases

Biofumigant cover crops for vineyards

A cover crop research program is being undertaken by South Australian seed company SEEDCO, with funding support from the Grape & Wine Research & Development Corporation (GWRDC).

A diverse range of plant species and varieties, including brassicas, is being screened for their potential as mid row ground cover in vineyards.

The observation of winter weed suppression by some fodder radish species has created a lot of interest among grape growers in south eastern Australia.

In addition, widespread interest in biofumigation, particularly the potential for root knot nematode suppression, has created a demand for *Brassica juncea* (Indian mustard) seed where this pest is prevalent.

The 1997 cover crop technology extension program will see over forty demonstration trials established in autumn, including in the Margaret River and Hunter Valley regions for the first time.

Fodder radish, fodder rape and oilseed mustard varieties, all of which have been analysed for glucosinolates by **John Kirkegaard** and **Muhammad Sarwar**, will feature in each of these trials.

Collaboration with other researchers will continue this season, and will detemine the impact on nematode populations and weed densities.

SEEDCO has produced a range of brochures on cover crops for Australian vineyards.

Further information on the research program and availability of seed is available from SEEDCO, 78 Burbridge Rd, Hilton, SA 5033, or phone 08 8234 9333.

*-**Richard Porter***, Cover Crops Technology Project Manager, SEEDCO, Adelaide (08 8234 9333).

Brassicas active against citrus nematode

Citrus nematode (*Tylenchulus semipenetrans*) is an important parasite of citrus and grapevines.

Greg Walker, SARDI, Adelaide has tested the biological activity of a range of brassicas against it. Whole plants of commercial and weed brassicas grown in a greenhouse were chopped into 2-4 cm long pieces and 8 or 16 g was mixed with 200 cc of naturally-infested sandy loam soil and incubated for four weeks. Nematodes were then extracted and counted.

Significant reductions in the population of citrus nematode larvae compared with the uninoculated control were observed with all brassicas (Table 1).

Humus rape, an imported US cultivar, was the most effective, and was equally effective at both 80 and 40 g/kg of soil. The effectiveness of all other cultivars tended to decline at the lower amount. At the high level, Hobson, Winifred, Arran and Rangi rapes were effective (69 – 77% reduction in numbers), as was Simax hybrid (68% reduction in numbers). Wild turnip from two locations was more effective than several of the commercial cultivars.

Preliminary experiments indicate that reductions of this magnitude are harder to achieve in the field. Breeding to improve efficacy may be required.

The biological activity can be used in the meantime by growers to select the most effective current cultivars.
- *Greg Walker*, SARDI Plant Research Centre, Adelaide (08 8303 9355)

Could cover crops suppress garden weevil?

University of Western Australia Plant Science student **Matthew Bowden** is undertaking an Honours project aimed at assessing the growth and survival of garden weevil (*Phlyctinus callosus*) on the roots of plant species common in the mid-row of orchards and vineyards.

Garden weevil is a significant pest in orchards and vineyards in south-western Australia and South Africa.

There is increasing interest in cover crops in vineyards generally for pest and disease suppression and green manuring. Matthew's study will include mustard and canola with various grasses and legumes.

The project is being co-supervised by **Stewart Learmonth**, (Agriculture WA, Manjimup (08 9771 2444), who works on garden weevil biology and management, **Ian Dadour**, UWA, and **John Matthiessen**, CSIRO.

Table 1. Effects of soil amendment with fresh Brassica on abundance of ctrus nematode.

Brassica	% reduction (at 80 or 40 g/kg soil) (80/40)
Barkant turnip	66/36
Polybra turnip	57/38
Simax hybrid	68/53
Wild turnip (Loxton)	64/32
Wild turnip (Mypolonga)	63/41
Indian mustard	54/25
Winifred rape	76/48
Hobson rape	77/55
Arran rape	70/43
Rangi rape	69/57
Humus rape	78/81

Desktop publishing by John Matthiessen, CSIRO Entomology, Perth

Appendix 3. WA POTATO PEST AND DISEASE SURVEY 1996/97

CULTURAL HISTORY INFORMATION FORM

GROWER: ..... PH: .....
ADDRESS: ..... FAX: .....
..... POSTCODE: .....

SITE DETAILS
"CROP(S)" IN PREVIOUS 12 MONTHS : .....
.....
HOW MANY YEARS AGO WERE POTATOES LAST GROWN ON THIS SITE:
.....
SOIL TYPE: .....
ESTIMATED NO. PREVIOUS POTATO CROPS ON THIS PADDOCK : .....

CROP DETAILS
SEED: VARIETY: .....
SOURCE - LOCATION: .....
ROW SPACING: .....
CUT SEED OR WHOLE SEED (CIRCLE).
DATES: PLANTED: .....
MATURE: .....
HARVESTED: .....

PESTICIDES:

TIMING/ OPERATION	TARGET PEST(S)	PESTICIDE	RATE	HOW APPLIED
PRE PLANT				
SEED TREATMENT				
IN-CROP / FOLIAR				

IRRIGATION: NOT IRRIGATED
RAINFED
OVERHEAD AND TYPE : .....

Appendix 4. WA POTATO PEST AND DISEASE SURVEY 1996/97

Ware potatoes Pest and disease damage graded according to Western Australia's Potato Marketing Corporation. A total of 100 crops assessed.

Insect damage (5m row sampling unit) - These are assessed on site and sorted into categories of:

marketable whole tubers

reject non-insect whole tubers are rejected if < 50g, or with damage by machinery, disease, knobs, greening, misshapen.

reject insect whole tubers are rejected and each type of insect damage to be categorised and weighed separately. Divided into **level 1 and level 2** damage categories if < 8mm deep and > 8mm deep respectively to conform with class 2 and waste potatoes respectively.

Disease damage (25 tubers sampling unit) - These are to be graded by proportion of surface affected by disease after approx. 21 days shelf storage in calico bags. Each type of disease is to be identified and given a severity score: 0 = healthy; 1 = < 5%; 2 = 5-30%; 3 = > 30% of tuber surface with disease symptom.

Processing potatoes Pest and disease damage graded according to Simplot Australia.
A total of 20 crops assessed and compared to data collected for the same crops at Simplot's shed, as a form of calibration.

Collate Simplot's data for other crops for the season, supplemented by a sample of 100 crops in total for disease scored as for ware potatoes. These samples collected by Simplot at the factory delivery point.

Insect damage (5m of row sampling unit and Simplot sampling unit) - These are to be assessed on site and sorted into categories of:

marketable whole tubers (including insect damage and surface disease < 0.8mm deep)
sliced tubers ≥ 50g (remainder of the reject, insect and disease damage tuber after these sections removed).

reject non-insect whole tubers < 40g
sections of tubers that are rejected because of damage by machinery, disease, knobs, greening, or sections that are < 50g.

reject insect sections of tubers that have been damaged by insects to a depth > 0.8mm deep and each type of insect damage to be categorised and weighed separately.

Disease damage (25 tuber sampling unit and Simplot sampling unit) - These are to be assessed at or near time of harvest and graded by proportion of surface affected by disease (refer to grading for ware potatoes) and then into categories of:

marketable whole tubers (including insect damage and surface disease < 0.8mm deep);
sliced tubers ≥ 40 g (remainder of the reject, insect and disease damage tuber after these sections removed).

reject non-disease whole tubers < 40g;
sections of tubers that are rejected because of damage by machinery, insects, knobs, greening, or sections that are < 50g.

reject disease sections of tubers that have been damaged by diseases > 0.8mm deep and each type of disease damage to be categorised and weighed separately.