GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

Screening of crucifer crops for glucosinolate (nematicide) levels by HPLC chemical analysis and field assessment in Semillon plantings

DAN 95/2

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Dr C. C. Steel and R. W. McLeod, Charles Sturt University, Wagga Wagga, NSW

Telephone (Dr C. C. Steel) 02 6933 2721.

Note: R. W. McLeod was Principal Researcher and project located with NSW Agriculture from July 1995 to January 1997.

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Introduction

Project DAN 95/2 followed on from DAN 92/2 – Covercrops for management of nematode infestations in vineyards. That project identified biofumigation, use of brassica crops for pest and disease control, as a potentially useful tool for managing nematode infestation in vineyards. Brassica crops produce glucosinolates, secondary metabolites that release toxic and volatile isothiocyanates when brassica tissues decompose. Crops that produce high concentrations of glucosinolates in their tissues should perform better than crops producing lower concentrations.

The leading objective of DAN 95/2 was to scan brassica crops potentially useable as cover crops in vineyards and so identify crops that provide high glucosinolate concentrations. This objective was extended to further examining the relationship between glucosinolate concentration and antinematode activity brassica green manures. Project DAN 92/2 highlighted the problem of brassica crops hosting root knot nematodes. DAN 95/2's second major objective was to investigate the relation between glucosinolate concentration and nematode hosting. Lastly, DAN 95/2 moved to its third objective, testing biofumigation in vineyards.

This, final, report on DAN 95/2 is conveniently divided into four parts, as follows:
Part 1 – Screening of covercrop-suitable brassica crops for glucosinolate concentration and types.
Part 2 – Antinematode activity of brassica green manures and its relation to glucosinolates.
Part 3 – Egg production on brassicas by root knot nematode and its relation to glucosinolate concentration in roots.
Part 4- Notes on growth habit and pest attack on brassica crops and testing of biofumigation in vineyards.
Each part summarizes its findings. Introductions, materials and methods, results and discussion sections set out background, research objectives, research methods, research results in detail, analyze the research outcomes in relation to objectives, and discuss the implications for industry.

OVERALL SUMMARY

Part 1 reports on glucosinolate concentrations and profiles in a total of 15 brassica crops. The crops, grown in spring 1995, autumn-winter 1996 and late autumn-winter 1996, included seven forage rapes, two forage turnips, two hybrid turnips, one kale, one fodder radish, one Indian mustard and one white mustard. Glucosinolate concentrations in crops differed by as much as fourfold. Plant stems contained higher concentrations than leaves. Concentrations in roots were as high as, or higher than concentrations in leaves and, in the case of the turnips, considerably so. Season of the year was identified as an important determiner of glucosinolate concentration. Arran forage rape is highlighted as a biofumigant crop because of its relatively high glucosinolate content. Forage turnips are identified as having potential because of high glucosinolate concentrations in roots. Indian mustards also merit attention because of high glucosinolate concentrations in the leaves.

Part 2 reports on the antinematode activity of 15 brassica crops and its connection with glucosinolate concentration. Green manures of Arran, Humus and Korina forage rapes, Barkant and Polybra turnips and Indian mustard 99Y-1-1 were particularly effective and this correlated with high glucosinolate concentrations. Green manures of crops and plant parts selected for high glucosinolate concentration performed better than green manures with lower glucosinolate concentration. Volatiles from re-wetted freeze dried brassica tissues killed high percentages of the nematodes exposed to them. The results confirm the role of glucosinolates in the antinematode action of brassica tissues and support use of the term biofumigation.

Part 3 reports nematode increase factors, the ratio of eggs produced to number initiating infection, for 25 different brassica crops. Increase factors exceeded 1.5 on all but four of the crops. The highest was 12.6, on Polybra turnip. Increase did not relate to glucosinolate concentrations of roots. Four radishes, all cultivars bred in Europe for resistance to nematodes, returned increase factors of less than one. Crops with high increase factors may increase nematode levels, during the growth stage, if grown in warm

districts. Adagio fodder radish is identified as a biofumigant crop that should avoid this problem. It seems that factors other than glucosinolate concentration in roots control nematodes increase in brassicas.

Part 4 reports observations on the growth habit and pest attack on 15 brassica crops in spring 1995, autumn-winter 1996 and late autumn-winter 1996. Cover crops of Rangi forage rape and Coolabah oats were grown in five vineyards, two in the outer Sydney area, one in the Hunter Valley and two in the Murrumbidgee Irrigation Area, to assess biomass production, impact on nematode levels and production of glucosinolate. Drought limited biomass at all but one site and nematode levels were low at all sites. No-crop, Rangi-cropped and Coolabah-cropped plots had similar nematode levels three months after plough-in at all sites. Analysis of data for all sites combined, however, showed a significant difference between no-crop and cropped plots, but not between Rangi and Coolabah. Glucosinolate concentrations were similar at all sites, suggesting that suggesting that glucosinolate production will be similar over the range of district conditions covered. Needs for more drought resistant crops and more field assessment of biofumigation are highlighted.

PART 1

Screening of Covercrop-suitable Brassica Crops for Glucosinolate Concentration and Types

Summary

Glucosinolate concentrations in 15 brassica crops grown in spring 1995 and autumn - winter 1996 were determined by measuring glucose released by the enzyme myrosinase. Glucosinolate types (glucosinolate profiles) were assayed in three of the crops by HPLC. In 1995, in spring grown crops, glucosinolate concentrations in leaves of the six crops gown ranged from 13.1 μM/g in of Winfred forage rape to 47.3 μM/g in Indian mustard 99Y-1-1. In stems the range was 20.3, in of Winfred rape, to 40.3, in of Humus rape. In roots the range was 10.7, in Indian mustard 99Y-1-1 to 24.2 in Polybra turnip. In 1996, in autumn-winter grown crops, glucosinolate concentrations in leaves of the same cops were lower, ranging from 7.6 in Winfred rape to 22.9 in leaves of Indian mustard 99Y-1-1. In late autumn-spring crops grown in 1996, glucosinolate concentrations in leaves of the nine crops grown ranged from 5.9 in of Gruner kale, to 20.3 in Korina rape; in roots the range was 3.4, in Maxi white mustard, to 38.0, in Korina rape.

Aliphatic glucosinolates predominated in leaves of Humus and Rangi rapes and Barkant turnip, the three crops assayed by HPLC. In roots, in contrast, aromatic and indolic types made up 46% of the total glucosinolate content in Humus rape, 72% in Rangi rape and 48% in Barkant turnip.

Introduction

When applied at similar rates, green manures of brassica crops kill more nematodes than do green manures of non-brassica crops (Mojtahedi et al. 1991; McLeod and Da Silva 1994; Crow et al.1996). The most widely accepted explanation is that brassica green manures release toxic volatile isothiocyanates (ITCs)) and so the concept of growing brassica crops to control pests and diseases has been termed biofumigation (Kirkegaard et al. 1993). ITCs form when glucosinolates, secondary metabolites produced by members of the brassica family, are hydrolysed by myrosinase, an enzyme contained in special cells in brassica plants. Brassica crops that produce large amounts of glucosinolates should be more effective biofumigant crops than brassicas crops that produce only small amounts. Identificating brassica crops with higher concentrations of glucosinolates should enhance the efficacy of biofumigation. At least10 different glucosinolates commonly occur in brassica tissues and the particular glucosinolates present (glucosinolate profile) also strongly influences biological activity (Kirkegaard et al. 1996).

The objectives in this part of DAN 95/2 were (1) to assess the range of glucosinolate concentrations in brassica crops potentially useful as cover crops in vineyards (2) to identify crops with high glucosinolate concentrations and (3) gather information on the types of glucosinolates present in these crops.

Materials and Methods

Growing of crops. A series of three sowings provided leaves, stems and roots of a total of fifteen brassica crops for determination of glucosinolate concentration and type. In each sowing, crops were grown outdoors in two randomized blocks of 1m square plots at the Biological and Chemical Research Institute, Rydalmere. Plots were fertilized before

sowing with 100 kg/ha superphosphate and 100 kg/ha Startaphos ® (11% N, 23% P, 1.5% S) and watered as required.

Sowing 1, sown in late winter, on 17 August 1995, included the forage rapes Humus, Rangi and Winfred, the turnips Barkant and Polybra and the Indian mustard 99Y-1-1. Samples for glucosinolate determinations were collected from six plants in late spring, on 17 November 1995. Samples of leaves, plant stems and roots were taken.

Sowing 2, sown in mid autumn, on 16 April 1996, included the same crops as sowing 1. Samples for glucosinolate determination were collected from six plants in mid winter, on 12 July 1996. Samples of leaves only were taken.

Sowing 3, sown in late autumn, on 17 May 1996, included the forage rapes Arran, Bonar, Hobson and Korina, the kale Gruner, the turnip hybrids Simax and Pasja, the fodder radish Adagio and the white mustard Maxi. Samples for glucosinolate determination were collected from six plants in early spring, on 27 September 1996. Samples of leaves and roots were taken.

Freeze drying and determination of glucosinolate concentration. At sampling, plant-parts were collected from six plants. Samples were frozen immediately on collection and were stored frozen until they could be freeze dried. For freeze drying, subsamples of 300g of frozen material were chopped into 2.5 cm by 2.5 cm pieces, freeze dried in a Dynavac ® freeze drier at -33°C for 24-48 hours and then ground using a Glen Creston ® Type DFH 48 mill. The ground samples were then stored in screw-cap plastic containers in desiccators, over silica gel. Glucosinolate concentrations were determined by a method that measures the amount of glucose released by enzymatic breakdown of glucosinolates (Mailer 1989). In this method, glucosinolates are extracted in water and purified on DEAE-Sephadex A-25 in Bio-Rad Econo columns. Glucose cleavage is by adding myrosinase enzyme and glucose is measured using glucose oxidise-peroxidase enzymes. Determinations were done on three subsamples of 100 mg of freeze dried material.

HPLC analysis. Freeze dried tissues of Humus and Rangi forage rapes and Barkant turnip from sowing 1 were analysed by HPLC to provide determinations of individual glucosinolates, by Dr M. Sanwar, CSIRO, Canberra. Dr Sanwar is currently processing samples of tissues from sowing 3, results are not available yet.

Results

In sowing 1 (Table 1), glucosinolate concentrations in leaves of forage rapes ranged from 13.1 to 19.8 μM / g, Winfred contained the lowest level and Humus the highest. Levels in stems were higher than in leaves, and reflected leaf levels. Levels in roots of Humus and Rangi were similar to levels in leaves, roots of Winfred contained almost twice the level in leaves. The turnips Barkant and Polybra showed glucosinolate levels and distribution in plant-parts close to those found in the forage rapes. Barkant turnip, like Winfred forage rape, had twice as much glucosinolate in roots compared to leaves. Leaves of mustard 99Y-1-1 contained over twice as the concentration of any of the other crops, 47.3 μM/g, mustard roots, however, had the lowest concentration of all the crop roots.

In sowing 2 (Table 2), glucosinolate levels (in leaves) ranged from 6.6 to 22.2 μM/g, and were lower than in sowing 1 in most crops. Humus again produced the highest level of the forage rapes. Levels in the turnips were only marginally lower than in sowing 1. Mustard 99Y-1-1 again produced more glucosinolate than any other crop, but formed only half the concentration produced in sowing 1. Table 2.1 compares the results of the two sowings.

In sowing 3 (Table 3), glucosinolate concentrations in leaves ranged from 5.9 μM/g, in Gruner kale, to 27.0 μM/g, in Simax turnip hybrid. In roots the range was 3.4, in Maxi white mustard, to 38.0, in Korina forage rape. All forage rapes, except Hobson, produced more glucosinolate in roots than in leaves. In contrast, the turnip hybrids, the fodder

radish Adagio and the white mustard Maxi formed more glucosinolate in leaves than in roots. Gruner kale formed similar concentrations in leaves.

Analysis of glucosinolate kinds (Table 4) showed that aliphatic types predominated in the leaves and the stems of the forage rapes Rangi and Humus and the turnip Barkant. In roots, aromatic and indolic types made up around 40 percent of the total glucosinolate content in Humus rape and Barkant turnip, in Rangi rape the percentage reached 72 percent.

Discussion

The results show that there is a considerable range of glucosinolate concentrations across the crops tested. Of the forage rapes, over the three sowings, Korina, Arran and Humus provided the highest glucosinolate concentrations, and Bonar the lowest. Korina is a European variety not available in Australia and Humus is a variety from USA, also not available in quantity and subject to plant breeders'rights. Arran, a forage cultivar available in Australia, is identified as the forage rape cultivar of choice for sowing as a biofumigant crop. The results suggest that cool conditions may especially depress glucosinolate formation in Rangi, currently the most sown biofumigant crop.

The glucosinolate levels found in the turnips Barkant and Polybra and the turnip hybrids Pasja and Simax indicate that these have potential as biofumigants crops, although the low levels in the roots of the hybrids should be noted. The highest concentration of all crops occurred in leaves of the Indian mustard, which is in line with outstanding biological activity of mustard tissues (Kirkegaard et al. 1996). Indian mustards are identified as worthy of specific development for use as cover crops in vineyards. Low levels in the kale Gruner, particularly in the roots, adds to data (Kirkegaard and Sanwar, person communication) indicating low concentrations in cultivars of this crop. The results for Maxi white mustard and Adagio radish indicate no distinct promise for these as biofumigant crops.

Season of the year and plant-part and are identified as important determiners of glucosinolate concentration. Cooler growing conditions reduced glucosinolate levels in the forage rapes and the Indian mustard. The turnip crops, however, varied only slightly. Season and district temperature regimes need to be considered if biofumigant crops are to be grown to best advantage. In cool districts, a short-term spring crop could be a better option than an autumn crop. In view of the differences in glucosinolate concentration in leaves, stems and roots, full assessment of the glucosinolate contribution of a crop would need to take account of these concentrations and the amounts of these plant-parts produced.

Table 1 (sowing 1). Glucosinolate concentrations in leaves, stems and roots of brassica crops grown at Rydalmere, spring 1995

Crop	Leaf	Stem	Root
		Glucosinolate (μM/g) (1)	
Forage rapes			
Humus	19.8 ± 0.4	40.3 ± 1.6	19.9 ± 2.9
Rangi	15.5 ± 3.5	32.5 ± 3.4	15.7 ± 1.6
Winfred	13.1 ± 1.8	20.3 ± 3.9	22.3 ± 0.7
Turnips			
Barkant	14.9 ± 0.32	30.8 ± 4.0	31.9 ± 1.3
Polybra	18.9 ± 0.4	22.3 ± 3.9	24.2 ± 2.7
Indian mustard			
99Y-1-1	47.3 ± 3.5	32.4 ± 3.2	10.7 ± 2.0

(1) μMoles of glucosinolate/g freeze dried material, mean and standard error, three determinations.

Table 2 (sowing 2). Glucosinolate concentrations in leaves of brassica crops grown at Rydalmere, autumn-early winter, 1996

Crop	Glucosinolate in leaves (μM/g) (1)
Forage rapes	
Humus	13.0 ± 1.3
Rangi	6.6 ± 0.3
Winfred	7.6 ± 0.5
Turnips	
Barkant	11.9 ± 1.5
Polybra	15.4 ± 0.9
Indian mustard	
99Y-1-1	22.9 ± 1.0

(1) μM glucosinolate /g freeze dried material, mean and standard error, three determinations.

Table 2.1 (sowings 1 and 2). Glucosinolate concentrations in leaves of spring brassica crops (1995) compared to autumn- winter crops (1996)

Crop	Glucosinolate concentration	
	Spring 1995 (μM/g) (1)	Autumn 1996 (μM/g) (1)
Forage rapes		
Humus	19.8 ± 0.4	13.0 ± 1.3
Rangi	15.5 ± 3.5	6.6 ± 0.3
Winfred	13.1 ± 1.8	7.6 ± 0.5
Turnips		
Barkant	14.9 ± 0.3	11.9 ± 1.5
Indian mustard		
99Y-1-1	47.3 ± 3.5	22.9 ± 1.0

(1) μM glucosinolate /g freeze dried material, mean and standard error, three determinations.

Table 3 (sowing 3). Glucosinolate concentrations in leaves and roots of brassica crops grown at Rydalmere, autumn-winter, 1996

Crop	Leaf	Root
	Glucosinolate (μM/g)	
Forage rapes		
Arran	16.2 ± 0.7	20.2 ± 1.0
Bonar	9.4 ± 1.8	20.3 ± 2.2
Hobson	12.5 ± 2.0	11.4 ± 0.8
Korina	18.3 ± 2.5	38.0 ± 3.0
Kale		
Gruner	5.9 ± 1.0	6.0 ± 1.2
Turnip hybrids		
Pasja	20.3 ± 1.9	11.2 ± 1.0
Simax	27.0 ± 1.5	3.7 ± 1.2
Fodder radish		
Adagio	12.0 ± 1.7	3.0 ± 1.4
White mustard		
Maxi	12.3 ± 3.0	3.4 ± 1.0

(1)μM glucosinolate /g freeze dried material, mean and standard error, three determinations.

Table 4. Types of glucosinolates in leaves, stems and roots of three brassica crops grown at Rydalmere, spring 1995

Crop	Percent of total glucosinolates								
	Leaf			Stem			Root		
	Al (1)	Ar (2)	I (3)	Al	Ar	I	Al	Ar	I
Humus forage rape	88	3	9	91	5	4	54	33	13
Rangi forage rape	75	0	25	74	4	22	28	48	24
Barkant turnip	84	3	13	83	0	17	62	18	20

(1) Aliphatic glucosinolates

(2) Aromatic glucosinolates

(3) Indolic glucosinolates

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PART 2

Antinematode Activity of Brassica Green Manures and its Relation to Glucosinolates

Summary

Green manures of 15 brassica crops were incorporated into soils inoculated with second stage larvae of root knot nematode Meloidogyne javanica. Glucosinolate concentrations in the green manures ranged from 6 μM/g in leaves of Gruner kale to 40 μM/g in stems of Humus forage rape. With application rates of 20g/kg of soil and below, some green manures killed more nematodes than did others. Green manures of Arran, Humus and Korina forage rapes and of Indian mustard 99Y-1-1 were identified as very effective. These had high glucosinolate concentrations compared to the less effective green manures of Bonar, Hobson and Rangi forage rapes. Green manures of Barkant and Polybra turnips had glucosinolate concentrations close to those of the more effective forage rapes and slightly more effective. Green manures of the turnip hybrids Pasja and Simax performed less well than expected from their relatively high glucosinolate concentrations. Green manure of Gruner kale performed better than expected from its low glucosinolate concentration. Different glucosinolate profiles in different species may account for these variations.

Green manures from plant parts selected for high glucosinolate concentration performed better than green manures from plant parts with low glucosinolate concentrations.

Nematodes were exposed to volatiles from rewetted freeze dried brassica tissues by placing larvae in plastic rings on agar in sealed petri dishes together with the tissues contained in small plastic cups. Mustard seed meal at 5 to 25 mg, Humus leaf at 10 to

200 mg and Arran leaf, Arran leaf plus myrosinase and Barkant leaf, each at 76 to 600 mg killed a high percentage of the exposed nematodes. Similarly freeze dried and rewetted leaf tissue of Coolabah oats had little or no effect.

Introduction

Knowing that a brassica green manure has a high glucosinolate concentration is helpful only if release of GIL breakdown products is in fact the main nematode killing mechanism. If the term biofumigation is to be used accurately, we need to know that volatiles from brassica tissues do contribute to nematode kill. Brassica green manures could kill nematodes by releasing GIL breakdown products (GIL allelopathy), by releasing toxins other than those derived from GILs (non-GIL allelopathy) and by stimulating antagonistic soil flora and fauna (biological control). Measuring the relative importance of these three mechanisms presents a major challenge (Stirling 1991).

To date, the most convincing evidence for GIL allelopathy against nematodes is that green manures of brassicas incorporated in soil kill more nematodes than do the green manures of non-brassica crops similarly incorporated (Mojtahedi et al. 1991, Mojtahedi et al 1993, McLeod and Da Silva 1994, Crow et al. 1996). Kirkegaard et al. (1996) have shown that volatiles from brassica tissues suppress fungal root pathogens of cereals. Evidence is needed of volatile action against nematodes.

The objectives in this part of DAN 95/2 were (1) to assess the range of antinematode activity of green manures of brassica crops potentially useful as cover crops in vineyards (2) to identify very effective green manure crops (3) to examine the relation between GIL concentration and activity and (4) to determine whether brassica tissues release nematicidal volatiles.

Materials and Methods

Nematodes. A population of *Meloidogyne javanica,* originally from grape roots from a vineyard at Pokolbin, NSW, and maintained on tomato, Grosse Lisse, in pot culture provided the inocula used in all experiments. Inocula of second stage larvae were prepared by shaking chopped galled roots from three-month-old tomato culture plants in 0.5% sodium hypochlorite for four minutes (Hussey and Barker 1973) to disrupt the egg masses. The suspension of eggs was then poured through 1000, 150 and 25μm banked sieves. The 25 μm sieve, which retained the eggs, was then placed in a tray with water just covering the mesh for three days. Numbers of hatched larvae were then counted.

Green manures, glucosinolate data, freeze dried material and mustard seed meal. Green manures for the experiment were cut from the plots sown for glucosinolate determination, their glucosinolate concentrations are presented in the glucosinolate part. For ease of reference, the results tables in this part re-present this data. Freeze dried material was prepared as for glucosinolate determination. Cold pressed mustard seed meal was supplied by Yandilla Mustard Oil Enterprises, Wallenbeen, NSW. Green manure of Coolabah oats was cut from a crop grown at Rydalmere in late autumn-spring, 1996.

Antinematode activity experiments. Larvae were added to 1 kg amounts of potting soil (1 part coarse sand to 1 part peat, by volume) contained in plastic plant pots. Green manures, chopped into 5cm pieces, were added in the required amount to individual pots and mixed in by tipping from pot to pot. Each experiment included five replicates and these were arranged in randomized blocks on benches in a glasshouse. After two weeks, live nematodes were extracted, for five days, in Whitehead and Hemming (1995) trays.

Experiment 1 included green manures of Humus, Rangi and Winfred forage rapes, Barkant and Polybra turnips and 99Y-1-1 Indian mustard. These were cut from glucosinolate sowing 2 (grown autumn-winter, 1996), glucosinolate assay samples were

cut on 12 July 1996, three months after sowing, and green manures were cut on the same day. Amounts of 20 and 40 g/kg were incorporated and 1600 (SD 70, n=2) nematodes were added to each pot, in 1 ml of water.

Experiment 2 included the same green manures as in experiment 1, from the same plots, . Green manures were cut 5 August 1996, three weeks after cutting for experiment 1. The amounts of green manure added were 10 and 20 g/kg, and 1600 (SD 65, n=2) nematodes were added per pot, in 1.3 ml water. This experiment also included a treatment with Nemacur ® (fenamiphos), 0.1 g/kg of 5% granules.

Experiment 3 included the forage rapes Arran, Bonar, Hobson and Korina, the kale Gruner, the turnip hybrids Pasja and Simax, the fodder radish Adagio and the white mustard Maxi. These green manures were cut from glucosinolate sowing 3 (grown autumn-spring, 1996) on 3 September, four months after sowing, glucosinolate assay samples were cut on 17 September, two weeks later. Only one amount, 10 g/kg, was added and 1200 (SD 300, n=2) nematodes were added per pot in 0.75 ml water.

Experiment 4 investigated green manures made from crops and plant parts chosen to span a wide range of GIL levels. The experiment included stems of Humus forage rape, stems of Winfred forage rape, leaves of Winfred forage rape, roots (including the turnip) of Barkant turnip and roots of Maxi white mustard. Green manures were cut from glucosinolate sowing 2 on 1996 August, four months after sowing. GIL data for stems and roots was drawn from glucosinolate sowing 1 (grown spring 1995), data for Winfred leaf from sowing 2. The amounts of material added were 5 and 10 g/kg, and 1600 nematodes were added to each pot.

Exposure to volatiles. Experiments 5 and 6 were set up to look at the antinematode activity of volatiles from brassica tissues, using a method developed by Kirkegaard et al. (1996), to study the action of volatiles from brassicas against soil-borne fungi. Tissues were freeze-dried as in for GL determination. This was placed in 20 mm diameter plastic

vessels embedded in 1.5 % water agar in 8.5 cm petri dishes. Nematodes, second stage larvae, were placed on the surface of the agar, in a 12 mm diameter ring, cut from plastic tube, and located midway between tissue container and dish edge. To allow release of ITCs through the action of myrosinase enzyme present in the tissues, water, 10 µl per mg, was placed in the tissue containers. Dishes were then sealed with two layers of Parafilm 'M' ®. In experiment 1, leaf tissue of Humus forage rape, at 10, 50, 100 and 200 mg were compared with mustard seed meal, also freeze dried, at 2.5, 5, 10 and 25 mg. Nematode kill was assessed by counting dead, not moving and body straight and contracted, and live larvae after 24 hours of exposure to any volatiles released from the tissues. Experiment 6 compared leaf tissue Arran forage rape, Barkant turnip and, as a non-brassica crop, Coolabah oats. As additional treatment, Arran leaf tissue was moistened with a solution of myrosinase, 1 mg/ml, in place of water. Nematode kill was assessed as in experiment 5, after 24 and 48 hours exposure.

Results

Antinematode activity of green manures. In experiment 1 (Table 1), with 20 and 40 g/kg applied, there was no significant difference between green manures, all drastically lowered nematode numbers. In experiment 2 (Table 2), with 10 and 20 g/kg applied, significant differences between green manures were evident. Of the three forage rapes, Humus green manure was the most effective. Green manures of the turnips Barkant and Polybra were about as effective as Humus . Indian mustard 99Y-1-1 was the most effective green manure at 10 g/kg, but not at 20 g/kg. The nematicide fenamiphos applied at 5 mg/kg was as effective as Humus green manure applied at 20 g/kg.

In experiment 3 (Table 3), with a single rate of application of 10 g/kg, green manures of the forage rapes Arran and Korina and the kale Gruner were the most effective. Green manures of forage rapes Bonar and Hobson and the white mustard Maxi were less effective than these. Green manures of the turnip hybrids Pasja and Simax were less effective again.

In experiment 4 (Table 4), green manures made from stems of Humus and Winfred forage rapes were the most effective and root material of Maxi white mustard was least effective.

Exposure to volatiles. Exposure to volatiles released from brassica tissues killed high percentages of exposed nematodes. In experiment 5, mustard seed meal at 2.5 - 25 mg was clearly more active than Humus leaf at 10 - 200 mg. In experiment 6, nón-contact exposure to brassica tissue again caused high levels of kill. Addition of myrosinase to Arran leaf did not materially increase its effectiveness. There was no indication of significant killing of nematodes through non-contact exposure to Coolabah oat tissue.

Discussion

The results identify Arran, Humus and Korina forage rapes and Indian mustard 99Y-1-1 as the most effective of the green manures tested. All of these were also found to have relatively high glucosinolate contents. Arran is available in Australia and the results suggest that it should be considered in place of Rangi, the currently used cultivar, for nematode control. Humus and Korina are not available to industry in Australia. The results also focus interest on Indian mustards, the one tested here is an oil seed cultivar. Indian mustards mature early and are drought tolerant (Kirk and Oram 1981). Development of Indian mustards specifically for cover cropping would improve the prospects for adoption of biofumigation.

The turnip cultivars Barkant and Polybra provided effective antinematode green manures, and their glucosinolate content was substantial. Turnips are autumn-winter crops that can be sown into permanent pasture. Both of these attributes make them potentially useful as cover crops. Interestingly, the green manures of the hybrid turnips tested were not particularly effective although the glucosinolate content was quite high. Particular note attaches to the fodder radish Adagio because of its potential for weed suppression (Porter and Grow 1997).

Effective nematode control linked with relatively high glucosinolate content is evident, with some exceptions, in the results of experiments 2,3 and 4, but not so in experiment 1. Notable exceptions are the good performance of Gruner kale relative to its glucosinolate concentration and the moderate performance of the turnip hybrids relative to their glucosinolate concentrations. Differences in the kinds of glucosinolate in different species could explain these variations (Kirkegaard et al.1996). It is suggested that in experiment 1, with 20 and 40 g/kg added, because of the large amounts of organic matter, biological and non-glucosinolate allelopathic effects masked differences due to different glucosinolate concentration. However, adding 20 g/kg in experiment 2 gave significant differences. Green manures were cut one month after those in experiment 1, glucosinolate concentrations may have increased as the crops aged so that glucosinolate activity became more pronounced (Mojtahedi et al 1993). Control from relatively low biomass applications may be an important advantage of brassica green manures compared to non-brassica green manures.

Strong evidence that brassica tissues release volatiles that kill nematodes emerges from experiments 5 and 6. The powerful kill from mustard seed meal is in line with its strong antifungal activity and is in line with its high glucosinolate concentration (Kirkegaard et al. 1996). The results suggest that the LD 50 for mustard seed meal is between 2.5 and 5 mg and for Humus leaf it is between 100 and 200 mg. The kills from Arran and Barkant in experiment 6 were in line with their glucosinolate concentrations . Barkant acted more slowly than Arran, this could be due to differences in glucosinolate types. Adding myrosinase to Arran tissue did not improve kill, suggesting that the amount naturally present is sufficient. The results support use of the term biofumigation to refer to pest and disease control through growing brassica crops.

Table 1 (experiment 1). Antinematode activity of brassica green manures against root knot nematode *Meloidogyne javanica* and glucosinolate content

Green manure	Survival (nematodes / kg) (1)		Glucosinolate µM / g (2)
None	486 a		
	20 g / kg added	20 g / kg added	
Forage rapes			
Humus	51 b	4 b	13.0 ± 1.3
Rangi	14 b	14 b	6.6 ± 0.6
Winfred	24 b	21 b	7.6 ± 0.5
Turnips			
Barkant	16 b	6 b	11.9 ±1.5
Polybra	39 b	49 b	15.4 ± 0.9
Indian mustard			
99Y-1-1	19 b	16 b	22.9 ± 1.0

(1) Means of five replicates, means followed different letters are significantly different, P=0.05.

(2) µMoles of glucosinolate / g freeze dried material, means (three determinations) and standard error. Crops grown autumn-winter, 1996 at Rydalmere.

Table 2 (experiment 2). Antinematode activity of brassica green manures against root knot nematode *Meloidogyne javanica* and glucosinolate content

Green manure / nematicide	Nematode survival (nematodes / kg) (1)		Glucosinolate µM / g (2)
None	555 a		
	10 g / kg added	20 g / kg added	
Forage rapes			
Humus	157 c	47 f	13.0 ±1.3
Rangi	233 b	79 e	6.6 ± 0.3
Winfred	163 c	119 d	7.6 ± 0.5
Turnips			
Barkant	127 d	93 e	11.9 ± 1.5
Polybra	133 d	105 d	15.4 ± 0.9
Indian mustard			
99Y-1-1	95 e	113 d	22.9 ± 1.0
Fenamiphos	35 f		

(1) Means of five replicates, means followed by different letters are significantly different, P=0.05.

(2) µMoles of glucosinolate /g freeze dried material, means (three determinations) and standard error. Crop grown autumn-winter, 1996 at Rydalmere. Green manures were cut three weeks after sampling for glucosinolate determination.

Table 3 (experiment 3). Antinematode activity of brassica green manures against root knot nematode *Meloidogyne javanica* and glucosinolate content

Green manure 10 g / kg added	Survival (nematodes / kg) (1)	Glucosinolate µM / g (2)
None	343 a	
Forage rapes		
Arran	31 d	16.2 ± 0.7
Bonar	44 c	9.4 ± 1.8
Hobson	59 c	12.5 ± 2.0
Korina	16 d	18.3 ± 2.5
Kale		
Gruner	19 d	5.9 ± 1.0
Turnip hybrids		
Pasja	79 b	20.3 ±1.9
Simax	69 b	27.0 ± 1.5
Fodder radish		
Adagio	84 b	12.0 ± 1.7
White mustard		
Maxi	56 c	12.3 ± 3.0

(1) Means of five replication, means followed by different letters are significantly different, P=0.05.

(2) µMoles of glucosinolate /g freeze dried material, means (three determinations).

Crops grown late autumn-spring 1996, at Rydalmere. Green manures cut three weeks before sampling for glucosinolate determination.

Table 4 (experiment 4). Antinematode activity of brassica green manures <u>from different plant parts</u> against root knot nematode *Meloidogyne javanica* and glucosinolate content

Green manure	Survival (nematodes / kg) (1)		Glucosinolate μM / g (1)
None	299 a		
	5 g / kg added	10 / kg added	
Forage rapes			
Humus stem	74 e	45 f	40.3 ± 1.6
Winfred stem	100 d	62 e	20.3 ± 3.9
Winfred leaf	118 c	52 f	13.1 ± 1.8
Turnip			
Barkant root	89 d	55 f	34.6 ± 1.0
Indian mustard			
99Y-1-1 root	168 b	98 d	10.7 ± 2.0

(1) Means of five replicates, means followed by different letters are significantly different, P=0.05.

(2) μMoles of glucosinolate /g freeze dried material, means (three determinations) and standard error. Crops grown autumn-winter 1996, at Rydalmere. Glucosinolate data from crops grown spring 1995.

Table 5 (experiment 5). Antinematode activity, against root knot nematode *Meloidogyne javanica*, of volatiles from wetted freeze dried brassica tissues

Freeze dried tissue	Amount (mg)	Percent kill
None, water only		7
Humus leaf	10	11
	50	24
	100	41
	200	83
Mustard seed meal	2.5	47
	5.0	77
	10.0	100
	25.0	95

Table 6 (experiment 6). Antinematode activity, against root knot nematode *Meloidogyne javanica*, of volatiles from wetted freeze dried brassica and non-brassica leaf tissues

Freeze dried tissue	Amount (mg)	Percent kill	
		24 hour exposure	48 hour exposure
None		6	7
Arran	38	31	30
	75	50	99
	150	62	88
	300	80	100
	600	53	85
Arran + myrosinase	38	7	69
	75	52	88
	150	73	100
	300	41	100
	600	79	100
Barkant	38	4	16
	75	4	69
	150	5	100
	300	2	78
	600	16	96
Coolabah oats	38	7	6
	75	3	5
	150	2	30
	300	6	9
	600	11	11

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PART 3

Egg Production by a Root Knot Nematode on Brassicas and its Relation to Glucosinolate Concentration in Roots

Summary

Egg production on 25 different brassica crops by root knot nematode *Meloidogyne javanica* was assessed by inoculating three week old seedlings with second stage larvae and after eight to nine weeks counting numbers of eggs produced.

In the initial experiment, in which tomato (Grosse Lisse) was included as a control, the nematode produced more eggs on tomato than on any of the seven brassicas, although the number on Polybra turnip was not significantly lower than on tomato. Polybra had the second highest concentration of glucosinolate in its roots. Checking of the egg recovery method used indicated that non-recovery of eggs was minimal.

Nematode increase factors exceeded 1.5 on all but four of the brassicas, the highest recorded being 12.6, on Polybra turnip. Four brassicas, the forage radishes Adagio, SCO 07024, Nemex and Pegletta, noted for resistance to beet cyst nematode (*Heterodera schactii*), recorded increase factors of less than one.

The results indicate that egg production on brassica crops by this nematode is not related simply to concentration of glucosinolate in the roots.

The radishes Adagio, SCO 07024, Nemex and Pegletta are identified as biofumigant crops that should not increase *Meloidogyne javanica* infestation by hosting it.

Introduction

Identifying brassica crops that produce high concentrations of glucosinolates in their leaves and stems and green manures that have high antinematode activity highlights them as potential biofumigant crops. Their potential is not so bright, however, if the target pest nematode is able to enter, develop, grow and lay eggs on the roots as the crop grows. There is much evidence showing that many brassicas are hosts of root knot nematodes. Nematode increase factors (ratio of number of eggs laid to initial number of nematodes) of well above one are common (Mojtahedi et al. 1991, Bernard and Montgomery-Dee 1993, McLeod and Warren 1993, McSorley and Frederick 1995). Increase factors vary across cultivars and crops (McLeod and Warren 1993). Development of resistance to root knot through plant selection has been reported (Bunte 1994).

Brassicas support less egg production than do very favourable hosts such as tomato, tobacco and many legumes (McLeod and Warren 1993). Also, root knot nematodes do not normally cause major problem on brassica crops in the field (Anon 1996, McSorley and Frederick 1995, McLeod personal observations), one factor being that they are usually grown as winter crops. Nevertheless, nematode reproduction as the crop grows would offset the benefit of biofumigation and remains a major concern if brassicas are to be used as biofumigant crops to manage root knot nematodes in vineyards.

The presence of appreciable concentrations of glucosinolates in the roots of brassicas suggests that the glucosinolate / isothiocyanate (ITC) system, whereby nematode toxic ITCs may be formed, suggests that the system may be involved in suppressing egg laying by root knot nematodes on brassicas. Lazzeri et al. 1993 hypothesized that release of ITCs may inhibit giant cell formation. For the system to work, nematodes would have to disrupt host tissues and so release myrosinase enzyme to catalyze ITC formation.

The objectives in this part of DAN 95/2 were (1) assess the range of root knot nematode increase on brassica crops potentially useful as cover crops in vineyards (2) to identify brassica crops that support less egg production, and (3) to test the hypothesis that glucosinolate concentration in brassica roots influences egg production.

Materials and Methods

Nematode inoculum. A population of *Meloidogyne javanica* originally from grape roots from a vineyard at Pokolbin, NSW, and maintained on tomato, Grosse Lisse, in pot culture was used in all experiments. Hatched second stage larvae were collected on 25 μm sieves by shaking roots bearing egg masses in 0.5% sodium hypochlorite, as described in the Part on antinematode activity.

Glucosinolate data. The data showing glucosinolate concentration in roots is drawn from the Part on glucosinolate screening. As root knot nematodes enter and live in root tissues, concentrations in roots are selected as more relevant than concentrations in leaves and stems. The concentrations are those in roots of crops three months after sowing.

Egg production experiments. Each of the four experiments included at least four randomized replicates of the treatments (different crops). Plants were grown in a glasshouse where temperatures ranged from 15 to 30 °C. Seeds of crops (treatments) were sown in pasteurized potting soil (one part coarse sand: one part peat) in 15 cm pots holding 800 g of potting soil. Pots were thinned to two seedlings two a pot, at three weeks from emergence pots were inoculated with nematodes by pipetting second stage larvae in approximately 1 ml water to a pot and placing the nematodes in 1 cm holes made within 2 cm of a seedling. Number of nematodes added was estimated, based on four counts. At 8 to 9 weeks from inoculation, plant roots were washed free of soil and the number of nematode eggs produced on the roots estimated by shaking them for 4 minutes in 0.8 % sodium hypochlorite to disperse eggs from egg masses (Hussey and

Barker 1973), retaining the eggs on 25 μm sieves and counting using Doncaster counting dishes. Nematode increase factors (NCFs), the ratio of eggs produced to number of nematodes added at inoculation, were calculated in each experiment (Mojtahedi et al. 1991).

Experiment 1 included the forage rapes Humus, Rangi and Winfred, the turnips Barkant and Polybra, the turnip hybrid Simax and the Indian mustard 99Y-1-1. The turnips of Barkant and Polybra were peeled and the peel included with the roots for recovery of any eggs on them. This experiment included a sowing of Grosse Lisse tomato to provide a very-favourable-host control. There were five replicates, number of nematodes added was 1600 (SD ± 400, n = 4) to a pot and egg numbers were counted eight weeks after inoculation. Also in this experiment, soil and root fragments remaining after removal of intact root systems, and so not included in the root egg count process, was tested to determine if it contained many eggs. The method, modified from Byrd et al. (1972), was as follows- the soil/root material was suspended by stirring with a flat paddle 4 L of water; the suspension allowed to settle for 5-10 second to remove sand; after decanting, sodium hypochloride (final concentration of 0.5 percent) added to disperse egg masses; suspension then sieved, eggs collected on 25 μm opening sieve and counted.

Experiment 2 included the forage rapes Humus and Rangi (as a standard control), the turnip hybrid Pasja, the fodder radish Adagio and the Indian mustards Claret, Ebony, Ruby and Topaz. There were seven replicates, 1800 (SD ± 300, n = 4) nematodes were added per pot and egg numbers counted 8 weeks after inoculation. Soil / root remaining after removal of roots was again checked for the presence of eggs.

Experiment 3 included the forage rapes Bonar, Korina, Rangi (standard control) and Striker, the kale Gruner, the Swede turnip Highlander, the turnip hybrid Pasja and the white mustard Maxi. There were four replicates, 1000 (SD ± 300, n = 4) nematodes were added to a pot and egg numbers counted 9 weeks after inoculation.

Experiment 4 included the local garden radishes Cherry Belle, Long Scarlet and White Icicle and the European bred fodder/oil radishes Adagio, SCO 7024, Nemex and Pegletta. Rangi forage rape was included as a standard control. There were five replicates, an increased inoculum of 5000 (SD ± 800, n = 3) nematodes was added per pot, to severely test Adagio, and egg numbers counted 8 weeks after inoculation.

Results

In experiment 1 (Table 1), the nematode produced many more eggs on tomato than on any of the brassicas, although the number on Polybra turnip was not significantly lower. The brassicas' NIFs ranged from 4.3 on Indian mustard 99Y-1-1, to 12.6 on Polybra turnip. The only significant difference amongst the brassicas was between these two, at the extremes of the range. Residual soil and root contained only negligible numbers of eggs (Table 1.1).

In experiment 2 (Table 2), the nematode produced similar numbers of eggs (differences not significant) on all brassicas, with the notable exception of Adagio fodder radish. The NIF on Adagio was 0.6, NIFs on the other brassicas ranged from 5.4 on Humus forage rape to 15.7 on Pasja turnip hybrid.

NIFs in experiment 3 (Table 3) were lower than in the previous two experiments and there were significant differences between the various brassicas. The nematode produced more eggs on Striker forage rape than on the others, similar numbers on Bonar forage rape, Highlander Swede turnip and Pasja hybrid turnip and lower numbers on Gruner kale and Maxi white mustard. Maxi white mustard plants had soft roots and root systems much reduced by rotting.

In experiment 4 (Table 4), the nematode produced large numbers of eggs on all of the culinary vegetable radishes and low numbers on the fodder radishes Adagio, SC07024, Nemex and Pegletta.

Absolute egg numbers differed in from experiment to experiment and this related to inoculum level used. In experiments 1 and 2, inoculum levels were similar and egg numbers on crops common were comparable. Inoculum level was lower in experiment 3, egg numbers were lower, and in experiment 4 inoculum level was increased and egg numbers were high.

Discussion

Nematode increase factors were well above one, that is the nematode was able to materially increase its numbers under the test conditions, on 18 of the 25 brassicas tested. The 25 included six forage rapes, one Kale, two turnips, two turnip hybrids, one Swede turnip, five Indian mustards, one white mustard and three culinary radishes. The results suggest that these would all have the potential to increase infestation as the crop grows and so counter the control from incorporating their green manures. Egg production should be less on winter grown crops in the field (Roberts et al. 1981, Ahuja and Singh 1990). However, observations on autumn crops of Rangi forage rape in vineyards in the Hunter Valley (McLeod, Somers and Gendy 1995) show that root knot nematode can increase under field conditions in warm viticultural districts.

Nematode increase factors were less than one, indicating that the nematode would not be able to increase its numbers, on Adagio, SCO 7024, Nemex and Pegletta radishes. Results for the other radishes show, however, that low egg production is not a feature of radishes in general. Adagio, SCO 7024, Nemex and Pegletta are cultivars bred in Europe for use as trap crops for control of beet cyst nematode, they also resist *Meloidogyne hapla* and *M. chitwoodi* (Bunte 1994, Heijbroek and Munning 1994). Present results indicate that their resistance acts alsoagainst *Meloidogyne javanica.* Porter and Grow (1997) have identified Adagio as a promising vineyard cover crop with potential for allelopathic weed control.

The results do not sustain the hypothesis that egg production relates simply to glucosinolate concentrations in roots. In comparing different brassica species, it should be noted that GIL levels effects could be confounded with different species having different kind of GILs (different GIL profiles) (Kirkegaard et al. 1996). However, comparison within the one species, the fodder rapes, shows no clear relation. In experiment 1, similar numbers of eggs were produced on Humus, Rangi and Winfred, the GIL levels ranged from 15.7 to 22.3 μM / g. In experiment 3, the nematode produced more eggs on Striker, a cultivar noted for high GIL content (Kirkegaard, personal communication) than on Bonar and Rangi, cultivars with moderate levels. Korina's root GIL level was twice that of Rangi but similar numbers of eggs were produced on them. *Meloidogyne incognita* and *M. javanica* produce more eggs on very favourable hosts such as tomato and legumes than on many brassica crops (McLeod and Warren 1993). Fewer nematodes invading, slower growth, inhibited development and reduced fecundity may all contribute to this host status difference (Anwar et al. 1994). While glucosinolates could be involved in lowering invasion, present results do not implicate later action of glucosinolates in the reduced host status of brassicas compared to very favourable hosts.

Table 1 (experiment 1). Eggs produced by *Meloidogyne javanica* on brassicas and root glucosinolate concentrations

Crop	Eggs produced ,000 / pot and NIF (1)	Root glucosinolate level µM / g
Tomato (control)	52.3 a (32.7)	---
Forage rapes		
Humus	11.8 bc (7.4)	19.4 ± 2.9
Rangi	12.0 bc (7.5)	15.7 ± 1.6
Winfred	9.3 bc (5.8)	22.3 ± 0.7
Turnips		
Barkant	14.5 bc (9.1)	31.9 ± 1.3
Polybra	20.1 ab (12.6)	24.2 ± 2.7
Turnip hybrid		
Simax	10.1 bc (6.3)	3.7 ± 1.2 (1)
Indian mustard		
99Y-1-1	6.9 c (4.3)	10.7 ± 2.0

(1) Mean of five replicates, means followed by the same letters do not differ significantly, P=0.05. NIF (nematode increase factor), ratio of number of eggs produced to number of larvae added at inoculation (1600 per replicate).

(2) Data from Table 3 (sowing 3) glucosinolate Part, other data from Table 1 (sowing 1) glucosinolate Part.

Table 1.1 (experiment 1). Eggs in soil and root fragments not included in counting eggs on crop root systems

Crop	Eggs in residual soil / root fragments Eggs / pot (1)
Tomato (control)	330
Forage rapes	
Humus	400
Rangi	0
Winfred	0
Turnips	
Barkant	0
Polybra	200
Turnip hybrid	
Simax	0
Indian mustard	
99Y-1-1	70

(1) Means of three replications.

Table 2 (experiment 2). Eggs produced by *Meloidogyne javanica* on brassicas and root glucosinolate concentrations

Brassica	Eggs produced ,000 / pot and NIF (1)	Root glucosinolate level µM / g
Forage rapes		
Humus	9.5 a (5.3)	19.9 ± 2.9 (2)
Rangi	13.3 a (7.4)	15.7 ± 1.6 (2)
Turnip hybrid		
Pasja	27.5 a (15.2)	11.2 ± 1.0 (3)
Fodder radish		
Adagio	1.0 b (0.6)	-----
Indian mustards		
Claret	11.7 a 6.5)	-----
Ebony	9.6 a (5.3)	-----
Ruby	17.3 a (9.6)	-----
Topaz	18.7 a (10.4)	-----

(1) Means of seven replicates, means followed by same letter are not significantly different, P=0.05. NIF (nematode increase factor) ratio of number of eggs produced to number of larvae added at inoculation (1800 per replicate).

(2) Data from Table 1 (sowing 1) in glucosinolate Part.

(3) Data from Table 3 (sowing 3) in glucosinolate Part.

Table 3 (experiment 3). Eggs produced by *Meloidogyne javanica* on brassicas and root glucosinolate concentrations

Brassica	Eggs produced, 000 / pot and NIF (1)	Root glucosinolate level µM / g
Forage rapes		
Bonar	4.1 b (4.1)	20.3 ± 2.2
Korina	3.5 c (3.6)	38.0 ± 3.0
Rangi	3.3 c (3.4)	15.7 ± 1.6 (2)
Striker	7.8 a (8.0)	-----
Kale		
Gruner	1.6 d (1.6)	6.0 ± 1.2
Turnip (Swede)		
Highlander	3.8 b (3.9)	------
Turnip hybrid		
Pasja	4.4 b (4.5)	11.2 ± 1.0
White mustard		
Maxi	1.9 d (1.9)	3.4 ± 1.0

(1) Mean of five replicated, means followed by the same letter are not significantly different. NIF (nematode increase factor) ratio of number of eggs produced to number of larvae added at inoculation (1000 per pot).

(2) Data from Table 1 (sowing 1) Part, other data from Table 3 (sowing 3) glucosinolate Part.

Table 4 (experiment 4). Eggs produced by *Meloidogyne javanica* on radish cultivars

Crop	Eggs produced ,000 per pot, and NIF (1)
Rangi forage rape (known control)	21.8 (3.8)
Local garden radishes	
Cherry Belle	19.8 (3.4)
Long Scarlet	11.5 (2.0)
White Icicle	18.8 (3.2)
European fodder/oil radishes	
Adagio	3.7 (0.6)
SCO 07024	5.5 (0.9)
Nemex	3.9 (0.7)
Pegletta	4.2 (0.7)

(1) Mean of five replicates. NIF (nematode increase factor), ratio of number of eggs produced to number of larvae added at inoculation (5000 per pot).

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PART 4

Observations on Brassicas as Cover Crops and Testing of Biofumigation in Vineyards

Summary

Observations on growth habit and pest attack and biomass production are noted for 15 brassica crops potentially useful as cover crops in vineyards. Observations are based on crops grown in three sowings: the first in late winter, 1995, the second in mid autumn 1996 and the third in late autumn, 1996.

Cover crops of Rangi forage rape and Coolabah oats were grown in autumn-winter, 1996 at Rydalmere (western Sydney), Broke (Hunter Valley), Camden (south-west Sydney), Yenda (Murrumbidgee Irrigation Area) and Nericon (Murrumbidgee Irrigation Area). Measurements were recorded on biomass (top growth) production, impact on nematode levels and glucosinolate concentration. At Rydalmere, where crops were watered, Rangi produced 5.8 t/ha dry matter and Coolabah oats produced 5.5 t/ha. Drought restricted growth at all other site, biomass of Rangi ranged from 1.7 t/ha at Broke to 3.5 t/ha at Camden and biomass of Coolabah oats ranged from 2.1 t/ha at Broke to 3.9 t/h at Camden.

At all sites, at three months after plough-in, nematode levels under no crop, Rangi and Coolabah were low and differed insignificantly. Data for all sites combined, however, showed a significant difference between no crop and either crop but not between Rangi and Coolabah. Glucosinolate concentrations varied only slightly, from 5.4 μM/g at Camden to 9.0 μM/g at Broke.

Introduction

Application, or otherwise, of biofumigation in viticulture will ultimately flow from assessment in relation to a complex of factors. Amongst these are impact on nematode infestation levels, cost and efficacy relative to other control options, how easy it is to manage, availability of cultivars suiting differing local conditions, potential to become weeds, pest and disease resistance and impact on grapevine pests and diseases (Brown et al. 1997, Smart 1997). Critical final assessment will occur through 'production run' testing, that is by growers trying it on a small scale under actual vineyard conditions and it is important that this happen early in the development process (Peters 1989). However, field testing to extend laboratory and glasshouse experimentation is a necessary part of the assessment process. Only limited work (McLeod et al. 1995) has been done in this area so far. A major, ongoing investigation of cover crops for viticulture now proceeding includes assessment of brassicas in relation to viticultural requirements (Porter 1996, Porter and Grow 1997).

The objectives in this part of DAN 95/2 were (1) to record observations on potential biofumigant crops relevant to cover cropping (2) to assess the impact on inter-row nematode numbers of growing and ploughing in a brassica cover crop, under vineyard conditions (2) to measure biomass production of the crop in different viticultural districts and (3) to compare production of glucosinolate (GIL) in different viticultural districts.

Materials and Methods

Observational plots. Observations on biomass production, crop growth habit and pest and disease attacked are based on plots of crops grown for assay of glucosinolate concentrations. These crops were grown in three sowings, the first started in late winter 1995, the second in autumn 1996 and the third in late autumn 1996 (see glucosinolate Part for details of crop culture). Plots were cut to estimate biomass at three or four months from sowing.

Vineyard trial procedure. Vineyard trials were initiated in autumn 1996 at five locations, locations which represented both coastal and inland viticultural districts. At four of the five locations, the vines were Semillon on own roots, at least eight years old and with a history of root knot nematode (RKN) damage. Only at one location, Rydalmere, was it possible to water the inter-row. Each trial conformed to the same design. Three treatments, Rangi forage rape at 8 kg / ha, Coolabah oats at 80 kg / ha and uncropped were established on 10 m^2 plots in six randomised blocks. Fertiliser application was 100 kg / ha of Progras Startaphos ® (N 11%, P 23 %, Ca 0.4% and S 1.5%) broadcast at sowing.

In autumn, three months after sowing, crop biomass, fresh and dry weight were estimated by cutting and weighing top growth from 1 m^2 squares. Five plants were dug from each Rangi plot and the roots inspected for the presence of galls. Leaves of three Rangi rape plants were collected per plot for determination of GIL, these samples were frozen in a portable refrigerator in the field, freeze dried and stored in a desiccator in the laboratory pending analysis. At this stage, top growth was ploughed in.

Three months after ploughing in of top growth, soil samples were collected for estimation of nematode numbers. Five 300 g, subsamples, down to 20 cm, were collected on each plot, bulked, mixed and 1 kg processed to determine nematode numbers, by the Whitehead and Hemming (1965) tray method. Root knot nematode larvae and any other plant parasitic nematodes were counted. Glucosinolate determinations followed the method of Mailer (1989), as outlined in the glucosinolate Part.

Trial 1, sited at Rydalmere, western Sydney, was on sown on 10 May, top growth ploughed in on 23 August and soil samples for nematode counts collected on 13 November. The vines were two year old Semillon on own roots. Soil type was a clay loam (64% sand, 8% silt, 28% clay, pH 6.6). There was no history of nematode infestation, so plots were inoculated by digging galled tomato roots and soil from a 20 cm

pot into the centre of each plot. Soil samples for nematode counts were collected from the sites of inoculation.

Trial 2, sited at Broke, in the Hunter Valley, was sown on 16 May, top growth was ploughed in on 27 August and soil samples for nematode counts collected on 19 November. Soil type was sandy loam (88% sand, 2% silt, 2% clay, pH 5.8). Here, drought stressed and less stressed Rangi plants were sampled separately for GIL determination.

Trial 3, sited at Camden, 50 km south west of Sydney, was sown on 20 May, top growth was ploughed in on 4 September and soil samples for nematode counts collected on 21 November. Soil type was loam (71% sand, 13% silt, 16% clay, pH 6.2).

Trial 4, sited at Yenda, in the Murrumbidgee Irrigation Area, was sown 20 May, top growth was ploughed in on 10 September and soil samples for nematode counts were collected on 21 November. Soil type was sandy loam (85% sand, 2% silt, 13% clay, pH 6.9).

Trial 5, sited at Nericon, in the Murrumbidgee Irrigation Area, was sown on 21 May, top growth was ploughed in on 10 September and soil samples for nematode counts were collected on 22 November. Soil type was sandy clay loam (70% sand, 6% silt, 24% clay, pH 6.6).

Results

Observational plots. Arran, Bonar, Hobson, Humus and Winfred forage rapes all formed good early ground cover, produced compact growth to 70 cm, produced at least 4 t / ha dry matter, attracted varying aphid infestation and remained vegetative for at least three months. Grower experience with Hobson at Broke in 1994 indicates that it can become rank and may regrowth be difficult to control. Humus and Korina rapes produced less

biomass than the others. Turnips and turnip hybrids formed adequate ground cover, Barkant, Pasja, Polybra produced around 4 t/h dry matter. At four months, growth was rank and seed setting had begun. Adagio fodder radish stood out as it produced the most biomass, but it became rank at four months and was setting seed. Indian mustard 99Y-1-1 and White mustard Maxi set seed after two months and then formed rank long stems, which then formed most of its biomass.

Vineyard trials. Generally dry weather followed sowing at all locations. This was reflected in low biomass production at all sites, except Rydalmere (Table 1). Rangi rape produced more fresh weight biomass than Coolabah oats at Rydalmere (under irrigation) and less at Yenda, at the other locations production was similar. At all locations, except Rydalmere, Coolabah produced more dry weight biomass than Rangi. Dry seasonal conditions may have restricted nematode egg hatching, so that only low nematode numbers were recorded). Low numbers at Rydalmere could be related to difficulties in establishing nematodes in the soil, a previous attempt to introduce nematodes at this location met with limited success (McLeod et al. 1995). Only root knot larvae were found.

In each of the trials, nematode numbers under the various treatments differed insignificantly. However, analysis of combined results for all sites (Table 2) indicated a significant difference between no crop and the other two treatments. The difference in nematode numbers under Rangi forage rape and Coolabah oats was insignificant.

Glucosinolate concentrations ranged within narrow limits, from 5.4 μM/g at Camden to 9.0 μM/g at Yenda.

Discussion

It should be noted that the observations recorded were made at Rydalmere, in a warm, coastal environment; crop behaviour could be different in cooler regions. The forage

rapes generally demonstrated attributes wanted in cover crops and, on the basis of experience with Rangi rape (McLeod and Da Silva 1994) produce sufficient biomass to lower nematode levels. Turnips, turnip hybrids and Adagio fodder presented problems of rank growth and seed setting at three to four months. Careful management, particularly turning in at the first indication of flowering should overcome these problems. Indian mustard 99Y-1-1 and white mustard became rank and began setting seed at two months, again these problems may be overcome but increased management would be needed.

Under the dry conditions experienced in the vineyard trials, Coolabah oats produced more dry weight biomass than did Rangi rape. Under more favourable conditions however, Rangi's production can exceed that of Coolabah (McLeod and Da Silva 1994). Even so, a need for biofumigant crops more productive under dry conditions is highlighted. Indian mustards offer possibilities in this regard (Kirk and Oram 1981, Angus et al. 1991). Determinations on drought stressed and unstressed plants from Broke suggest that dry conditions may depress glucosinolate synthesis. Otherwise, GIL concentrations varied only within a narrow range, suggesting similar GIL production in coastal and inland viticultural districts.

While the results demonstrate nematode control from either crop, better control from the brassica crop was not demonstrated. Clearly, low biomass production and low nematode numbers negated decisive testing in this series of trials. Much more field trial scale work remains to be done.

Table 1. Notes on growth habit and pest attack on brassica crops, Rydalmere, spring 1995

Crop	Observations
Forage rapes	
Humus	Closed canopy, up to 50 cm. Light snail damage.
Rangi	Dense closed canopy, up to 60 cm. Light snail damage.
Winfred	Dense closed canopy, up to 70 cm. Light snail, severe aphid.
Turnips	
Barkant	Dense closed canopy, up to 50 cm. Some snail and aphid. Large bulbs, displayed above ground.
Polybra	Dense closed canopy, up to 50 cm. Some snail and aphid. Large bulbs, displayed above ground.
Indian mustard	
99Y-1-1	Open canopy, up to 1.5 m. Light snail and aphid. Flowered at 7 weeks.

Table 2. Biomass production (top growth) and notes on growth habit of brassica crops, Rydalmere, autumn-winter, 1996

Crop	Biomass		Observation
	Fresh weight (t/ha)	Dry weight (t/ha)	
Forage rapes			
Humus	28	2.3	Closed canopy, up to 50 cm, no flowering.
Rangi	59	6.7	Dense closed canopy, up to 70 cm, no flowering.
Winfred	42	4.6	Dense closed canopy, up to 60 cm, no flowering.
Turnips			
Barkant	54	5.4	Dense closed canopy, up to 40 cm, no flowering.
Polybra	27	2.3	Closed canopy, up to 30 cm, no flowering.
Indian mustard			
99Y-1-1	39	3.8	Open canopy. Long stems and small leaves, flowering at 8 weeks.

Table 3. Biomass production (top growth) and notes on growth habit and pest attack brassica crops, Rydalmere, autumn-winter, 1996

Crop	Biomass		Observations
	Fresh weight t/ha	Dry weight t/ha	
Forage rapes			
Arran	52	6.3	Dense closed canopy, to 60 cm. Light aphid and snail damage, flowering.
Bonar	23	4.8	Closed canopy, to 30 cm, severe aphid, not flowering
Hobson	31	4.0	Closed canopy, to70 cm, severe aphid, not flowering
Korina	13	2.0	Openings in canopy, to 20 cm, aphids, flowering
Kale			
Gruner	52	7.2	Closed canopy, to 70 cm, slight aphid, not flowering.
Turnip hybrids			
Pasja	51	5.3	Closed canopy, to 30 cm, slight aphid, seeding.
Simax	33	3.8	1 m, good ground cover, flowering.
Fodder radish			
Adagio	85	10.0	Closed canopy, up to 2 m and rank at maturity, seeding.
White mustard			
Maxi	30	7.0	Open canopy, up to 1.5 m, mostly stem at maturity.

Table 4. Biomass, impact on root knot nematode and glucosinolate production of a brassica and an oat inter-row cover crop in Semillon plantings, autumn 1996

Location and crop	Biomass (top growth)		Nematodes / kg (1)	Glucosinolate µM / g (2)
	Fresh weight t/ha	Dry weight t/ha		
Rydalmere				
No crop	2.0	0.5	31	----
Rangi rape	46.0	5.8	15	8.3 ± 1.7
Coolabah oats	37.0	5.5	10	----
Broke				----
No crop	1.8	0.5	60	
Rangi	9.8	1.7	20	9.0 ± 1.1
Coolabah oats	7.5	2.1	13	----
Camden				
No crop	nil growth	----	13	----
Rangi rape	32.0	3.5	15	5.4 ± 0.5
Coolabah oats	30.0	3.9	8	----
Yenda				
No growth	nil growth	----	48	----
Rangi rape	6.5	1.4	12	7.8 ± 1.0
Coolabah oats	14.0	3.1	30	----
Nericon				
No crop	8.4	1.4	73	----
Rangi rape	17.0	2.4	32	6.5 ± 1.0
Coolabah oats	18.0	3.4	25	----

(1) In 1 kg of inter-row soil. Mean of six replicates, differences not significant.

(2) µM of glucosinolate / g freeze dried material. Mean and standard error, n = 3.

Table 5. Impact of a brassica and an oat inter-row cover crop on root knot nematodes in Semillon plantings, combined results from five locations, autumn 1996

Crop	Nematodes / kg (1)
No crop	47 a
Rangi rape	18 b
Coolabah oats	18 b

(1) In 1 kg of inter-row soil. Mean of six replicates, means followed by the same letter are not significantly different.

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