GRAPE AND WINE RESEARCH AND DEVELOPMENT

FINAL REPORT

Covercrops for management of nematode infestations in vineyards

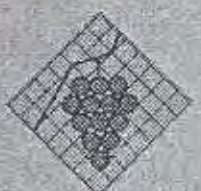
DAN 92/2

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R. W. McLeod, Biological and Chemical Research Institute, Rydalmere (02–843–5777)



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SUMMARY

Project DAN 92/2 focussed on the management of covercrops in relation to nematode infestation in viticulture. There were two main areas of research, one covered the impact of crop residues on nematode survival and the other covered nematode increase on both traditional and innovative crops. The results of the project are reported here in 7 parts (Parts 2–8), summaries of each of theses follow in turn. A summary is also included at the start of each part.

Increase of the rootknot nematodes *Meloidogyne incognita* and *M. javanica* on legume, cereal and brassica crops was studied under glasshouse conditions with introduction of known numbers of eggs and determination of new egg production after 8 weeks. Brassica crops were included because of their potential to more effectively reduce infestation when ploughed in. Both nematodes increased more on the legumes than on the cereals or brassicas, increases of *M. incognita* on Faba bean being comparable to increase on tomato and large increases occurred also on cultivars of field pea, vetch and lupin. Final numbers of both nematodes were less than initial number on 7 of the 15 barleys and oats tested. *M. javanica* produced no eggs on Coolabah oats. Neither nematode produced eggs on Petite Yellow and Cracker Jack marigolds. Both nematodes increased on the six brassica crops tested, this is a concern if they are to be used for control by fumigation.

In two further experiments sod clovers and grasses and selected other covercrops were infected with known numbers of rootknot nematode (*Meloidogyne incognita* and *M. javanica*) eggs and the amount of nematode increase determined by estimating new egg production. There was no difference between the two nematodes. The greatest increase occurred on O'Connors strawberry clover (almost fiftyfold). More than tenfold increase occurred on Kopu

and Tahora white clovers and from sixfold to ninefold increase occurred on Seaton Park, Trikkala and Woogenellup subterranean clovers. No increase and no, or minor reproduction, occurred on Brumby, Citation II, Concord, Duet, Jazz, KV, Tetila or Wimmera ryegrasses or on Tudor creeping, Shadow chewings or Victory chewings fescue grasses. Increase on two barrel medics and four lucernes was less than on the clovers, with least egg production on WL Southern Special lucerne. There was no increase on Swan and Wallaroo oats but the Triticale Tahara supported threefold increase. The brassica crops Hobson forage rape and Humus green manure rape and Rauola oilseed radish supported threefold to tenfold increase, least increase occurring on Rauola radish. There was no reproduction on the marigolds African King or French Minuet.

In an experiment with citrus nematode (*Tylenchus semipenetrans*), sixteen covercrops, representing legumes, cereals, grasses, brassicas and marigold were grown in soil infested with citrus nematode. The nematode survived only on citrus seedling grown as controls. Thus brassica crop green manures can be used for control of citrus nematode without the risk of citrus nematode increase while the crop is in the ground. Legume and cereal crops can also be grown without risk.

Chopped green material (leaves and stems) or chopped roots of covercrops were mixed with infested soil and effects on survival of the rootknot nematodes *Meloidogyne incognita* and *M. javanica* measured to assess and compare biofumigation potential. Green material of Coolabah oat (but not Cooba oat), Dun field pea, Rangi forage rape and Humus green manure rape significantly lowered infestation. Green material of Rangi rape was more effective than green material of Coolabah oat or Dun field pea and green material of Humus rape was more

effective than green material of Rangi forage rape. Chopped roots of Rangi and of Humus rapes were as effective as the chopped green material and chopped tap root of Rauola radish was also effective. Green material of Rangi rape controlled hatched larvae more effectively than it controlled unhatched eggs. Green material of Rangi rape was equally effective when soils from three different sources (bushland, citrus and a vineyard) were used to supply microorganisms to start breakdown of the green material.

In an experiment with citrus nematode *Tylenchulus semipenetrans*, green material of Coolabah oat, Dun field pea and Rangi forage rape substantially lowered infestation, Rangi was signficantly better than the other crops. In a second experiment green material of Rangi and Humus were equally and highly effective against citrus nematode.

When 4 kg/m^2 (40 t/ha) green material was turned into 1 m^2 plots, residual nematode control activity against rootknot, equivalent to that in plots treated with fenamiphos nematicide, was detected 4 weeks after turned–in in soil from plots of Humus rape but not from plots of Coolabah oat, Dun pea, Rangi rape or Rauola radish.

Trial sowings involving 13 covercrops were made from 1992 to 1994 on sites in the Griffith and Hunter Valley districts infested with rootknot (*Meloidogyne incognita*, *M. javanica*) and/or citrus nematode (*Tylenchulus semipenetrans*). The crops were the oat varieties Cooba, Coolabah and Saia, the legumes Dun field pea, Languedoc vetch and Ultra lupin and the brassicas Arran forage rape, Barossa canola, Hobson forage rape, Humus green manure rape, Indian mustard, Rangi forage rape and Winfred forage rape. Brasica crops were emphasised because of their potential for biofumigation.

The observations suggest that autumn sowing of brassicas is a feasible option within the viticultural system. Most experience has been with Rangi forage rape; it was found to have characteristics suiting it to use as a covercrop and was found to be the most successful of the brassica crops tested.

At Griffith there was evidence of rootknot nematode (*M. incognita*) reproduction on autumn sown Languedoc vetch. There was no reproduction on Barossa canola or Rangi forage rape, the brassica covercrops sown there. Ploughed-in residues of Cooba oats and Rangi forage rape controlled citrus nematode more effectively than did residues of Hobson and Winfred forage rapes.

In the Hunter Valley trials there was evidence of rootknot nematode (*M. javanica*) reproduction by mid August on autumn sown Dun field pea and Ultra lupin crops. Some nematode development occurred also on Rangi forage rape sown in early April.

In a small-plot trial at Rydalmere, in which infestation with *M. javanica* was contrived, residues of Coolabah oats, Humus green manure rape and Rangi forage rape lowered numbers of nematodes significantly, residues of Dun field pea and Arran forage rape did not significantly reduce nematode numbers.

Infestation levels of *Meloidogyne javanica*, a major rootknot nematode pest of viticulture in Australia, were significantly lowered by 1.5-60g/kg of mustard seed meal mixed into soil and also by 1.5-8g placed on the surface of 15cm pots watered by overhead sprinklers. However application of effective amounts in the field would probably be

uncompetitive with application of conventional nematicide. Surface applied fresh green material, chopped leaves and stems, of the brassica crops Rangi forage rape and Humus green manure rape significantly lowered infestation, suggesting that biofumigation by mulching of crop topgrowth could be an effective alternative to full incorporation. Surface applied chopped green material of Dun field pea also significantly reduced infestation.

Rangi forage rape and Humus green manure plants sown in the field in mid autumn were not infected by rootknot nematode *Meloidogyne javanica* whereas tomato plants were infected.

Removing tops of Rangi rape plants 7 weeks old and later and removing tops and applying glyphosate herbicide increased rather than decreased nematode egg production.

Infection of undisturbed Rangi plants when 5 and 7 weeks old resulted in less egg production than infection of plants 3 weeks old.

Egg production on Rangi plants transplanted into infested soil was related to root growth. Most eggs were produced on 2 month old transplants, but with good root growth considerable egg production occurred on 3, 4 and 5 month old transplants.

In an experiment whereby hatched second stage larvae of root knot nematode *Meloidogyne javanica* were placed in sand on seedlings more larvae infected the favourable host crops tomato (Grosse Lisse), Dun field pea and Trikkala subterranean clover than infected the less favourable hosts Rangi forage rape and Cooba oats. Infection of all crops was much

reduced in the temperature range 10–15°C and all crops were heavily infested at 15–20°C and 20–25°C. The results are discussed on relation to infection of favourable and less favourable hosts in the field at low soil temperatures. A soil temperature of 18°C is suggested as a guide for autumn sowing of less favourable host cover crops in infested situations.

Seedlings of tomato, Trikkala clover, Rangi rape and Cooba oats growing in 4 x 14 cm tubes, were exposed to infestation levels of 25–200 larvae/tube (tomato and clover) and 50–1000 (Rangi rape and Cooba oats) 25 to 1000 larvae/tube. In the range 25–200 /tube, percent infection (number in–root as a percentage of number added) in tomato was 54 to 77% and percent infection was maintained at higher levels. In Trikkala clover percent infection was from 18–45% percent infection, reduced percent infection occurring with 200/tube. In the range 50–1000 per tube percent infection of Rangi rape was 26–72%, highest percent infection, occurring with 50/tube, percent infection, although lower, was then maintained. Percent infection in Cooba oats was 5–12%, highest percent infection, occurring with 50/tube, percent infection, although lower, was then maintained.

Second stage larvae kept in water at 20°C for 1–3, 4–7, 8–11 and 11–14 days successfully infected tomato seedlings but in a second experiment larvae kept for 8–11 days and longer became parasitised.

In summer and autumn in a glasshouse on Grosse Lisse tomato and Dun field pea, favourable hosts, rootknot nematode *Meloidogyne javanica* produced eggs in 5 to 5.5 weeks while on Rangi forage rape, and Cooba oats, less favourable hosts, egg production took 1 week longer to start. In autumn–winter on tomato and Dun pea egg production started after

7 weeks, on Rangi rape after another 3 weeks and on Cooba oats small numbers of eggs were detected only after 17 weeks. In winter on tomato and Dun pea, egg laying started after 8.5 weeks, on Rangi rape after another 5 weeks while on Cooba oats no eggs were detected over a 13.5 week observation period.

Growth, as measured by nematode profile area, was faster in favourable hosts than in less favourable hosts. At 4 and 6 weeks from infestation favourable hosts contained many more maturing nematodes than unfavourable hosts. Comparison of numbers of larvae infecting with numbers maturing gave maturation percentages of 55–100%, maturation percent was not closely related to host status. Rate of egg production on tomato was severely slowed in winter. Because of slower maturation on Rangi rape, start of egg laying on it in winter was delayed until early spring when temperatures were rising so egg laying proceeded normally.

It is proposed that in a wide range of crops, reduced egg production (less favourable host status), slower growth and reduced infection in less favourable hosts result from the same defence barrier or resistance process in the host. It is suggested that reduced infection is the main contributor to lowered egg production in many unfavourable hosts.

PART 1. BACKGROUND

Introduction

Rootknot disease, caused by nematodes of the genus *Meloidogyne*, has been noted in viticulture in Australia since the early development of the industry (Manuel, 1924). Today a range of nematodes occur in vineyards in all mainland states (McLeod, Reay and Smyth, 1995). The rootknot nematode *Meloidogyne javanica* is the most prevalent, occurring widely in the Hunter Valley, Murrumbidgee and Murray River vineyard districts in New South Wales, the Granite Belt in Queensland, Riverland and Barossa Valley areas in South Australia, Murray River districts in Victoria and the Swan Valley region of Western Australia. A second rootknot nematode *incognita* is also prevalent, occurring in Hasting River, Hunter Valley and Murrumbidgee River regions in New South Wales, Riverland, Barossa Valley and Central areas in South Australia, Murray River districts in Victoria and in Western Australia. A third parasite of grape, citrus nematode *Tylenchulus semipenetrans,* is spread widely in the Murrumbidgee and Murray River districts in New South Wales, in northern and southern districts in Queensland, in the Riverland, Central and Barossa Valley regions in South Australia, in irrigation districts in Victoria and in Western Australia. Several other parasitic nematodes, notably root lesion nematodes (species of *Pratylenchus*) and dagger nematode (*Xiphinema* species) and a few additional rootknot species, occur but are of localised distribution and, except for *Xiphinema index*, which transmits fanleaf virus, there is little evidence to connect them with damage or significant loss of production. Table 1 lists Parasitic nematodes found on grape in various states.

Evidence that nematode damage reduces yield

There is much observational and grower experience to support that nematode damage causes significant loss of production in the field. However evidence from formal field experimentation is limited. Sauer (1966), in a trial of DBCP nematicide on Gordo Blanco infested with citrus nematode in Sunraysia, found an increased yield of 30% in the second, third and fourth seasons after fumigation. In follow-up trials with Sultana significant yield increases of about 12% occurred in the second and third seasons after fumigation. Hedberg et al (1986), in a trial comparing performance of Shiraz on different rootstocks with rootknot and citrus nematode present at Griffith, found yield increases averaging up to 48% over 6 years. Edwards (1988), in a trial comparing performance of Carina on different rootstocks with citrus nematode present in Sunraysia, recorded yield increases averaging 80% over three seasons. Edwards (1989), in trials looking at performance of Shiraz, Chardonnay and Cabernet Sauvignon on different rootstocks with citrus and rootknot nematodes present at Rutherglen, found yield increases of 25% from Shiraz, 93% from Cabernet Sauvignon and 100% from Chardonnay. Walker (1990), in a trial of Nemacur nematicide on established vines with citrus and rootknot present at Loxton, found yield increases averaging 26% over three seasons. Edwards (1991), in trials of nematicides on Sultana in Sunraysia, found significant yield increases in the fourth season after start of treatment with citrus nematode present and in the second season with rootknot present. It should be noted that Harris (1986), in a two trials of nematicides on Sultana with citrus and rootknot nematodes present, found no significant yield increase. It should also be noted that part of the yield increases recorded in these trials could be due to factors other than reduced nematode damage. In roostock trials increased vine vigour may be an innate characteristic of a rootstock. Table 2 lists the work referred to above

and summarises the findings. Table 3 presents an estimate of the monetary cost of nematode damage to the grape crop Australia wide.

Rootstocks and nematode control

There has been much research on using rootstocks to reduce nematode damage. Extensive collections of rootstocks have been screened for resistance and/or tolerance to rootknot, citrus and to a lesser extent to root lesion nematodes, *Pratylenchus spp,* (Sauer 1967, Stirling and Cirami 1984, Wachtel 1986). These trials have established that substantial yield increases can be got from planting vines on rootstocks in nematode infested areas. Rootstocks are now widely planted in Australian viticulture (Cirami 1993). Although mainly used to counter root diseases, rootstocks have many other applications such as providing tolerance to salinity, increasing or decreasing vine vigour, providing tolerance to drought or to acid or alkaline soils (Hardie and Cirami 1988). There are however problems with rootstocks. The high initial cost requires that cost/benefit must be carefully assessed. The method cannot be used to lessen losses in existing plantings. Excess vine vigour induced by the stock can slow ripening of fruit. Winemaking can be affected by changes in juice quality and wine quality may be impaired (McCarthy and Glen 1984, Hedberg *et al.* 1986, Cirami *et al.*, Ruhl *et al.* 1988).

Chemical products and nematode control

Application of chemical products to control nematodes in grape in Australia began with the use of fumigants especially dibromochloropropane (DBCP) (Sauer 1966). Regulatory

action now prevents the application of DBCP. Non-fumigant products for nematode control then became available and several of these have been trialed in vineyards (Harris 1986, Walker 1990, Edwards 1991). At present one antinematode product, Nemacur 400® (400g/L fenamiphos), is registered for control of parasitic nematodes. Nemacur is either sprayed along the interrow or injected into the vine row with drip irrigation water. Registration is limited to Queensland, New South Wales, Western Australia and Tasmania. Although yield increases from Nemacur have been recorded (Walker 1990), it is not used widely. High cost and the current thrust to minimise use of antipest products are major factors which inhibit wider adoption of Nemacur and other antinematode chemicals.

Covercrops in viticulture in Australia

Growing interrow covercrops in autumn-winter is a recommended practice, widely adopted in southern grape areas (Davidson, 1992). Crops most favoured are the cereals such as barley, oats, cereal rye (ryecorn) and triticale and annual legumes such as field peas, tic bean (broad bean) and lupin. These annual covercrops are either ploughed in, mulched or sprayed with desiccant herbicide in spring before they pose a risk of frost damage to vines. Sod culture, where a sward, usually of grass and clover, is maintained in the interrow without cultivation is also recommended, particularly in wet, cool districts and on steep slopes. Although it is known that rootknot nematodes are hosted by many crops, including of the crops grown as covercrops (McLeod, Reay, and Smyth, 1994) and it is know that incorporation of crop residues can lower infestation levels (Singh and Sitaramaiah, 1970), the possible impact of interrow covercrops and swards on nematode infestation in vineyards has received scant attention.

Objectives of project DAN 92/2

The mission of project DAN92/2 has been to gain a better understanding of the relation between covercrops and rootknot and citrus nematode infestation in viticulture with the further aim of identifying ways whereby covercrop management could be varied so that nematode problems are controlled or reduced. The specific topics of investigation were:

*Increase of rootknot nematode and citrus nematode on cereal, legume and brassica covercrops and on rod culture grasses and legumes (**Part 2**).

* Biofumigation – the effects of residues of cereal, legume and brassica covercrops on survival of rootknot nematode and of citrus nematode (**Part 3**).

* Field observations on covercrops, including brassica crops and nematode infestation in vineyards (**Part 4**).

* Biofumigation – Efficacy of mustard seed meal and efficacy of surface applied covercrop topgrowth (**Part 5**).

* Reproduction of rootknot nematode on Rangi and Humus rapes: influence of soil temperature at sowing and of end-of-viticulture covercrop practices (**Part 6**).

* Influence of temperature, infestation level and age of larvae on infection by rootknot nematode of viticultural covercrops of different host status (**Part 7**).

* Influence of season fo the year on maturation of rootknot nematode in viticultural covercrops of different host status (**Part 8**).

Table 1. Nematode parasites of grape in Australia, listed by state.

PEST NEMATODE DISTRIBUTION

Queensland	**NSW**	**Victoria**
M. javanica	M. arenaria	M. incognita
Paratrichodorus	M. hapla	M. javanica
Pratylenchus	M. incognita	Paratrichodorus
T. semipenetrans	M. javanica	Pratylenchus
Xiphinema	M. thamesi	T. semipenetrans
	Paratrichodous	Xiphinema X. index
	Pratylenchus	
	T. semipenetans	
	Xiphinema	

South Australia	**Western Australia**	**Tasmania**
M. arenaria	M. arenaria	
M. incognita	M. incognita	
M. javanica	M. javanica	
Paratrichodorus	Paratrichodorus	
Pratylenchus	Pratylenchus	
T. semipenetrans	T. semipenetrans	
Xiphinema	Xiphinema	

Reference: McLeod, Reay and Smyth, 1994.

Table 2. Yield increases from nematode control measures, field trial results

Reference Type of control	District Grape Variety	Nematode	Yield Increase from control	
Sauer 1966 nematicide	Sunraysia Sultana	citrus	Year 4	12%
Hedburg *et al* 1986 rootstock	Griffith Shiraz	rootknot + root lesion	Over 6 years	48%
Harris 1986 nematicide	Sunraysia Sultana	Rootknot	No significant increase	
Edwards 1988 rootstock	Sunraysia Carina	citrus	Year 1 Year 2 Year 3	86% 75% 82%
Edwards 1989 rootstocks	Rutherglen Shiraz, Cab. Sav.	citrus + rootknot	Shiraz Cab. Sav. Chard.	25% 93% 100%
Walker 1990 nematicides	Loxton	rootknot + citrus	Year 1 Year 2 Year 3	9% 42% 27%
Edwards 1991 nematicides	Sunraysia Sultana	citrus rootknot	Year 4 Year 2	20% 22%

Table 3. An estimate of losses Australia wide resulting from nematode damage to the grape crop.

Crop	Gross value of production ($M)[A] xz	Crop losses (with present control practices)		Nematode of known importance present
		%	~Value ($M)	
Grape	354	7	25	*Meloidogyne* spp. *Tylenchulus semipenetrans, Pratylenchus* spp.

Reference: Stirling, Stanton and Marshall (1992).

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Part 2. INCREASE OF ROOTKNOT NEMATODES AND CITRUS NEMATODE ON ANNUAL COVERCROPS AND ON SOD CULTURE GRASSES AND LEGUMES CULTIVATED IN AUSTRALIAN VITICULTURE

Summary

Increase of the rootknot nematodes *Meloidogyne incognita* and *M. javanica* on legume, cereal and brassica crops was studied under glasshouse conditions with introduction of known numbers of eggs and determination of new egg production after 8 weeks. Brassica crops were included because of their potential to more effectively reduce infestation when ploughed in. Both nematodes increased more on the legumes than on the cereals or brassicas, increases of *M. incognita* on Faba bean being comparable to increase on tomato and large increases occurred also on cultivars of field pea, vetch and lupin. Final numbers of both nematodes were less than initial number on 7 of the 15 barleys and oats tested. *M. javanica* produced no eggs on Coolabah oats. Neither nematode produced eggs on Petite Yellow and Cracker Jack marigolds. Both nematodes increased on the six brassica crops tested, this is a concern if they are to be used for control by fumigation.

In two further experiments sod clovers and grasses and selected other covercrops were infected with known numbers of rootknot nematode (*Meloidogyne incognita* and *M. javanica*) eggs and the amount of nematode increase determined by estimating new egg production. There was no difference between the two nematodes. The greatest increase occurred on O'Connors strawberry clover (almost fiftyfold). More than tenfold increase occurred on Kopu and Tahora white clovers and from sixfold to ninefold increase occurred on Seaton Park,

Trikkala and Woogenellup subterranean clovers. No increase and no, or minor reproduction, occurred on Brumby, Citation II, Concord, Duet, Jazz, KV, Tetila or Wimmera ryegrasses or on Tudor creeping, Shadow chewings or Victory chewings fescue grasses. Increase on two barrel medics and four lucernes was less than on the clovers, with least egg production on WL Southern Special lucerne. There was no increase on Swan and Wallaroo oats but the Triticale Tahara supported threefold increase. The brassica crops Hobson forage rape and Humus green manure rape and Rauola oilseed radish supported threefold to tenfold increase, least increase occurring on Rauola radish. There was no reproduction on the marigolds African King or French Minuet.

In an experiment with citrus nematode (*Tylenchus semipenetrans*), sixteen covercrops, representing legumes, cereals, grasses, brassicas and marigold were grown in soil infested with citrus nematode. The nematode survived only on citrus seedling grown as controls. Thus brassica crop green manures can be used for control of citrus nematode without the risk of citrus nematode increase while the crop is in the ground. Legume and cereal crops can also be grown without risk.

Introduction

A covercrop growing between vine rows will increase pest nematode infestation if the nematodes present can infect the roots of the crop, mature and produce more eggs than present before sowing. *Meloidogyne incognita* and *M. javanica*, the most widespread and damaging nematode pests of vine in Australia (Stirling 1976, McLeod 1978, Harris 1984, Davidson 1992), can reproduce on a very large number of plant species (Khair 1987). While

it is known that many crops are hosts, information is needed on how much these rootknot nematodes reproduce and increase on the various covercrops used in Australian viticulture.

Certain crops in the genus *Brassica*, most notably winter rapeseed cultivars of *B. napus*, have been found to have an enhanced disinfesting effect when residues of them are incorporated into soil (Mojtahedi *et al.* 1991) and this has been attributed to the levels of glucosinolates in these plants the concept of incorporating residues of brassica crops to control pests and diseases has been termed biofumigation (Kirkegaard *et al* 1993).

As with covercrops, a sod culture which includes a grass or legume which is a favourable host, one that allows a nematode to increase by producing more eggs than present before the cover crop is sown, will increase nematode infestation in the interrow and so eventually increase infestation on vines.

After the two rootknots *M. incognita* and *M. javanica* the most prevalent pest nematode of grapes is the citrus nematode *Tylenchulus semipenetrans*. Especially prevalent in vineyards in districts where grapes and citrus grow close to each other (Sauer 1962, Stirling 1976, McLeod 1978), citrus nematode is primarily a parasite of citrus. Other important crops attached, besides citrus and grape, are olive and persimmon (Inserra, Vovlas and O'Bannon, 1980). Although the host range of *T. semipenetrans* is considered to be very restricted, the possibility of citrus nematode increasing on viticultural covercrops cultivated in Australia has not been tested.

The purposes of Part 2 of DAN 92/2 were firstly to determine the relative increase of

Meloidogyne incognita and *M. javanica*, the two major rootknot pests of vines on traditional annual legume and cereal covercrops and secondly, because of the potential for biofumigation to determine relative increase of these nematodes on brassica crops, which are not traditional covercrops in viticulture. Further experiments extended the project to determine rootknot nematode increase on sod culture grasses and legumes and selected other covercrops. A final experiment investigated the possible increase of citrus nematode on covercrops.

Materials and Methods

Supplies of *M. incognita* and *M. javanica* were established by growing tobacco or tomato seedlings in UC mix (1 part coarse river sand, 1 part shredded peat) mixed with vineyard soil (*M. incognita* from Griffith and *M. javanica* from the Hunter Valley). Nematode species were identified by examination of perineal patterns. To prepare inoculum for adding nematodes in an experiment, galled roots bearing egg masses, on 3 months old plants, were chopped and shaken in 0.5% NaOCl for 4 minutes (Hussey and Barker 1973). Root pieces and liquid were then washed on a bank of three sieves, 1 mm, 250 μm and 25 μm opening size. Eggs, retained on the 25 μm sieve, were washed into a 200 ml beaker and the number of eggs/ml estimated by direct counting of four 1 ml samples.

M. incognita and *M. javanica* were tested in separate experiments, experiment 1 (*M. incognita*) beginning 24 November 1992 and experiment 2 (*M. javanica*) beginning on 4 December, 1992.

Seed of each cover crop was sown in five 8 cm diameter plastic plant tubes holding 800

g of pasteurizing potting medium (1 part coarse river sand, 1 part loam soil, 1 part shredded peat). Legume seed was inoculated with the appropriate nodule bacterium culture. After emergence seedlings were thinned leaving three to a pot and the pots arranged in five randomized blocks on glasshouse benches. Three weeks after emergence each pot in experiment 1 was infested with 8000 ± 1800 eggs of *M. incognita*. In experiment 2 each pot received 7000 ± 1200 eggs of *M. javanica* 3 weeks after emergence.

Experiment 3, on the increase of the two rootknot nematodes on sod clovers and grasses, was sown on 11 May ,1993. The treatments, 18 crops by two nematodes (26 treatments), were arranged in five randomised blocks on a glasshouse bench. Legumes were inoculated with appropriate nodule bacteria cultures. Nematodes were added three weeks after sowing, 1400 eggs to a pot. Winter temperatures delayed nematode development so numbers of new eggs were counted 13 weeks after nematodes were introduced.

Experiment 4, on the increase of the two rootknot nematodes on additional selected cover crops, was sown on 29 November, 1993. The treatments, 17 crops by two nematodes (34 treatments), were arranged in five randomised blocks and legumes were inoculated with appropriate nodule bacteria. Three weeks after sowing 1700 nematode eggs were added per pot.

Rootknot nematode increase was determined 8 weeks after nematode infestation except in experiments when 13 weeks was allowed. Roots in each pot were washed free of soil, shaken in 0.8% NaOCl, the extracted eggs collected as already described and counted. Increase factors (If) were calculated by dividing final number of eggs by the initial number (Mojtahedi

et al. 1991).

The If values were then subjected to analysis of variance. Statistical analyses were done using generalised linear models with log link function and with Poisson error assumption.

Experiment 5, on the increase of citrus nematode, was sown on 20 May, 1993. A range of crops was chosen to represent legume, cereal, grass, crucifer and marigold crops (listed in Table 7). Soil collected from orange trees on rough lemon stock at the Horticultural Research and Advisory Station, Griffith, was mixed one parts to three by volume with potting medium (one part peat to one part sand, by volume). Estimates of nematode numbers by tray extraction (Whitehead and Hemming 1965) showed a citrus nematode level of 900 larvae in 800g of the mix. Pots, 8 cm diameter, holding 800g, were filled with the soil mix. The 16 crops (listed in Table 7) were sown in five randomised blocks. Five pots were planted to sweet orange seedlings as controls. All pots were fertilised with Osmocote slow release granules.

Six months after sowing all plants were removed from their pots, the root balls shaken free of loose soil and roots with adhering soil put in a misting chamber for 5 days, to wash out nematodes on the roots or in the soil for counting.

Results

Increases on individual legume, cereal and brassica crops are reported in tables 1 to 3, table 4 compares increase on the three crop groups. Table 5 reports increases on sod cultured

clovers and grasses, table 6 shows results of further tests and table 7 lists crops tested against citrus nematode.

Discussion

Increase factors ranged from 0 to 36.4 indicating extensive scope for minimizing build-up of rootknot nematode infestation through selection of covercrop. In experiments 1 and 2 legumes as a group supported a much larger increase than did the cereal or the brassica groups (Table 4). Considered over all crops, *M. incognita* (in experiment 1) increased more than did *M. javanica* (in experiment 2) (Table 4). It should be noted that these nematode effects would be confounded with any effect due to different experimental conditions. However both experiments were done in the same glasshouse, the only obvious difference being the small difference in starting time. While *M. incognita* increased more than did *M. javanica* on most crops there were exceptions, the increase of one relative to the other also differed from crop to crop and the crop by experiment interaction was highly significant ($P<0.01$).

On the legumes (table 1) *M. incognita* increased most on Faba bean. The increase on Faba bean was as much as on tomato plants included in the experiment as controls. Both nematodes increased more than fourfold on Dun pea, Languedoc vetch and Gungurru lupin. Paraggio medic supported the lowest increase of all the legumes.

Increase on cereals (Table 2) as a group was much less than on either legumes or brassicas. Many cereals had If values well below 1, indicating that moderate or high

infestation levels should be substantially reduced by growing these particular cultivars. *M. javanica* produced no eggs on Coolabah oats. It is also worth noting that neither nematode reproduced on two marigolds, Petite Yellow and Cracker Jack, included in the experiments.

While increase on brassicas (Tables 3 and 4) as a group was much less than on legumes, the increases are of concern if they are to be used for biofumigation. The risk of increase could be reduced if these crops can be sown after soil temperatures cool in autumn and ploughed in before they start to rise in spring (Pung, Barbetti and Sivasithamparam, 1992).

The nematodes increased least on the three mustards tested but the growth habit of these crops may not meet the need for cover crops to produce a dense cover. The ideal brassica control crop would combine low nematode increase with high glucosinolate level, quick growth and production of a large bulk of plant material.

Considering only nematode control, the obvious strategy would be to use the cover crop with the lowest If. Many factors need to be considered when selecting a cover crop, however, and the need for nematode control must be balanced against other requirements.

In experiments 3 and 4 there were again very large differences in the extent to which the rootknot nematodes increased on individual crops. In the previous experiments, the two nematodes increased by different amounts on some crops, and measured over all tested crops, *M. incognita* increased more than *M. javanica*. However statistical analyses showed no difference between the two nematodes, in both experiment 3 and experiment 4. Results for the two nematodes were therefore combined and a single rootknot increase factor calculated for each crop.

All the clovers promoted substantial increases (Table 5), the largest rise, almost fiftyfold, occurring on O'Connors strawberry clover. More than tenfold increase occurred on Kopu and Tahora white clovers. On the subterranean clovers Seaton Park, Trikkala and Woogenellup the increases were from sixfold to ninefold. The ryegrasses and fescue grasses, on the other hand, allowed no increase, indeed there was no, or minor, reproduction (production of new eggs) on them. Inter-row swards in which clovers dominate can thus be expected to maintain high inter-row infestations of rootknot nematodes. Grass swards, in contrast, should be insignificant as sources of vine infection.

Most of the crops in experiment 4 were included because of some special interest. Wilson (1992) noted that the legume Sharnae lotus has resistance to rootknot nematode. Experiment 4 results (Table 6) confirm this in that there was almost no increase on it (increase factor of 0.9, Table 6). Sharnae lotus is summer growing and suited to subtropical coastal conditions, so of limited potential as a vineyard cover crop. Two barrel medics and four lucernes were included to extend the range of Medicago types tested. In general these allowed much less increase than the clovers tested in experiment 3. The increase factor on WL Southern Special lucerne (0.07) is the lowest recorded on a legume in our tests to date. Testing of lucerne types suitable for use as cover crops, such as prostrate forms, could be rewarding.

Swan and Wallaroo oats were included because of uncertainty over the identity of the seed lot tested in experiment 2. There was no nematode increase on either Swan or Wallaroo. They are ranked with Saia and Coolabah as suitable for maintaining low rootknot levels. Tahara Triticale was included because no Triticale cultivar had been tested, threefold increase occurred on it, making it of no special value for management of rootknot infestation.

Increases on Hobson forage rape and Humus green manure rape were much the same as on the forage rapes tested in experiments 1 and 2. While green manure of Humus may be effective for nematode control (J. Brown pers. comm.) the risk of nematode increase on its roots as the crop grows needs to be addressed if it is to be grown for rootknot nematode control. Rauola oilseed radish supported the lowest increase of the crucifers tested so far. Rauola is however not suited to covercropping because of its vigorous, rank growth. More investigation is needed to see whether low nematode increase and especially effective control are features of crucifers, such as radishes and turnips, which have enlarged tap roots.

Marigolds were tested because of the popular interest in them for nematode control. No reproduction occurred on African King or French Minuet marigolds. All of the four marigolds tested so far have totally resisted rootknot reproduction. The test range has included two African (*Tagetes erecta*) types, Cracker Jack and African King, and two French (*T. patula*) types, French Minuet and Petite Yellow.

At the end of experiment 5, with citrus nematode, the sweet orange seedlings had an average 3,200 citrus nematode larvae extracted from them. No larvae were present in the extractions from any of the other plants in the test. These results confirm that covercrops are non-hosts of citrus nematode, thus inter-row covercrops should not increase or maintain infestations of this nematode in vineyards. Brassica crop green manures could be used for control of citrus nematode without the risk of citrus nematode increase while the crop is in the ground.

Table 1. Relative increase of rootknot nematodes *Meloidogyne incognita* and *M. javanica* on legume cover crops (experiments 1 and 2).

Crop	Cultivar/Line	Increase Factor[1] *M. incognita*	*M. javanica*
Faba bean	Tic Bean	36.4 (3.55)[2]	2.4 (1.08)[2]
Field pea	Dun Pea	26.4 (3.23)	12.9 (2.52)
Vetch	Languedoc	15.7 (2.76)	2.4 (1.17)
Lupin	Gungurru	8.0 (2.13)	4.3 (1.61)
Medic	Paraggio	3.5 (1.47)	2.2 (1.17)
	Parabinga	2.9 (1.32)	4.7 (1.70)
	L.s.d. (P=0.05)	(0.40)	(0.35)

[1] Number of nematode eggs on roots after 8 weeks ÷ number added initially, values are averages of 5 estimates.

[2] Average derived after transforming data to ln (x+1) for statistical comparison.

Table 2. Relative increase of rootknot nematodes *Meloidogyne incognita* and *M. javanica* on cereal cover crops (experiments 1 and 2).

Crop	Cultivar/Line	Increase Factor[1] *M. incognita*	*M. javanica*
Oats	Echidna	7.7 (2.14)[2]	1.30 (0.80)[2]
	Cooba	4.2 (1.59)	1.33 (0.81)
Barley	Yerong	2.2 (1.13)	0.31 (0.26)
	Galleon	2.1 (1.08)	0.14 (0.13)
	Chebec	2.0 (1.00)	0.15 (0.14)
Ryecorn	South Australian	1.9 (1.06)	1.00 (0.67)
Barley	Yarran	1.2 (0.78)	1.05 (0.68)
	Skiff	1.2 (0.76)	0.32 (0.27)
	O'Connor	0.96 (0.66)	0.13 (0.12)
	Lara	0.89 (0.57)	0.10 (0.09)
	Schooner	0.69 (0.48)	0.27 (0.23)
Oats	Saia	0.66 (0.47)	0.08 (0.08)
Barley	Clipper	0.40 (0.31)	0.03 (0.03)
Oats	Coolabah	0.27 (0.23)	0 (0)
	Swan/Wallaroo[3]	0.12 (0.17)	0 (0)
	L.s.d. (P=0.05)	(0.40)	(0.35)

[1] Number of nematode eggs on roots after 8 weeks ÷ number added initially, values are averages of 5 estimates.

[2] Average derived after transforming data to ln $(x+1)$ for statistical comparison.

[3] Identity of cultivar not certain but result included to show that there are other oat cultivars as resistant as Coolabah.

Table 3. Relative increase of rootknot nematodes of *Meloidogyne incognita* and *M. javanica* on brassicas (experiments 1 and 2).

Crop	Cultivar/Line	Increase Factor[1] *M. incognita*	*M. javanica*
Forage rape	Emerald	7.6 (2.13)[2]	1.6 (0.93)[2]
	Rangi	7.3 (2.07)	1.1 (0.73)
	Arran	6.5 (1.98)	1.6 (0.93)
Canola/rapeseed	Yickadee	6.3 (1.96)	1.9 (1.03)
	Jupiter[3]	4.0 (1.60)	1.5 (0.78)
	Barossa	3.6 (1.43)	0.81 (0.59)
Mustard	CPI 81792	2.8 (1.34)	–[4]
	Siromo	1.7 (0.96)	2.5 (1.18)
	994–1–1	1.2 (0.74)	2.1 (1.10)
	L.s.d. (P=0.05)	(0.40)	(0.35)

[1] Number of nematode eggs on roots after 8 weeks ÷ number added initially, values are averages of 5 estimates.

[2] Average derived after transforming data to ln $(x+1)$ for statistical comparison.

[3] This cultivar was included in the study of Mojtahedi *et al* (1991) and similar increase of *M. chitwoodi* reported.

[4] Plants did not grow well enough to give reliable result.

Table 4. Relative increase of *Meloidogyne incognita* and *M. javanica* on legumes compared to increase on brassicas and cereals, and increase of *M. incognita* (experiment 1), compared to increase of *M. javanica* (experiment 2).

Crop Group	Increase Factor	
	M. incognita	*M. javanica*
Legumes	13.3 (2.09)	4.13 (1.33)
Brassicas	4.2 (1.44)	1.35 (0.77)
Cereals	1.8 (0.83)	0.48 (0.33)
L.s.d.	(0.21)	(0.20)
Experiment 1, *M. incognita*	6.4 (1.45)[1]	
Experiment 2, *M. javanica*	2.0 (0.76)[1]	

[1] Difference significant, P = 0.01

Table 5. Relative increase of rootknot nematodes *Meloidogyne incognita* and *M. javanica* on sod clovers and grasses (experiment 3).

Crop	Cultivar	Increase Factor[1]
White clover	Haifa	4.5 (2.4–8.3)[2]
	Kopu	12.3 (8.5–17.9)
	Tahora	11.6 (7.9–17.0)
Strawberry clover	O'Connors	49 (40–59)
Subterranean clover	Seaton Park	7.6 (4.5–12.6)
	Trikkala	8.7 (5.3–14.3)
	Woogenellup	5.8 (3.2–10.8)
Ryegrass	Brumby	0.0 *
	Citation II	0.0 *
	Concord	0.03 *
	Duet	0.0 *
	Jazz	0.0 *
	KV	0.03 *
	Tetila	0.02 *
	Wimmera	0.02 *
Fescue grass	Tudor creeping red	0.0 *
	Shadow chewings	0.0 *
	Victory chewings	0.0 *

1. Number of eggs at end of experiment ÷ number introduced to start infestation. As the results for the nematodes were not significantly different, the results were pooled to give a single average.

2. 95% confidence interval.

* Not included in statistical analysis because no eggs were present in most of the replicates.

Table 6. Relative increase of rootknot nematodes (*Meloidogyne incognita* and *M. javanica* on additional selected covercrops (experiment 4).

Crop	Increase Factor[1]
Legumes	
Sharnae lotus	0.9 (0.2–3.5)[2]
Mogul barrel medic	1.6 (0.5–4.4)
Sephi barrel Medic	3.3 (1.6–6.8)
Aurora lucerne	1.5 (0.5–4.4)
Hunter River lucerne	4.2 (2.3–8.0)
WL 516 lucerne	1.1 (0.3–2.8)
WL Southern Special lucerne	0.07 *
Ultra lupin	4.0 (2.0–8.0)
Woollypod vetch	8.0 (5.0–12.7)
Cereals	
Swan oats	0.2 *
Wallaroo oats	0.3 *
Tahara Triticale	3.3 (1.6–6.8)
Brassicas	
Hobson forage rape	9.6 (6.2–14.6)
Humus green manure rape	6.0 (3.5–10.2)
Rauola oilseed radish[3]	2.7 (1.2–6.0)
Marigold	
African King	0.0 *
French Minuet	0.0 *

1. Number of eggs at end of experiment ÷ number introduced to start infestation. As the results for the nematodes were not significantly different, the results were pooled to give a single average.

2. 95% confidence interval.

3. *Raphanus sativus*, a crucifer but not in the genus *Brassica*.

* Not included in statistical analysis because no eggs were present in most of the replicates.

Table 7. Crops tested for increase of citrus nematode, *Tylenchulus semipenetrans* (experiment 5).

Crop
Legumes
Dun field pea
Faba (Tic) bean
Haifa white clover
Languedoc vetch
Paraggio medic
Sharnae lotus
Ultra lupin
Woogenellup subterranean clover
Cereals
Clipper barley
Coolabah oats
Swan oats
Grasses
KV perennial ryegrass
Wimmera ryegrass
Crucifers
Rangi forage rape
Rauola oilseed radish
Marigold
Petite Yellow

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PART 3. BIOFUMIGATION FOR CONTROL OF NEMATODES IN VITICULTURE EFFECTS OF RESIDUES OF CEREAL, LEGUME AND BRASSICA COVERCROPS ON SURVIVAL OF ROOTKNOT AND CITRUS NEMATODES

Summary

Chopped green material (leaves and stems) or chopped roots of covercrops were mixed with infested soil and effects on survival of the rootknot nematodes *Meloidogyne incognita* and *M. javanica* measured to assess and compare biofumigation potential. Green material of Coolabah oat (but not Cooba oat), Dun field pea, Rangi forage rape and Humus green manure rape significantly lowered infestation. Green material of Rangi rape was more effective than green material of Coolabah oat or Dun field pea and green material of Humus rape was more effective than green material of Rangi forage rape. Chopped roots of Rangi and of Humus rapes were as effective as the chopped green material and chopped tap root of Rauola radish was also effective. Green material of Rangi rape controlled hatched larvae more effectively than it controlled unhatched eggs. Green material of Rangi rape was equally effective when soils from three different sources (bushland, citrus and a vineyard) were used to supply microorganisms to start breakdown of the green material.

In an experiment with citrus nematode *Tylenchulus semipenetrans*, green material of Coolabah oat, Dun field pea and Rangi forage rape substantially lowered infestation, Rangi was signficantly better than the other crops. In a second experiment green material of Rangi and Humus were equally and highly effective against citrus nematode.

When 4 kg/m^2 (40 t/ha) green material was turned into 1 m^2 plots, residual nematode control activity against rootknot, equivalent to that in plots treated with fenamiphos nematicide, was detected 4 weeks after turned–in in soil from plots of Humus rape but not from plots of Coolabah oat, Dun pea, Rangi rape or Rauola radish.

Introduction

Inter–rows infested with nematodes will be sources of infection of vines in adjacent rows. If nematicide is applied in bands along the row (Edwards, 1991) or is applied by drip irrigation re–infestation from untreated inter–rows could quickly negate the effect of the treatment. If nematode resistant rootstocks are used, chances of a resistance breaking type (Walker, 1992) arising are increased if inter–row infestations are high.

Application of conventional synthetic nematicides to the whole inter–row is not a realistic option because of the high cost and it also goes against the current pressure for reduced use of industrially made materials.

Brassica and related crops of the family Cruciferae produce appreciable quantities of glucosinolates, these breakdown in soil to release volatile isothiocyanates, chemicals that kill or inhibit insect pests, disease–causing fungi and nematodes. (Kirkegaard *et al.,* 1993) have reviewed pest and disease control from brassicas and have coined the term biofumigation for the concept of incorporating residues of brassica crops to control pests and diseases. Control of a rootknot nematode *Meloidogyne chitwoodi* by ploughing in of green material of Jupiter rapeseed, has been demonstrated in experiments (Mojtahedi *et al.,* 1991, 1993). Research results are thus encouraging more investigation of management of pest nematodes in

agricultural and horticultural systems, through biofumigation.

In part 3 of DAN 92/2 the objective was to investigate the effects of green material and of roots of both brassica and other non-brassica crops on rootknot nematodes *Meloidogyne incognita* and *M. javanica* and on citrus nematode *Tylenchulus semipenetrans*, the nematodes most important in Australian viticulture. Three experiments tested whether or not green material of crucifer crops reduce infestation more than green material of cereal or legume crops. Two experiments compared the effects of the brassica rape cultivars Rangi and Humus. Three experiments compared the effects of green material and of roots of crucifer crops. Two experiments tested residual effects of crops incorporated into small field plots. One experiment compared effects against eggs and against hatched larvae of rootknot (*M. javanica*) and one experiment investigated the effects of using field soil from different sources to start decomposition.

Materials and Methods

Crops and nematodes: The investigation included the oat cultivars Cooba, a popular vineyard cover crop and Coolabah, which is highly resistant to nematode increase (Part 2, McLeod and Warren, 1993), Dun field pea, also a popular vineyard covercrop, and the crucifer crops Rangi, a low-cost, long-used brassica forage rape, Rauola oilseed radish (CPI 58403) brassica, grown in Swiss vineyards (J.A. Kirkegaard, personal communication), and Humus (Brassica napus), a winter rape developed by the University of Idaho, USA for green manure purposes and selected for high glucosinolate levels (J. Brown, personal communication).

Cultures of *M. incognita* and *M. javanica*, originally from vineyard soil (Part 2,

McLeod and Warren, 1993), were maintained separately on tomato or tobacco plants. Soil infested with citrus nematode was collected as required directly from the field.

Procedures: 500g lots of pasteurised UC potting soil (1 part coarse river sand to 1 part shredded peat) were placed in plastic bags. Each bag was infested by adding 2–4g of a mixture of chopped roots infected with *M. incognita* and roots infected with *M. javanica*, which had been incubated for 1 week to provide an inoculum of egg masses, eggs and hatched larvae. 50g of sandy loam vineyard soil from Pokolbin, Hunter Valley was then added to provide micro-organisms to start decomposition.

Green material (leaves and stems) or roots, from 2 – 3 month old crops, were chopped in a peat shredder, reducing the material to pieces of 3 – 5 cm in size. Amounts of 10, 20 or 40g were added to a bag, the total contents shaken and the bags tied closed with twisties. Five bags of each treatment were prepared and the bags were placed in a single layer on trays, in 5 randomized blocks, in a room at 18 – 20°C. After one month each bag's content was transferred to an 8 cm diameter plastic plant tube and a 3-week-old Grosse Lisse tomato seedlings planted. After growing for 3 weeks in a glasshouse the roots of the test tomato plants were washed and the knots on them counted.

In experiments investigating effects on citrus nematode, naturally infested sandy loam dug from under the canopy of rough lemon citrus trees growing at the Horticultural Research and Advisory Station at Gosford was used instead of UC potting soil and no starter soil was added. After one month each bag's contents was transferred to a Whitehead and Hemming (1965) tray and after 5 days the number of emerged active larvae counted.

Results were analysed using a generalised linear modelling statistical method. Treatment groups were compared, for example the three levels (10, 20, 40 g) of one crop compared to the three levels of another, by partitioning of the variance.

Comparison of crucifer and other crops: Experiment 1 compared green material of Cooba oat, Dun field pea and Rangi rape at one rate only, 40g to a bag, against rootknot. Experiment 2 compared green material of Coolabah oat, Rangi rape and Rauola radish at rates of 10, 20 and 40g, against rootknot. Experiment 3 compared green material of Coolabah oat, Dun field pea and Rangi rape at 10, 20 and 40g, against citrus nematode.

Comparison of Rangi and Humus rapes: Experiment 1 compared green material of Rangi and Humus rapes at 10, 20 and 40g, against rootknot, and included a treatment of 8mg of the nematicide fenamiphos (Nemacur Granular Nematicide, 5% fenamiphos). Experiment 2 tested the same rates of the two green materials, against citrus nematode.

Comparison of green material and roots: Experiment 1 compared green material and root material of Rangi and of Humus, at 10, 20 and 40g, against rootknot. Experiment 2 compared roots only of Rangi and Humus at 10, 20 and 40g, against rootknot. Experiment 3 compared roots of Rangi and Rauola radish (swollen tap root) at 10, 20 and 40g, against rootknot.

Small plot experiments: Crops were grown in 1m x 1m plots, spaced 0.5m. apart, at Rydalmere in a clay loam (64% sand, 8% silt, 28% clay, 8.5% organic matter, pH 6.6), not infested with nematodes. Plots were fertilized with 80g/m^2 of Osmocote (14–6.1–11.6) for

oat, rape and radish and 50g/m^2 superphosphate plus 50g/m^2 lime for Dun pea. Oat and pea seed (inoculated with nodule bacteria) was sown at 10g/m^2 and rape and radish at 1g/m^2.

Experiment 1, started on 19 April 1993, compared Coolabah oat, Dun field pea, Rangi rape, Rauola radish and an uncropped control in six randomized blocks. Three months after sowing plots were mown, the fresh yield of green material recorded, and a count taken for dry matter estimation. 4kg of each plot's green material (2 Dun field pea plots yielded 3.0 and 3.6kg only) was chopped to pieces of 10 – 20cm length, returned to the plot and turned in with a small rotary hoe to a depth of 10cm. Four weeks after turn-in 800g of soil was collected in plastic bags from each plot, 3g of chopped galled root (*M. incognita* plus *M. javanica*) was mixed with each soil sample, sample plus nematode inoculum were transferred to an 8cm plant tube and a bioassay done as previously described.

Experiment 2, started on 1 July 1993, compared Coolabah oat, Rangi rape and Humus rape, an uncropped control and plots treated with fenamiphos (Nemacur Granular Nematicide, 5% fenamiphos at 1g fenamiphos per m^2) in six randomized blocks. Crops were turned in 3 months after sowing and fenamiphos was applied at this time. Experimental procedure was then as in experiment 1.

Effect on eggs compared to effect on hatched larvae: Eggs were obtained by shaking chopped galled roots of tomato (*M. incognita* mixed with *M. javanica*) in 0.5% NaOCl for 4 minutes. The suspension was then poured through 1000, 150 and 25μm banked sieves, after thorough washing eggs were washed off the top of the 25μm sieve and refrigerated.

Hatch larvae were obtained by collecting eggs as above on a 25μm sieve, then placing the sieve with the mesh just under water in a dish for 5 days and then collecting the hatched larvae on a 5μm sieve.

The experiment, with green material of Rangi rape at 10, 20 and 40g, compared effects on eggs and effects on hatched larvae. Eggs were introduced by adding 4ml of a suspension containing 1800 ± 400 eggs to a bag. Larvae were introduced by adding 4ml of a suspension containing 1600 ± 560 active hatched larvae to a bag.

Comparison of different starter soils: Three soils, all sandy loams, one from a vineyard at Pokolbin, Hunter Valley (77% sand, 7% silt, 16% clay, 1.6% organic matter, pH 4.7), another from under Rough Lemon trees at Gosford (85% sand, 15% clay, 2.3% organic matter, pH5.5) and the third from under native vegatation at Somersby (90% sand, 10% clay, 1.3% organic matter, pH 6.3) were collected. The Somersby soil was later found to have a very heavy natural infestation of *M. javanica*. The experiment, with green material of Rangi rape at 10, 20 and 40g, compared UC potting soil plus one or other of the soils as the starter soil.

Results

Crucifer compared to other crops: All crops, except Cooba oats (Table 1, experiment 1) in these experiments significantly controlled rootknotting on test plants, and significantly reduced survival of citrus nematode. In experiment 1 Rangi rape was significantly better than the

other crops, in experiment 2 the Rangi rape and the Rauola radish treatment groups were both significantly better than Coolabah oat treatment group and in experiment 3, against citrus nematode, the Rangi rape treatment group was significantly better than the Dun field pea and Coolabah oat groups. The superior effect of green material of crucifer crops compared to other crops against rootknot nematodes (Mojtahedi *et al* 1991) is thus confirmed. A similar enhanced effect against citrus nematode is demonstrated.

Rangi rape compared to Humus rape: In experiment 1 (Table 2), against rootknot, the Humus rape treatment group was significantly better than the Rangi rape group. At 20 and 40g it was as effective as the 8 mg of fenamiphos nematicide treatment. In experiment 2, against citrus nematode, both crops gave a high level of control and there was no significant difference between them.

Green material compared to roots: In all three experiments (table 3), both green material and root material reduced rootknot significantly. In experiment 1 there was no significant difference between the green material and the root material treatment groups. In experiment 2 there was no difference between Rangi root material and Humus root material treatment groups. In experiment 3 there was no difference between Rangi root material and Rauola radish tap root. All treatments in experiment 1 allowed considerable knotting. However, the test infestation in this experiment (shown by the nil treatment) was extreme, such levels would be extremely rare in the field.

Small-plot experiments: Treatment differences (Table 4) were not significant in experiment 1. In experiment 2, the Humus rape and fenamiphos results differed significantly from the

uncropped control. Both experiments tested treatment effects remaining 4 weeks after turning in, also the extreme test infestation level in experiment 1 (shown by the no crop treatment) could have obscured treatment differences. In experiment 2, the effects of Coolabah oat and of Rangi rape had become undetectable (not significantly different from uncropped control) while control activity of Humus rape was detectable (significantly different from uncropped control) and equivalent to the residual activity of fenamiphos nematicide.

Effects on eggs and larvae and effect of different starter soils: The results (Table 6) show that hatched rootknot larvae are more vulnerable to the action of green material of Rangi rape than eggs.

On the basis of individual treatment results (Table 7) control appeared best with Hunter vineyard soil and least with Somersby bushland soil. With Somersby soil test infestation (shown by its nil control) was much higher than with the other soils. When this was adjusted for in statistical analysis there was no signfiicant difference between the soil treatment groups.

Discussion

The results confirm the potential for better control of nematode infestation through ploughing in of a crucifer crop. The superior effect of Rangi rape is perhaps not as great as the superior effect of Jupiter rape against *M. chitwoodi* demonstrated by Mojtahedi *et al.,* (1991). However, comparison of results is problematic because they assessed effects differently, by staining and counting numbers of larvae entered into roots of test tomato plants. When rootknot load was extreme (Table 3, experiment 1) considerable knotting of test plants occurred. Nevertheless, with typical infestation levels, the effect should be of value in the field. However crucifer crops, may support nematode increase (Part 2, McLeod and Warren, 1993). They should be sown and grown when soil temperatures are low (below 16°C, Roberts, Van Gundy and McKinney, 1981) so that rootknot infection is inhibited.

Although Coolabah oat was less effective than Rangi rape or Rauola radish (Table 1), the effect demonstrated, both against rootknot and citrus nematodes, is worth underlining since this crop also allows virtually no reproduction of rootknot as the crop grows (Part 2, McLeod and Warren, 1993). Rauola radish grew very tall, it produced rank growth that was difficult to turn in.

Humus rape was significantly more effective than Rangi rape against rootknot and was very effective against citrus nematode (Table 1 and 2), and its effect persisted longer in field plots (Table 4). These results are consistent with a higher glucosinolate level in Humus. Further trialing of Humus as a specific nematode control crop is warranted.

In a comparison of effects roots and leaves, Mojtahedi *et al* (1993) found that although roots of Jupiter rape containing more glucosinolate than leaves, chopped roots were less effective than similarly chopped leaves. When homogenised however roots were highly effective and they suggested in explanation that roots may breakdown more slowly than leaves and so release isothiocyanates later. A longer exposure period (4 weeks instead of the 10 days in Mojtahedi's *et al*, experiment) may account for the similar effects of chopped green material and chopped roots noted in our experiments.

Control from both green material and roots should be a significant plus in the field. One should add to the effect of the other and control from roots should provide a deeper and a more even effect than would be the case if roots were not effective. Delayed release from roots should prolong control.

In the small plot experiments, $4kg/m^2$ (40t/ha) of green material was turned in to a depth of 10cm (that is into a volume of 100L of soil). Soil bulk density was estimated to be $1.33g/cm^3$ (1.33kg/L), so 4kg of green material was added to 133kg soil, that is 30 g of green material to 1kg of soil. In the soil–in–bag experiments amounts added were 18g/kg (10g to 550g) to 72g/kg (40g to 550g). Thus $4kg/m^2$ (40t/ha) of green material is within the range needed, on the basis of the soil–in–bag experiments, to give significant control. Green material yields of this order are quite feasible (Table 5).

Control activity in soil after 4 weeks from green material releasing volatile isothiocyantes is unlikely (Brown *et al.*, 1991). However Mojtahedi *et al* (1993) found leaves of Jupiter rape protected soil for 6 weeks. Delayed release from root material could have

contributed to the effect detected at 4 weeks in our small-plot experiment.

As eggs are less vunerable than hatched larvae, plough-in should be 1 to 2 weeks after rain or irrigation and after soil temperature start to rise in spring so that eggs are hatched or about to hatch. However ploughing in should not be delayed more than this because infection and new egg production could occur.

All three of the soils compared as starter soils were low in organic matter (1.3-2.3%), nevertheless each promoted significant control. This suggests that low organic matter, mineral soils have sufficient microorganisms to facilitate release of isothiocyanates.

Table 1. Effects of green material of cereal, legume and crucifer crops on survival of rootknot nematodes and of citrus nematode, soil-in bag experiments.

Crop	g/550g	Experiment 1 Knots/test plant[1]	Experiment 2 Knots/test plant[1]	Experiment 3 Surviving citrus[1] larvae/550g
None	0	146a[2]	38a[2]	16464a[2]
Cooba Oat	40	101ab	–	–
Coolabah oat	10	–	22b	5392b
	20	–	23b	3672b
	40	–	15bc	2072cd
Dun pea	10	–	–	5616b
	20	–	–	2976c
	40	67b	–	1128e
Rangi rape	10	–	29a	3520bc
	20	–	13bcd A	1280de B
	40	14c	3e	920e
Rauola radish	10	–	12bcd	–
	20	–	10cd A	–
	40	–	6de	–

[1] averages of 5 observations.

[2] averages with the same letter in a column do not differ significantly, P>0.05.

A. Rangi rape and Rauola radish treatment groups differ from Coolabah oat group, P<0.01.

B. Rangi rape treatment group differs from Coolabah oat and Dun pea group, P<0.01.

Table 2. Effects of green material of Rangi rape and of Humus rape on survival of rootknot nematodes and citrus nematode, soil-in bag experiments.

Crop	g/550g	Experiment 1 Knots/test plant[1]	Experiment 2 Surviving citrus larvae/500g[1]
None	0	139a[2]	5388a[2]
Rangi	10	90ab	1332b
	20	68bc	674c
	40	66bc	605c
Humus	10	73bc	1288b
	20	39cd A	928bc
	40	39cd	577c
Fenamiphos[3]	8mg	22d	–

[1] averages of 5 observations.
[2] averages with the same letter in a column do not differ significantly, P>0.05.
[3] As Nemacur Granular Nematicide, 5% fenamiphos.
A. Humus treatment group differs from Rangi group P<0.01.

Table 3. Effects of green material and of chopped roots of Rangi rape, Humus rape and Rauola radish on survival of rootknot nematodes, soil-in bag experiments.

Crop	g/550g	Experiment 1 Knots/test plant[1]	Experiment 2 Knots/test plant[1]	Experiment 2 Knots/test plant[1]
None	0	334a[2]	112a[1]	26a[2]
Rangi green	10	191bc	–	–
	20	163bcde	–	–
	40	128cde	–	–
Rangi root	10	165bcde	34b	13b
	20	96e	50b	2c
	40	145bcde	32b	2c
Humus green	10	170bcd	–	–
	20	134cde	–	–
	40	193bc	–	–
Humus root	10	127cde	24b	–
	20	120de	45b	–
	40	220b	28b	–
Radish root	10	–	–	2c
	20	–	–	5c
	40	–	–	4c

[1] averages of 5 observations.
[2] averages with the same letter in a column do not differ significantly, P>0.05.

Table 4. Survival of rootknot nematodes added to soil taken from small plots 4 weeks after turning in cereal, legume and crucifer crops, Rydalmere 1993.

Crop[1]	Experiment 1 Knots/test plant[2]	Experiment 2 Knots/test plant[2]
None	257a[3]	153ab[3]
Coolabah Oat	215a	164a
Dun pea[1]	268a	–
Rangi rape	204a	110bc
Rauola radish	180a	–
Humus rape	–	72c
Fenamiphos nematicide[4]	–	63c

[1] 4 kg/m^2 of green material turned in, but 2 Dun pea plots yielded 3.0 and 3.6 kg only.
[2] averages of 6 plots.
[3] averages with the same letter in a column do not differ significantly, P>0.05.
[4] As Nemacur Granular Nematicide, 5% fenamiphos, 1g fenamiphos/m^2.

Table 5. Yields of green material of cereal, legume and crucifer crops, small plots, Rydalmere, 1993.

Crop	Experiment 1		Experiment 2	
	Fresh weight[1] kg/plot	Dry matter[1] kg/plot	Fresh weight[1] kg/plot	Dry matter[1] kg/plot
Coolabah Oat	7.1b[2]	0.67b[2]	4.5b[2]	0.52b[2]
Dun pea	4.0c	0.44c	–	–
Rangi rape	9.9a	0.80a	7.9a	0.67a
Humus rape	–	–	5.2b	0.47b
Rauola radish	6.3b	0.49c	–	–

[1] averages of 5 observations.
[2] averages with the same letter in a column do not differ significantly, P>0.05.

Table 6. **Effects of green material of Rangi rape on survival of eggs and of hatched larvae of rootknot nematodes, soil–in bag experiment.**

Green material added g/550g	Eggs added, knots/ test plant[1]	Hatched larvae, knots/ test plant[1]
None	21.0a[2]	17.0a
10	17.0a	2.4d
20	9.4b A	1.6d B
40	2.8c	0c

[1] averages of 5 observations.
[2] averages with the same letter in a column do not differ significantly, P>0.05.

A, B. Egg treatment group differs from hatched larvae group, P<0.01.

Table 7. Effects of green material of Rangi rape on survival of rootknot nematodes in UC potting soil with different starter soils, soil-in bag experiments.

Starter soil	Green material, g/550g	Knots/test plant[1]
Hunter vineyard	0	62bc[2]
	10	42cde
	20	35de
	40	7f
Gosford citrus	0	53cd
	10	25e
	20	44cde
	40	29e
Somersby bushland	0	106a
	10	64bc
	20	81b A
	40	58bcd

[1] averages of 5 observations.

[2] averages with the same letter in a column do not differ significantly, $P>0.05$.

A. Somersby bushland treatment group differs from the other groups, $P<0.01$. When difference in test infestation (nil control) is allowed for, difference is not significant.

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PART 4. FIELD OBSERVATIONS ON COVERCROPS, INCLUDING BRASSICA CROPS, AND NEMATODE INFESTATION IN VINEYARDS

Summary

Trial sowings involving 13 covercrops were made from 1992 to 1994 on sites in the Griffith and Hunter Valley districts infested with rootknot (*Meloidogyne incognita*, *M. javanica*) and/or citrus nematode (*Tylenchulus semipenetrans*). The crops were the oat varieties Cooba, Coolabah and Saia, the legumes Dun field pea, Languedoc vetch and Ultra lupin and the brassicas Arran forage rape, Barossa canola, Hobson forage rape, Humus green manure rape, Indian mustard, Rangi forage rape and Winfred forage rape. Brasica crops were emphasised because of their potential for biofumigation.

The observations suggest that autumn sowing of brassicas is a feasible option within the viticultural system. Most experience has been with Rangi forage rape; it was found to have characteristics suiting it to use as a covercrop and was found to be the most successful of the brassica crops tested.

At Griffith there was evidence of rootknot nematode (*M. incognita*) reproduction on autumn sown Languedoc vetch. There was no reproduction on Barossa canola or Rangi forage rape, the brassica covercrops sown there. Ploughed-in residues of Cooba oats and Rangi forage rape controlled citrus nematode more effectively than did residues of Hobson

and Winfred forage rapes.

In the Hunter Valley trials there was evidence of rootknot nematode (*M. javanica*) reproduction by mid August on autumn sown Dun field pea and Ultra lupin crops. Some nematode development occurred also on Rangi forage rape sown in early April.

In a small-plot trial at Rydalmere, in which infestation with *M. javanica* was contrived, residues of Coolabah oats, Humus green manure rape and Rangi forage rape lowered numbers of nematodes significantly, residues of Dun field pea and Arran forage rape did not significantly reduce nematode numbers.

Introduction

Research in Part 2 of Project DAN 92/2 (McLeod and Warren, 1993; McLeod, 1994) has shown that legume crops such as peas, lupins, vetches, tic beans and clovers are very favourable hosts of rootknot nematodes (*Meloidogyne javanica, M. incognita*) and are likely to increase infestation if grown as covercrops in viticulture. It has also shown that most cereal crops and grasses allow little or no nematode reproduction. Growing barleys, most oat varieties and ryegrasses should lead to less nematode infestation in vineyards.

Research in Part 3 of DAN 92/2 (McLeod and DaSilva, 1994) has shown that residues, tops and roots, of covercrops reduce nematode infestation when mixed into soil. Residues of some brassica crops, and other crucifer crops, were more effective than residues of cereal or legume crops, probably because brassica crop residues release volatile,

nematode–toxic isothiocyanates. The concept of using brassica residues to reduce soilborne pests and diseases is termed biofumigation (Kirkegaard *et al.* 1993).

Much of the research referred to above relies on laboratory and glasshouse tests. Part 4 of DAN 92/2 looked at field performance of legume, cereal and also brassica crops, in vineyards where there were well established rootknot or citrus nematode infestations. Information on crop performance and nematode development is recorded. Observations are based on trials in the Griffith and Hunter Valley districts, sown in 1992, 1993 and 1994. Results from a field experiment with small plots at a non– vineyard site are also included.

Growing of crops and nematode assessment

Sowings were either demonstration sowings, along the entire length of interrows, or were trials with plots of various crops within interrows. Sowing times were from end of March to third week of May. Sowing was by hand broadcasting or by machine drilling. Large seeded crops, such as peas, lupins, oats, were sown at 100 kg/ha, brassica crops were sown at 10 kg/ha. All crops received 200 kg/ha of single superphosphate or 400 kg/ha of lime/superphosphate, peas were inoculated with appropriate nodule bacteria cultures and oats sowings received 100 kg/ha of Starter 15 (14:10:15) to supply nitrogen. Crops were grown without irrigation.

At most sites nematode levels were assessed before planting by collecting soil samples of 1kg at a depth of 5 to 25cm and estimating numbers of active larvae (tray

extraction method, Whitehead and Hemming 1965). Nematode development in crop roots was looked at by digging plants in August and examining stained roots to determine nematode presence and stage of development. When crops produced enough top growth to provide a worthwhile addition of topgrowth soil samples were taken one month after plough-in, to assess nematode control from ploughed-in crop residues.

Griffith district trials and observations

On 31 March 1992 at Yoogali interrows (rootknot nematode *M. javanica* and citrus nematode present) were sown to Barossa canola, Languedoc vetch and Coolabah oats. Sampling before sowing indicated interrow nematode levels of 150 rootknot and 900 citrus nematode/1kg soil (average of 10 samples). Within row levels were 600 rootknot and 50 citrus nematodes/1kg soil. In this trial soil samples were taken again on 12 May, 6 weeks after sowing (Table 1). Crop growth was patchy and restricted by early dry conditions. The May soil samples, 5 from each crop, showed active rootknot and citrus nematode larvae in soil under all crops (Table 1). Digging of 10 plants of each crop on 24 August, 5 months after sowing, detected rootknot nematodes in the roots of Languedoc vetch but not in the roots of Coolabah oats or Barossa canola. Five soil samples were taken on 10 October, one month after plough-in, from each crop and from adjacent uncropped interrows. Nematode numbers were low in all samples (less than 100/1kg soil) so no assessment of nematode control from ploughing in of the crop residues eventuated.

On 21 May 1993 interrow plots of Rangi forage rape, Barossa canola and Cooba oats were sown on the site of the 1992 trial. Crop growth was restricted by lack of water,

brassica crops showed patches of small purplish plants probably due to uneven fertiliser application. Rangi rape still provided good ground cover. Barossa canola was more stemmy, allowing more weed growth, and was in flower in August whereas Rangi rape was not. Digging of 10 plants of each crop on 30 August, 3 months and 10 days after sowing, indicated no nematode development on any crop. The trial was not further assessed because of the restricted growth of crops.

Also on 21 May 1993 interrows at Corbie Hill were sown by drill to Cooba oat, ryegrass, Hobson forage rape, Rangi forage rape and Winfred forage rape. Sampling of interrow soil before sowing indicated absence of rootknot nematode but a citrus nematode level of 1000/1kg soil (average of 10 samples). All forage rapes produced growth 30–80 cm high and provided good ground cover. At the ends of rows, where fertiliser may have been missed, plants were small and purpled, indicating nutrient deficiency. On 16 November, one month after plough-in, three 1 kg soil samples, made up of 10 x 100 g subsamples were taken from under each crop. Estimates of nematode numbers (Table 2) indicated substantially lower citrus nematode levels where Rangi rape had grown than where the other rape crops had grown. Numbers were also low under Cooba oats, indicating an effect from residues of this crop as well.

Hunter Valley trials and observations

On 3 April and 7 April 1992 interrows were drill sown to covercrops at two sites

near Pokolbin. The rootknot nematode at both sites was identified as *M. javanica*. Cooba oats, Dun field pea, Ultra lupin and Rangi forage rape were sown at one site (Site 1). Soil sampling before sowing indicated an interrow rootknot nematode level of 2200/1kg soil at Site 1. All crops suffered severe weed competition and severe grazing damage at this site so that there was not enough top growth to warrant assessing nematode levels after plough-in. On 12 August, 4 months and 3 weeks after sowing, 10 plants of each crop were dug to check nematode development. Small knots were found on Dun field pea, Ultra lupin and Rangi forage rape. The knots on Dun pea and Ultra lupin had mature females and egg masses. Knots on Rangi rape contained immature females but no eggs. Small knots were found on Cooba oats but no nematodes were found in them.

At Site 2 interrows were sown to Ultra lupin and Rangi rape. Nematode numbers were not estimated before sowing but soil samples taken on 19 May, 6 weeks after sowing, indicated an interrow level of 1200 rootknot nematodes/1kg soil (average of 5 samples) and a vine row level of 800/1kg soil. Rangi rape grew well, to 50 cm high, providing dense ground cover and a bulk of green matter. Digging of plants on 12 August, 4 months after sowing, revealed knots on Ultra lupin plants and small knots also on Rangi rape plants. The Ultra lupin knots contained adult females and eggs, Rangi rape knots contained females but no eggs. Ten soil samples were taken under each crop on 26 August, 2 weeks after ploughing in. No nematodes were found in the samples from under Rangi rape, an average of 10 per 1kg of soil occurred under Ultra lupin.

On 22 April 1993 interrow plots, 45m long, at Site 1 of 1992, were sown to Saia oats, Dun field pea, Barossa canola, Humus green manure rape and Rangi forage rape by

hand broadcasting. Stand establishment was uneven and in June growth of all crops was showing drought stress. Brassica crops showed nutrient deficiency in patches, probably because of uneven distribution of fertiliser. Growth of Humus green manure rape was obviously inferior to growth of other crops. Barossa canola growth was stemmy, allowed weed growth and it flowered in August, well ahead of Rangi and Humus rapes. Digging of 10 plants of each crop on 11 August, 3 months after sowing, revealed knots only on Dun field pea, females but no eggs occurred in the knots.

In 1993 there was opportunity to observe interrows sown to Hobson forage rape at Broke. The sowing made very strong growth. Plants grew to 90 cm, ploughing in was difficult because of the thick, tough stems and regrowth required ploughing in again.

At Broke in 1994 mustard (oilseed variety 99Y-1-1) sown interrow in autumn made good growth despite the exceptionally dry season. Trial sowings of other brassicas including Arran, Rangi and Humus rapes in the Hunter Valley failed because of the dry conditions and were attacked by cabbage aphid. The drought conditions appeared to favour cabbage aphid outbreak and damage. Rangi seemed better able to withstand aphid damage than the other rapes.

Small plot trial Rydalmere 1994/95

Dun field pea, Coolabah oat, Arran forage rape, Humus green rape and Rangi forage rape were sown in 1 m by 1 m plots in six randomised blocks on 25 October. On 17 January 1995, 2 months and 3 weeks after sowing, plots were mown, the cut plant tops

removed and on each plot 6 L of soil plus rootknot nematode (*M. javanica*) infected tomato plant roots was spread to provide a uniform nematode infestation. Four kg of material cut from the plot was then returned to each plot and the plots rotary hoed to mix in the nematode inoculum and to incorporate the cover crop residues. On 14 February, 1 month after rotary hoeing, 1 kg of soil was taken from the centre of each plot to assess the effects of the various cover crops on nematode numbers (tray extraction method, Whitehead and Hemming 1965). The results (Table 3) show that green materials of Coolabah oat, Humus green manure rape and Rangi forage rape all lowered nematode numbers, green materials of Dun pea and Arran rape were not so effective.

Discussion

Our observations suggest that autumn sowing of brassicas in vineyards is a feasible option. Most experience has been with Rangi forage rape. Humus, a cultivar developed in north America for biofumigation, has performed well in control of nematodes (Table 3 results and McLeod and Da Silva 1994) but did not grow as well as Rangi in most situations. Both are compact crops, growing to 50cm, with soft leaves and stems that are easy to plough in. Leafy, forage type brassicas provide good ground cover and a bulk of top growth, features needed in covercrops. Barossa canola, being a brassica type bred for seed production, produced much less leaf bulk, less ground cover and its early flowering may require early ploughing in. Brassica crops should be ploughed in before flowering to prevent seed set.

Important requirements for successful establishment of forage type brassicas include

thorough cultivation to control weeds and preparation of a fine, firm seedbed. Sowing depth should be no deeper than 25 mm and there should be enough moisture at sowing to ensure germination. Application of 200–400 kg/ha of single superphosphate or lime/super will usually be needed, soil pH should be above 5.5 and preferably 6.0 to 6.8. Nitrogen fertilisers should be considered where soil nitrogen is known to be low but these must not be applied in contact with seed because gemination can be severely reduced. Borated fertilisers are advisable where there is a history of boron deficiency, again these must not be placed in contact with the seed. Drought severely affected the growth of the forage type brassicas in our trials. This may have resulted partly from dry conditions at sowing. However leafy forage brassicas may be unsuitable where winter rainfall is unreliable, unless irrigation is possible.

Rangi rape controlled citrus nematode better than either Hobson or Winfred forage rapes at Corbie Hill in 1993. Rangi is an old, relatively unimproved forage rape, so it may contain higher levels of glucosinolates than more modern forage brassicas. Rangi also performed as well as Humus in the small plot trial in 1994/95, against rootknot nematode. It should be noted that continuing research is likely to lead to brassicas with improved nematicidal activity and types suiting a range of situations. It should also be noted that residues of Cooba oats (Table 2) and Coolabah oats (Table 3) controlled nematodes. Residues of these crops should also contribute to controlling nematodes.

Detection of reproducing nematodes on Dun pea and Ultra lupin in 1992 and on Dun pea in 1993 in the Hunter Valley indicates that winter temperatures there are warm enough to allow nematode reproduction on April sown legume crops by mid August. Nematodes

had maturated on Rangi rape by mid August but had not laid eggs. In 1993 sowing was later, on 22 April, nematodes had matured on Dun pea by mid August but no nematodes were found on Barossa canola, Humus rape or Rangi rape. Brassica crops, which are moderate hosts, should be sown later than mid April and ploughed in by mid August in the Hunter Valley to minimise the risk of nematodes reproducing and thereby offsetting their biofumigation action. At Griffith there was no indication of nematode development on brassicas.

Table 1. Numbers of nematodes under autumn sown covercrops 6 weeks after sowing, Griffith 1992

Crop	Larvae in 1 kg soil (averages of 5 estimates)		
	Rootknot[1]	Citrus[2]	Total
No crop	700	500	1200
Coolabah oats	650	900	1550
Languedoc vetch	150	700	830
Barossa canola	400	350	750

[1] *Meloidogyne incognita*

[2] *Tylenchulus semipenetrans*

Table 2. Numbers of citrus nematode *tylenchulus semipenetrans* one month after ploughing in of autumn sown covercrops, Griffith, 1993

Crop	Larvae in 1 kg soil (average of 3 estimates)
Cooba Oats	500
Hobson forage rape	3200
Rangi forage rape	120
Winfred forage rape	1400

[1] *Tylenchulus semipenetrans*

Table 3. Numbers of rootknot nematode *Meloidogyne javanica* one month after ploughing in of covercrops, Rydalmere 1994/95

Crop	Larvae in 1 kg soil (averages of six replications, numbers having the same letter do not differ significantly)
No crop	23[a]
Dun pea	13[ab]
Coolabah oats	3[b]
Arran forage rape	11[ab]
Humus green manure rape	3[b]
Rangi forage rape	3[b]

[1] *Meloidogyne javanica*

Acknowledgments

Frank Franco, Ken Bray, Mick Burgoyne and Neil Grosser made vineyard sites available and helped with field operations. Alan Boys and Grant Dell also assisted with field work. Dr. Idris Barchia assisted with statistical analysis. Wrightson Seeds Ltd provided information on growing forage rapes and donated seed. Valley Seeds Pty Ltd also donated seed. We thank each of these for the generous assistance we received.

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PART 5. BIOFUMIGATION FOR CONTROL OF NEMATODES IN VITICULTURE: EFFICACY AGAINST *MELOIDOGYNE JAVANICA* OF MUSTARD SEED MEAL AND EFFICACY OF SURFACE APPLIED COVERCROP TOPGROWTH

Summary

Infestation levels of *Meloidogyne javanica*, a major rootknot nematode pest of viticulture in Australia, were significantly lowered by 1.5–60g/kg of mustard seed meal mixed into soil and also by 1.5–8g placed on the surface of 15cm pots watered by overhead sprinklers. However application of effective amounts in the field would probably be uncompetitive with application of conventional nematicide. Surface applied fresh green material, chopped leaves and stems, of the brassica crops Rangi forage rape and Humus green manure rape significantly lowered infestation, suggesting that biofumigation by mulching of crop topgrowth could be an effective alternative to full incorporation. Surface applied chopped green material of Dun field pea also significantly reduced infestation.

Introduction

Biofumigation for pest and disease control is based on growing brassica crops, either as break crops in cereal rotations or, in horticulture and viticulture, growing brassica green manure crops in the interrows and, while the crop is still green, cultivating to incorporate the crop's topgrowth. In cereal rotations, improved yields following Indian mustard and canola are recorded (Angus, van Herwaarden and Howe, 1991; Kirkegaard *et al.*, 1994) and have been linked with control by these crops of cereal root pathogens (Kirkegaard *et al.*, 1993;

Angus *et al.*, 1994). McLeod and Da Silva (1994) have shown that brassica green material mixed into soil lowers infestations of the nematode pests important in Australian viticulture.

Glucosinolates in the roots and leaves are believed to be responsible for the pest and disease controlling effects of brassica crops (Kirkegaard *et al.*, 1993). Mustard seed meal, a by product of mustard seed oil production, has high levels of glucosinolates and strong antifungal activity (Kirkegaard and Wong, in press). Application of mustard meal is therefore a possible alternative to growing a covercrop for biofumigation control of nematodes in vineyards.

In growing interrow covercrops cultivation should be kept to a minimum, to reduce damage to soil structure and lessen the risk of erosion (Davidson, 1992). In vineyards where soil structure is fragile or there is a serious risk of erosion, mowing and mulching, leaving the cut material on the surface, is preferable to cultivation. In these situations a biofumigation process which relied on mulching rather than ploughing crops in would be more acceptable.

The experiments reported here had as their objectives (1) to test the efficacy of mustard seed meal for control of rootknot nematode and (2) to explore the use of surface application and watering of brassica green material for control of rootknot nematode.

Materials and Methods

Rootknot nematode, *Meloidogyne javanica*, originally from grape roots, was maintained in culture on tomato or tobacco plants growing in potting soil (1 volume coarse

sand: 1 volume peat). Soil from these cultures provided the nematode infested soil used in all experiments. Mustard seed meal, from Indian mustard (*Brassica juncea*, Line 99Y–1–1), came from a cold pressed mustard oil production process based at Wallendbeen, NSW. Fresh green material, leaves and stems, of Rangi forage rape, Humus green manure rape and Dun field pea was cut from field grown crops 3–4 months after sowing and chopped into pieces 5cm x 5cm. All experiments included five replicates, arranged in randomised blocks.

Mustard seed meal at 15 to 60g/kg (experiment 1)

1kg of amounts of infested soil were placed in plastic bags. Treatments were: control (no addition), 15, 30 , 60g of mustard seed meal and 60g of green material of Rangi forage rape. These amounts were mixed with the soil in the bags. The bags were tied closed, held for 4 weeks and then numbers of surviving nematodes assessed by the Whitehead and Hemming (1965) tray method.

Mustard seed meal at 1.5 to 6g/kg (experiment 2)

In this experiment, mustard seed meal was tested at much smaller doses than in experiment 1 and the effect of surface application was also tested. Amounts of 1.5, 3 and 6g of mustard seed meal were mixed with infested soil in plastic bags, as in experiment 1. The same quantities were then mixed with 1kg amounts of infested soil which were then transferred to 15cm plastic pots. The same amounts were also placed on the surface of 1kg amounts of infested soil in 15cm plastic pots. Treated pots were held on a glasshouse bench receiving about 10mm of overhead watering twice a day, to simulate, with the surface treated

pots, topdressing and watering–in. Two weeks after the start numbers of surviving nematodes were estimated by the tray method.

Mustard seed meal at 1 to 8g/kg (experiment 3)

Mustard seed meal was tested at levels down to 1g/kg either mix into soil or topdressed and watered in. Amounts of 1g, 2g, 4g were either mixed into infested soil or applied to the surface of infested soil in 15 cm pots and overhead watered as in experiment 2. One week from the start numbers of surviving nematodes were estimated by the tray method.

Efficacy of surface application and watering of green material of Rangi rape (experiment 4)

Amounts of 80g and 160g of chopped leaves and stems of Rangi forage rape were tested, either mixed into soil or placed on the soil surface, using 15cm overhead watered pots as in experiment 3. Numbers of surviving nematodes were estimated 2 weeks after the start by the tray method (500g) and also by bioassay (500g). Bioassay was by planting a 3 week old tomato seedling and counting numbers of knots on the roots 3 weeks later.

Comparison of green material of Rangi forage rape and Humus green manure rape, surface applied and watered (experiment 5)

Amounts of 80 and 160g of chopped leaves and stems of Rangi forage rape and Humus green manure rape placed on the surface were tested against mixing 80g into the soil.

Watering was as in experiments 3 and 4. Numbers of surviving nematodes were estimated 2 weeks after the start, by the tray method.

Comparison of brassica and non-brassica green material, surface applied and watered(experiment 6)

Amounts of 80 and 160g of chopped leaves and stems of Rangi forage rape and Humus green manure rape were compared with 80 and 160g of leaves and stems of Dun field pea. All materials were surface applied and watering was as in previous experiments.

RESULTS

The amounts of mustard seed meal used in experiment 1 (Table 1) completely eliminated nematode larvae. Some nematodes survived in soil treated with green material of Rangi rape. Rangi rape was added as fresh material, in terms of dry matter the amount applied would be lower than the amounts of mustard seed meal used. It was notable that there were few bacterial feeding nematodes in the mustard seed meal treated soil whereas massive numbers occurred in the green material treated soil.

In experiment 2 (Table 2) amounts of mustard meal of 1.5 to 6.0 g/kg soil significantly lowered nematode infestation. This was so with all three methods of application. It is of particular interest is that surface application and watering controlled as well as mixing in.

Amounts of 1 and 2 g/kg soil of mustard meal did not significantly control nematode in experiment 3 (Table 3) but amounts of 4 and 8 g/kg did. Again it is notable that, as in experiment 2, surface application gave control comparable to control from mixing in.

In experiment 4 (Table 4), 80 and 160 g/kg of green material of Rangi rape gave good control, whether applied to the surface or mixed in and whether measured by tray assay of surviving larvae or estimated by bioassay.

Comparison of Rangi forage rape and Humus green manure rape in experiment 5 (Table 5), on the basis of tray assay of larvae, indicated much the same control from each rape, and comparable control whether applied to the surface or mixed in. Bioassay results however suggest a better result from Humus at 80 g/kg than from Rangi at 80 g/kg when mixed in.

In experiment 6 (table 6) green material of pea controlled nematode numbers as well as the green material of the brassicas.

DISCUSSION

Amounts of mustard seed meal applied in experiment 1 were, on a dry weight basis, high relative to the amount of Rangi rape green material and to the amounts of fresh crop residues previously investigated (McLeod and Da Silva, 1994). However experiment 1 and the later experiments substantiate the strong biological activity of this material reported by Kirkegaard and Wong (in press). Mustard seed meal inhibited both rootknot and bacterial

feeding nematodes and in this respect its action differed from the action of the green material of Rangi rape, which reduced rootknot nematode numbers but simulated massive numbers of bacterial feeding nematodes. Selective action of plant residues against plant parasitic nematodes has been reported before (Patrick, Sayer and Thorpe, 1964) but the mechanism is not well understood. Bacterial feeding nematodes feed on microbial populations that decompose plant matter, thus they may be adapted to tolerate toxins, including isothiocyanates (ITCs) released from glucosinolates, during decay of plant residues. In experiment 1, the large amounts of mustard meal may have generated short term levels of ITCs high enough to affect both plant parasitic and bacterial feeding nematodes, whereas the green material did not.

The results of experiments 2 and 3 with mustard seed meal suggest that at least 4g/kg soil may be needed for reliable reduction of infestation. On a field scale, assuming a soil bulk density of 1 g/cm^3, this would be equivalent to 4 t/ha. At this level, with the cost of transport added, treatment with mustard seed meal in most situations would be more costly than treatment with a conventional nematicide such as fenamiphos. Fenamiphos has the additional advantages of easy application through drip watering systems and systemic antinematode activity (McLeod and Khair, 1975).

Control from surface application of both mustard seed meal and green plant material is particularly interesting. Formulated nematicides with ITCs as active agents, for example Vapam, are applied and dispersed in water (Christie, 1959), so the efficacy of surface applied brassica material may be explained by the leaching in of ITCs together with other water dispersed materials. The surface layer could also seal the surface, retaining volatiles, and so

enhance efficacy. The efficacy of surface applied pea residues is unexpected since earlier experiments, with pea residues mixed in soil (Part 3, McLeod and Da Silva, 1994), indicated weak activity of residues of this crop.

The efficacy of surface applied green material and then watering these experiments suggests that slashing and mulching, or cultivation leaving green material on the surface, followed by watering, could be an alternative to cultivation and full turning in of crop topgrowth for nematode control. Application in vineyard interrows would be limited by water supply as watering would be needed to move active materials into the soil. Another possible application could be mowing, with side delivery into the vine row, followed by watering in row, so that the vine row itself is treated. With any of these possibilities, however, it should be noted that disturbance of covercrop roots may be required to get the added benefit of release of ITCs from crop roots.

Table 1. **Survival of rootknot nematode *Meloidogyne javanica* in soil after mixing in 15–60 g/kg of mustard seed meal (experiment 1).**

Treatment	Larvae surviving after 4 weeks[1]
No treatment	430
15 g mustard seed meal	0
30 g mustard seed meal	0
60 g mustard seed meal	0
80 g Rangi green material	90

[1] Mean of 5 estimates.

Table 2. **Survival of rootknot nematode *Meloidogyne javanica* in soil after 1.5–6.0 g/kg of mustard seed meal mixed in or placed on the soil surface (experiment 2).**

How applied	Meal added to 1 kg soil	Larvae surviving after 2 weeks[1]
No treatment, pots with overhead watering	0	416 a
Mixed with soil kept in plastic bag	3.0 g	93 b
	6.0 g	25 c
Mixed with soil in pots with overhead watering	1.5 g	5 d
	3.0 g	11 cd
	6.0 g	5 d
On surface of pots with overhead watering	1.5 g	1 e
	3.0 g	7 cd
	6.0 g	19 cd

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 3. **Survival of rootknot nematode, *Meloidogyne javanica* in soil after 1–8 g/kg of mustard seed meal mixed in or placed on the soil surface (experiment 3).**

How applied	Meal added to 1 kg soil	Larvae surviving after 7 days[1]
No treatment	0	607 a
Mixed in	1 g	330 a
On surface	1 g	359 a
Mixed in	2 g	219 a
On surface	2 g	285 a
Mixed in	4 g	9 bc
On surface	4 g	39 b
Mixed in	8 g	5 c
On surface	8 g	9 bc

[1] Mean of 5 estimates means. Followed by different letters are significantly different, P = 0.05.

Table 4. **Survival of rootknot nematode *Meloidogyne javanica* in soil after green material of Rangi rape mixed in or placed on the soil surface (experiment 4).**

How applied	Amount added to 1 kg soil	Larvae surviving after 2 weeks[1]	Bioassay knots/plants[1]
No treatment	0	264 a	59 a
Mixed in	80 g	4 b	3 c
On surface	80 g	8 b	13 bc
Mixed in	160 g	1 b	7 cdc
On surface	160 g	13 b	8 cd

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 5: **Survival of rootknot nematode *Meloidogyne javanica* in soil after green materials of Rangi and Humus rapes mixed in or placed on the soil surface (experiment 5).**

Treatment	Amount added to 1 kg soil	Larvae surviving after 2 weeks[1]	Bioassay knots/plant[1]
No treatment	0	470 a	145 a
Rangi, mixed in	80 g	22 b	69 ab
Humus, mixed in	80 g	49 b	27 b
Rangi, on surface	80 g	37 b	41 ab
Humus, on surface	80 g	17 b	37 b
Rangi, on surface	160 g	44 b	31 b
Humus, on surface	160 g	24 b	39 b

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 6: **Survival of rootknot nematode *Meloidogyne javanica* in soil after green material of Rangi and Humus rapes and Dun pea placed on the soil surface (experiment 6).**

Crop	Amount added to 1 kg soil	Larvae surviving after 3 weeks[1]
None	0	567 a
Rangi	80 g	166 ab
Humus	80 g	162 abc
Dun pea	80 g	100 bc
Rangi	160 g	34 e
Dun pea	160 g	54 bc

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Acknowledgments

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4-1 White lupin covercrop, a very favourable host of rootknot nematodes



4-2 Cooba oats covercrop,most cereal crops are unfavourable hosts of rootknot nematodes



4-3 Rangi forage rape growing as a viticultural covercrop



4-4 Barossa canola growing as a viticultural covercrop. Stemmy growth and early flowering lessens its value as a covercrop



4-5 Left, Rangi forage rape and right, Humus green manure rape

PART 5. BIOFUMIGATION FOR CONTROL OF NEMATODES IN VITICULTURE: EFFICACY AGAINST *MELOIDOGYNE JAVANICA* OF MUSTARD SEED MEAL AND EFFICACY OF SURFACE APPLIED COVERCROP TOPGROWTH

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Introduction

Biofumigation for pest and disease control is based on growing brassica crops, either as break crops in cereal rotations or, in horticulture and viticulture, growing brassica green manure crops in the interrows and, while the crop is still green, cultivating to incorporate the crop's topgrowth. In cereal rotations, improved yields following Indian mustard and canola are recorded (Angus, van Herwaarden and Howe, 1991; Kirkegaard *et al.*, 1994) and have been linked with control by these crops of cereal root pathogens (Kirkegaard *et al.*, 1993;

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In growing interrow covercrops cultivation should be kept to a minimum, to reduce damage to soil structure and lessen the risk of erosion (Davidson, 1992). In vineyards where soil structure is fragile or there is a serious risk of erosion, mowing and mulching, leaving the cut material on the surface, is preferable to cultivation. In these situations a biofumigation process which relied on mulching rather than ploughing crops in would be more acceptable.

The experiments reported here had as their objectives (1) to test the efficacy of mustard seed meal for control of rootknot nematode and (2) to explore the use of surface application and watering of brassica green material for control of rootknot nematode.

Materials and Methods

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sand: 1 volume peat). Soil from these cultures provided the nematode infested soil used in all experiments. Mustard seed meal, from Indian mustard (*Brassica juncea*, Line 99Y-1-1), came from a cold pressed mustard oil production process based at Wallendbeen, NSW. Fresh green material, leaves and stems, of Rangi forage rape, Humus green manure rape and Dun field pea was cut from field grown crops 3-4 months after sowing and chopped into pieces 5cm x 5cm. All experiments included five replicates, arranged in randomised blocks.

Mustard seed meal at 15 to 60g/kg (experiment 1)

1kg of amounts of infested soil were placed in plastic bags. Treatments were: control (no addition), 15, 30 , 60g of mustard seed meal and 60g of green material of Rangi forage rape. These amounts were mixed with the soil in the bags. The bags were tied closed, held for 4 weeks and then numbers of surviving nematodes assessed by the Whitehead and Hemming (1965) tray method.

Mustard seed meal at 1.5 to 6g/kg (experiment 2)

In this experiment, mustard seed meal was tested at much smaller doses than in experiment 1 and the effect of surface application was also tested. Amounts of 1.5, 3 and 6g of mustard seed meal were mixed with infested soil in plastic bags, as in experiment 1. The same quantities were then mixed with 1kg amounts of infested soil which were then transferred to 15cm plastic pots. The same amounts were also placed on the surface of 1kg amounts of infested soil in 15cm plastic pots. Treated pots were held on a glasshouse bench receiving about 10mm of overhead watering twice a day, to simulate, with the surface treated

pots, topdressing and watering–in. Two weeks after the start numbers of surviving nematodes were estimated by the tray method.

Mustard seed meal at 1 to 8g/kg (experiment 3)

Mustard seed meal was tested at levels down to 1g/kg either mix into soil or topdressed and watered in. Amounts of 1g, 2g, 4g were either mixed into infested soil or applied to the surface of infested soil in 15 cm pots and overhead watered as in experiment 2. One week from the start numbers of surviving nematodes were estimated by the tray method.

Efficacy of surface application and watering of green material of Rangi rape (experiment 4)

Amounts of 80g and 160g of chopped leaves and stems of Rangi forage rape were tested, either mixed into soil or placed on the soil surface, using 15cm overhead watered pots as in experiment 3. Numbers of surviving nematodes were estimated 2 weeks after the start by the tray method (500g) and also by bioassay (500g). Bioassay was by planting a 3 week old tomato seedling and counting numbers of knots on the roots 3 weeks later.

Comparison of green material of Rangi forage rape and Humus green manure rape, surface applied and watered (experiment 5)

Amounts of 80 and 160g of chopped leaves and stems of Rangi forage rape and Humus green manure rape placed on the surface were tested against mixing 80g into the soil.

Watering was as in experiments 3 and 4. Numbers of surviving nematodes were estimated 2 weeks after the start, by the tray method.

Comparison of brassica and non-brassica green material, surface applied and watered(experiment 6)

Amounts of 80 and 160g of chopped leaves and stems of Rangi forage rape and Humus green manure rape were compared with 80 and 160g of leaves and stems of Dun field pea. All materials were surface applied and watering was as in previous experiments.

RESULTS

The amounts of mustard seed meal used in experiment 1 (Table 1) completely eliminated nematode larvae. Some nematodes survived in soil treated with green material of Rangi rape. Rangi rape was added as fresh material, in terms of dry matter the amount applied would be lower than the amounts of mustard seed meal used. It was notable that there were few bacterial feeding nematodes in the mustard seed meal treated soil whereas massive numbers occurred in the green material treated soil.

In experiment 2 (Table 2) amounts of mustard meal of 1.5 to 6.0 g/kg soil significantly lowered nematode infestation. This was so with all three methods of application. It is of particular interest is that surface application and watering controlled as well as mixing in.

Amounts of 1 and 2 g/kg soil of mustard meal did not significantly control nematode in experiment 3 (Table 3) but amounts of 4 and 8 g/kg did. Again it is notable that, as in experiment 2, surface application gave control comparable to control from mixing in.

In experiment 4 (Table 4), 80 and 160 g/kg of green material of Rangi rape gave good control, whether applied to the surface or mixed in and whether measured by tray assay of surviving larvae or estimated by bioassay.

Comparison of Rangi forage rape and Humus green manure rape in experiment 5 (Table 5), on the basis of tray assay of larvae, indicated much the same control from each rape, and comparable control whether applied to the surface or mixed in. Bioassay results however suggest a better result from Humus at 80 g/kg than from Rangi at 80 g/kg when mixed in.

In experiment 6 (table 6) green material of pea controlled nematode numbers as well as the green material of the brassicas.

DISCUSSION

Amounts of mustard seed meal applied in experiment 1 were, on a dry weight basis, high relative to the amount of Rangi rape green material and to the amounts of fresh crop residues previously investigated (McLeod and Da Silva, 1994). However experiment 1 and the later experiments substantiate the strong biological activity of this material reported by Kirkegaard and Wong (in press). Mustard seed meal inhibited both rootknot and bacterial

feeding nematodes and in this respect its action differed from the action of the green material of Rangi rape, which reduced rootknot nematode numbers but simulated massive numbers of bacterial feeding nematodes. Selective action of plant residues against plant parasitic nematodes has been reported before (Patrick, Sayer and Thorpe, 1964) but the mechanism is not well understood. Bacterial feeding nematodes feed on microbial populations that decompose plant matter, thus they may be adapted to tolerate toxins, including isothiocyanates (ITCs) released from glucosinolates, during decay of plant residues. In experiment 1, the large amounts of mustard meal may have generated short term levels of ITCs high enough to affect both plant parasitic and bacterial feeding nematodes, whereas the green material did not.

The results of experiments 2 and 3 with mustard seed meal suggest that at least 4g/kg soil may be needed for reliable reduction of infestation. On a field scale, assuming a soil bulk density of 1 g/cm^3, this would be equivalent to 4 t/ha. At this level, with the cost of transport added, treatment with mustard seed meal in most situations would be more costly than treatment with a conventional nematicide such as fenamiphos. Fenamiphos has the additional advantages of easy application through drip watering systems and systemic antinematode activity (McLeod and Khair, 1975).

Control from surface application of both mustard seed meal and green plant material is particularly interesting. Formulated nematicides with ITCs as active agents, for example Vapam, are applied and dispersed in water (Christie, 1959), so the efficacy of surface applied brassica material may be explained by the leaching in of ITCs together with other water dispersed materials. The surface layer could also seal the surface, retaining volatiles, and so

enhance efficacy. The efficacy of surface applied pea residues is unexpected since earlier experiments, with pea residues mixed in soil (Part 3, McLeod and Da Silva, 1994), indicated weak activity of residues of this crop.

The efficacy of surface applied green material and then watering these experiments suggests that slashing and mulching, or cultivation leaving green material on the surface, followed by watering, could be an alternative to cultivation and full turning in of crop topgrowth for nematode control. Application in vineyard interrows would be limited by water supply as watering would be needed to move active materials into the soil. Another possible application could be mowing, with side delivery into the vine row, followed by watering in row, so that the vine row itself is treated. With any of these possibilities, however, it should be noted that disturbance of covercrop roots may be required to get the added benefit of release of ITCs from crop roots.

Table 1. **Survival of rootknot nematode *Meloidogyne javanica* in soil after mixing in 15–60 g/kg of mustard seed meal (experiment 1).**

Treatment	Larvae surviving after 4 weeks[1]
No treatment	430
15 g mustard seed meal	0
30 g mustard seed meal	0
60 g mustard seed meal	0
80 g Rangi green material	90

[1] Mean of 5 estimates.

Table 2. **Survival of rootknot nematode *Meloidogyne javanica* in soil after 1.5–6.0 g/kg of mustard seed meal mixed in or placed on the soil surface (experiment 2).**

How applied	Meal added to 1 kg soil	Larvae surviving after 2 weeks[1]
No treatment, pots with overhead watering	0	416 a
Mixed with soil kept in plastic bag	3.0 g	93 b
	6.0 g	25 c
Mixed with soil in pots with overhead watering	1.5 g	5 d
	3.0 g	11 cd
	6.0 g	5 d
On surface of pots with overhead watering	1.5 g	1 e
	3.0 g	7 cd
	6.0 g	19 cd

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 3. **Survival of rootknot nematode, *Meloidogyne javanica* in soil after 1–8 g/kg of mustard seed meal mixed in or placed on the soil surface (experiment 3).**

How applied	Meal added to 1 kg soil	Larvae surviving after 7 days[1]
No treatment	0	607 a
Mixed in	1 g	330 a
On surface	1 g	359 a
Mixed in	2 g	219 a
On surface	2 g	285 a
Mixed in	4 g	9 bc
On surface	4 g	39 b
Mixed in	8 g	5 c
On surface	8 g	9 bc

[1] Mean of 5 estimates means. Followed by different letters are significantly different, P = 0.05.

Table 4. **Survival of rootknot nematode *Meloidogyne javanica* in soil after green material of Rangi rape mixed in or placed on the soil surface (experiment 4).**

How applied	Amount added to 1 kg soil	Larvae surviving after 2 weeks[1]	Bioassay knots/plants[1]
No treatment	0	264 a	59 a
Mixed in	80 g	4 b	3 e
On surface	80 g	8 b	13 bc
Mixed in	160 g	1 b	7 cde
On surface	160 g	13 b	8 cd

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 5: **Survival of rootknot nematode *Meloidogyne javanica* in soil after green materials of Rangi and Humus rapes mixed in or placed on the soil surface (experiment 5).**

Treatment	Amount added to 1 kg soil	Larvae surviving after 2 weeks[1]	Bioassay knots/plant[1]
No treatment	0	470 a	145 a
Rangi, mixed in	80 g	22 b	69 ab
Humus, mixed in	80 g	49 b	27 b
Rangi, on surface	80 g	37 b	41 ab
Humus, on surface	80 g	17 b	37 b
Rangi, on surface	160 g	44 b	31 b
Humus, on surface	160 g	24 b	39 b

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 5: **Survival of rootknot nematode *Meloidogyne javanica* in soil after green materials of Rangi and Humus rapes mixed in or placed on the soil surface (experiment 5).**

Treatment	Amount added to 1 kg soil	Larvae surviving after 2 weeks[1]	Bioassay knots/plant[1]
No treatment	0	470 a	145 a
Rangi, mixed in	80 g	22 b	69 ab
Humus, mixed in	80 g	49 b	27 b
Rangi, on surface	80 g	37 b	41 ab
Humus, on surface	80 g	17 b	37 b
Rangi, on surface	160 g	44 b	31 b
Humus, on surface	160 g	24 b	39 b

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

Table 6: **Survival of rootknot nematode *Meloidogyne javanica* in soil after green material of Rangi and Humus rapes and Dun pea placed on the soil surface (experiment 6).**

Crop	Amount added to 1 kg soil	Larvae surviving after 3 weeks[1]
None	0	567 a
Rangi	80 g	166 ab
Humus	80 g	162 abc
Dun pea	80 g	100 bc
Rangi	160 g	34 c
Dun pea	160 g	54 bc

[1] Mean of 5 estimates. Means followed by different letters are significantly different, P = 0.05.

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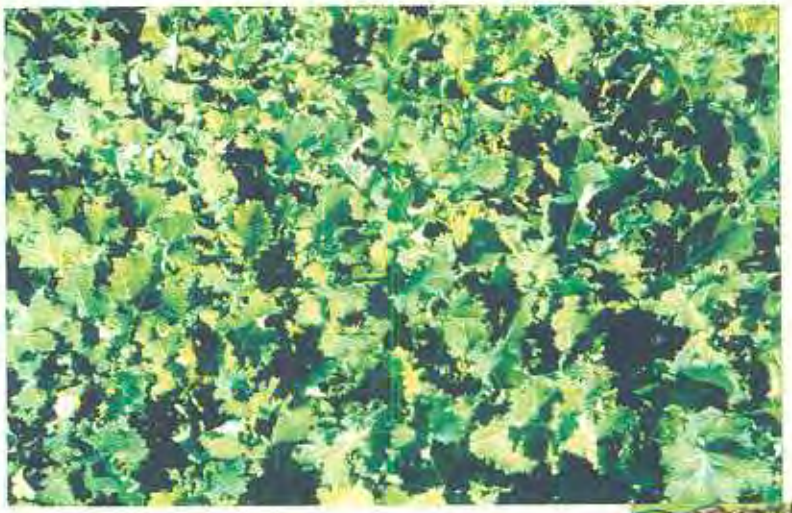
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5-1 A dense cover of Rangi forage rape



5-2 Rangi forage rape covercrop mulched in spring



5-3 Rangi forage rape covercrop fully incorporated in spring



5-4 A covercrop mown and side delivered to the vine row

PART 6. REPRODUCTION OF ROOTKNOT NEMATODE *MELOIDOGYNE JAVANICA* ON RANGI AND HUMUS RAPES: INFLUENCE OF SOIL TEMPERATURE AT SOWING AND OF END-OF-VITICULTURAL-COVERCROP MANAGEMENT PRACTICES

Summary

Rangi forage rape and Humus green manure plants sown in the field in mid autumn were not infected by rootknot nematode *Meloidogyne javanica* whereas tomato plants were infected. 18°C is suggested as guide soil temperature for sowing brassica covercrops in infested situations.

Removing tops of Rangi rape plants 6 weeks old but later slashing tended to increase egg numbers. Application of glyphosate herbicide to the cut surfaces of slashed stems tended to increase egg production.

Infestation of undisturbed Rangi plants when 5 and 7 weeks old resulted in less egg production than infection of plants 3 weeks old.

Egg production on Rangi plants transplanted into infested soil was related to root growth. Most eggs were produced on 2 month old transplants, but with good root regrowth considerable egg production occurred on 3, 4 and 5 month old transplants.

Introduction

Recent research has shown that green manures of brassica crops have promise for control of soil borne pest and diseases in agriculture and horticulture (Kirkegaard *et al*. 1993). Significant suppression of pest nematodes of viticulture by green material of brassica crops, including Rangi forage rape and Humus green manure rape, has been confirmed and trial sowings indicate that Rangi rape can be grown as a vineyard covercrop (McLeod and Da Silva, 1994; McLeod, Somers and Gendy, 1995). There is however a concern in that the crop is also a host of the rootknot nematodes *Meloidogyne incognita* and *M.javanica* (McLeod and Warren, 1993), both significant pests of grapevines.

In viticulture in Australia, covercrops are sown in autumn, when soil temperatures are falling, and covercrop growth is terminated in spring, to reduce risk of frost damage to the vines and to prevent completion for water and nutrients during the vine growing season (Davidson, 1992). Growth may be stopped by slashing and mulching, leaving topgrowth on the soil surface without disturbance of roots, or by cultivating, ploughing and turning in topgrowth. Slashing or cultivating may followed by spraying with a glyphosate based herbicide to control regrowth.

There are many studies which indicate that in, the field, rootknot nematodes do not infect when soil temperatures fall, during the onset of the cool season, to levels that may be different for crops of different host status and for different rootknot nematodes (Sauer and Giles, 1957; Thomason, 1962; Vrain, Barker and Holtzman, 1978; Roberts, Van Gundy and McKinney, 1981; Pung, Barbetti and Sivasithamparam, 1992; McLeod, Somers and Gendy,

1995).

One objective of the work reported here was to investigate infection of Rangi forage rape and Humus green manure rape by the rootknot nematode *Meloidogyne javanica* in the field at autumn soil temperatures. The other objective was to assess the impact of various end of covercrop practices on nematode reproduction.

Materials and Methods

Infection at autumn sowing temperatures in the field (experiment 1)

On 15 April 1994, 2.5m rows, on soil infested with *M. javanica*, were sown to Rangi forage rape, Humus green manure rape and Grosse Lisse tomato (3 week old transplants). On the same day, soil from the test site was put in 15cm pots, 4 pots were sown to each crop and placed in a glasshouse. Field soil temperature, at 15cm depth, was 17°C at sowing time. Daily readings with a dial thermometer until 9 June indicated soil temperatures consistently below 20°C and temperature logging with a Hobo data logger[1] from 9 June to 14 June indicated soil temperatures of 8.5 to 13°C. On 30 May and 26 June, 6 and 10 weeks after sowing, roots of 16 plants of each crop from the field and the glasshouse plants were examined for knots and any knots present stained with 0.05% cotton blue in lactoglycerol blue (Bridge, Page and Jordan, 1982) to verify rootknot infection.

On 14 June, all three crops were again sown into the *M. javanica* infested soil in the field. Panes of glass were placed over tomato plants to protect them from cold and frost.

[1]Hastings Data Loggers, Wauchope, NSW.

Logging of soil temperatures from 21 June to 7 August recorded soil temperatures of 8 to 14°C. Again, plants of each crop were grown in the same soil in a glasshouse and on 30 August, 11 weeks after sowing, plants were dug and the roots examined for infection.

Nematode reproduction after slashing (experiment 2)

A group of 35 15 cm pots, arranged in 5 blocks and filled with of rootknot nematode (*M. javanica*) infested potting soil (1 volume coarse sand :1 volume peat), was sown to Rangi forage rape and three plants allowed to grow on each pot. At 6, 7, 9, 10 and 11 weeks from sowing the stems of plants in five randomly chosen pots, one from each block, were cut just above soil level but leaving leaf nodes to provide top regrowth. Each time the roots of plants in a single uncut pot were washed, stained and stage of nematode development checked. Three weeks after the last cut numbers of new eggs were estimated by shaking roots in 0.8% NaOCl (McLeod and Warren, 1993).

Reproduction after slashing and application of glyphosate (experiment 3)

Rangi forage rape plants were grown as in experiment 2, except that hatch larvae, 980 (mean of 4 estimates) in 1ml of water to a pot, were used to infest the soil 3 weeks after sowing. At 6, 7 and 8 weeks after infestation plants in five pots were slashed as in experiment 2. At each of these times five more pots were slashed and glyphosate herbicide immediately painted onto the cut surfaces. Stage of nematode development was also checked, using extra sown for the purpose. Two weeks after the last slashing numbers of new eggs were estimated, as in experiment 2. To examine the effect of the glyphosate on hatchability

of eggs, five lots of about 60 eggs from glyphosate treated plants and from untreated slashed plants were placed in water in glass dishes and hatch after 8 days observed.

Reproduction on undisturbed roots (experiment 4)

Sequential sowings of Rangi forage rape were made on 21 July, 4 August and 17 August 1995. Each time, seven 4 by 20cm tubes and seven 15cm pots of potting soil were sown and one seedling a container let grow. Three weeks after the last sowing, when first sown plants were 7 weeks old, second sown plants were 5 weeks old and last sown plants were 3 weeks old, all containers were infested with 830 (mean of 4 estimates) hatched larvae in 1ml of water to a container. Ten days after infestation roots from the tubes were washed, stained and nematodes in them counted. Plants in the 15cm pots were left to grow for 9.5 weeks after infestation, then numbers of eggs were estimated, by the NaOCl method.

Reproduction on root regrowth (experiment 5)

In early March, April, May, June and July 1994, Rangi forage rape was sown outdoors in nematode free soil. On 1 September 1994, five plants from each sowing were uprooted and replanted in *M. javanica* infested potting soil in 20cm pots and maintained in a glasshouse. After 10 weeks in the glasshouse, numbers of eggs were estimated, by the NaOCl method. Root regrowth was also estimated, by weighing the trimmed off secondary root growth, before extraction of eggs.

Reproduction after complete removal of tops and chopping roots (experiments 6,7 and 8)

Rangi forage rape plants were grown in 15cm pots as in previous experiments. Experiment 6 was sown on 12 December 1993 and three weeks later infected with 300 hatched larvae (mean of 4 estimates) to a pot. At 2, 4, 6, 8, and 10 weeks after infection plants from 10 randomly pots were uprooted, the roots lightly washed and the tops cut of below soil level, leaving no shoot buds. The roots were then chopped, mixed into 1kg of fresh steamed potting soil and placed in plastic bags. One week after the last uprooting the soil from the plastic bags was transferred to pots, bioassayed by planting 3 week old tomato seedlings and 5 weeks later numbers of knots on bioassay plants were counted.

Experiment 7 was sown on 22 February 1994 and followed the same general procedure as in experiment 5. Pots were infected with 800 second stage larvae (mean of 4 estimates) to a pot. Plants were uprooted 3, 5 and 7 weeks after infection and in addition, at each of these times roots were stained to check stage of nematode development.

In experiment 8 roots of 6 week old tomato plants infected with *M. javanica* at the young female stage but without eggs were buried in potting soil and kept in the laboratory. Four and seven days later roots were stained to see if eggs had been laid.

Results

In experiment 1, examination 6 and 10 weeks after sowing of crops sown in the field on 15 April 1994 (mid autumn) revealed knots and developing nematodes in the roots of

tomato plants but no galls or nematodes were found on or in roots of either Rangi rape or Humus rape. Plants of same crops grown at the same in the same soil in a glasshouse showed galls with developing nematodes present. Plants of the three crops sown on 14 June (winter) in the field were free of knots when dug 11 weeks later. Plants grown in the same soil, over the same period, in the glasshouse were severely knotted.

In experiment 2 (Table 1), removing plant tops at 6 weeks from sowing in infested soil and with nematodes at swollen stage larval stage lowered numbers of eggs produced. Removing tops at 7 and 9 weeks, with nematodes in the swollen larval to pre-egg laying female stage, stimulated egg production. Removing tops at 10 and 11 weeks, when egg laying had started, caused no marked change in egg production.

In experiment 3 (Table 2), removing plant tops and applying glyphosate to the cut stems 6 weeks after infestation, when nematodes were at the swollen larval stage, if anything slightly lowered egg production compared to egg production on slashed only plants. Application of glyphosate at 7 and 8 weeks, when egg laying was in progress, increased egg production. Plants slashed and treated with glyphosate at 8 weeks yielded twice as many eggs as slashed, not treated plants. Eggs from plants treated with glyphosate and from not treated plants gave the same percent hatch over 8 days, 81% and 82%.

Fewer nematodes in experiment 4 (Table 3) infected the roots of 7 and 5 week old undisturbed plants than infected 3 week old plants. This was reflected in much lower egg production on the older plants.

On transplanted plants on which there was regrowth of roots most eggs were produced on 2 month old plants, although 4 month old Rangi rape plants sustained comparable numbers (experiment 5, Table 4). On 4 and 5 month old plants more eggs were produced on Rangi rape than on Humus rape. Root regrowth of the older Rangi plants was, as measured by root weight when eggs were counted, stronger than root regrowth of Humus plants of the same age.

With complete removal of plant tops and chopping of roots (experiments 6 and 7, Table 5), there was reduced nematode survival when plants were less than 4 weeks old and nematodes were in the late larval stage. On older plants, when mature female nematodes were present, there was considerable survival and reproduction. In experiment 8, young females on detached, buried roots, were without eggs when first examined, after 4 days, but at 8 days numerous egg masses were present.

Discussion

The results of the field sowing experiment confirm field observations, including studies in vineyard situations (McLeod and Gendy 1995), that mid autumn sowing of brassica crops can avoid rootknot infestation. The field infection of tomato but not Rangi and Humus rapes when sown in mid autumn also supports other field observations that less favourable host crops are less liable to infestation when temperatures fall. The results suggest 18°C as a guide soil temperature for sowing brassica covercrops to avoid infection. Soil temperatures were just below this when the mid autumn sowing of experiment 1 was made. Roberts (1981) found that wheat was not infected by *M. incognita* in the field until soil temperatures

were above 18°C. It should be noted however that extended delay of sowing should be avoided because the crop's growing season will be shortened (Davidson, 1992).

It would seem from experiments 2 and 3 that slashing an infected crop is more likely to increase than to decrease nematode reproduction. An explanation of this effect could be that root reserve nutrients are mobilised in response to removal of tops, resulting in increased nutrient supply for egg laying females. The results suggest that glyphosate may also alters plant activity to favour egg production.

The results of experiment 4 suggest that infection of crops nearing maturity, as may be the case with covercrops sown in mid autumn and still in the ground in spring when soil temperatures start to rise, is possible; but reproduction will be considerably curtailed. The same should apply when crops are slashed and mulched, without disturbing the roots. Increased resistance to rootknot and reduced egg production in older plants has been reported in other crops (Ritter and Ritter, 1958; Jadhav, and Kurundkar, 1991; Canals, Pinochet, Felipe, 1992).

If plants regrow after cultivation, the results of experiment 5 suggest that there could be considerable reproduction when the roots are exposed to infection. In this situation, the weaker root regrowth of older Humus plants noted here could be an advantage. Less infection and reproduction on older plants could be due to fewer active root tips, through which larvae can enter and low nutrient availability in roots nearing maturity.

If a crop is infected, the results of experiments 6, 7 and 8 suggest that cultivation,

even with drastic mechanical disruption of roots, is unlikely to have much impact on reproduction unless nematodes are in a preadult development stage. When soil temperatures are above winter levels the preadult period would be 4 to 5 weeks.

Table 1. Effect of crop slashing on reproduction of rootknot nematode *Meloidogyne javanica* on Rangi forage rape (experiment 2)

When slashed (weeks after sowing)[1]	Nematode development when slashed	Eggs Production (eggs/pot)[2]
Not slashed		4230
6	Swollen larvae	2620
7	Swollen larvae and young females	7310
9	Mature females	7000
10	Females and small eggs masses	4520
11	Females large egg masses	3530

[1] Soil was already infested when sown.

[2] Mean of 5 estimates.

Table 2: **Impact of crop slashing and slashing and application of glyphosate on reproduction of rootknot nematode *Meloidgyne javanica* on Rangi forage rape (experiment 3).**

When slashed (weeks after infestation)	Treatment	Nematode development when slashed	Egg production (eggs/pot)[1]
–	Not slashed	–	5540
6	Slashed only	Swollen larvae	8300
	Slashed and glyphosate		6450
7	Slashed only	Females, small egg masses	6200
	Slashed and glyphosate		9800
8	Slashed only	Females, egg masses	4800
	Slashed and glyphosate		9200

[1] Mean of 5 estimates.

Table 3. Reproduction of rootknot nematode *Meloidogyne javanica* on undisturbed roots of Rangi forage rape (experiment 4)

Age of plants when infested (weeks)	Nematodes in roots[1]	Egg production[2] (Eggs/pot)
3	36	1350
5	28	120
7	14	120

[1] 6 days after infestation, mean of 7 estimates.

[2] Mean of 7 estimates.

Table 4: Reproduction of rootknot nematode *Meloidogyne javanacia* on root regrowth of Rangi and Humus rapes (experiment 5)

Age of plants when transplanted	Egg Production (eggs/plant)[1]		Root regrowth (g)[1]	
	Rangi	Humus	Rangi	Humus
2 months	4320	4000	74	102
3 months	1360	1740	153	187
4 months	4160	610	52	37
5 months	1500	480	62	41
6 months	110	170	85	10

[1] Mean of 5 estimates

Table 5: **Effect of root chopping and complete removal of plant tops on reproduction of rootknot *Meloidogyne javanica* nematode on Rangi forage rape (experiments 6 and 7).**

When chopped (weeks after infestation)	Experiment 6[1]	Experiment 7[2]	
	Bioassay plants infected (out of 10)	Nematode development	Bioassay plants infected (out of 10)
2	1	–	–
3	–	Swollen larvae	0
4	1	–	–
5	–	Females	10
6	5	–	–
7	–	Females egg masses	10
8	6	–	–
10	8	–	–

[1] Pots infested with 300 second stage larvae

[2] Pots infested with 800 second stage larvae

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PART 7. INFLUENCE OF TEMPERATURE, INFESTATION LEVEL AND AGE OF LARVAE ON INFECTION BY ROOTKNOT NEMATODE *MELOIDOGYNE JAVANICA* OF VITICULTURAL COVERCROPS OF DIFFERENT HOST STATUS

Summary

In an experiment whereby hatched second stage larvae of root knot nematode *Meloidogyne javanica* were placed in sand on seedlings, more larvae infected the favourable host crops tomato (Grosse Lisse), Dun field pea and Trikkala subterranean clover than infected the less favourable hosts Rangi forage rape and Cooba oats. Infection of all crops was much reduced in the temperature range 10–15°C. Tomato and Dun pea were more infected at 15–20°C, than at 20–25, trikkala clover and Rangi rape were similarly infected while Cooba oats was more infected at 20–25°C than at 15–20°C. The results are discussed in relation to infection of favourable and less favourable hosts in the field at low soil temperatures.

Seedlings of tomato, Trikkala clover, Rangi rape and Cooba oats growing in 4 x 14 cm tubes, were exposed to infestation levels of 25–200 larvae/tube (tomato and clover) and 50–1000 (Rangi rape and Cooba oats). In the range 25–200 /tube, percent infection (number in-root as a percentage of number added) in tomato was 54 to 77% and percent infection was maintained at higher levels. In Trikkala clover percent infection was from 18–45% percent infection, reduced percent infection occurring with 200/tube. In the range 50–1000 per tube percent infection of Rangi rape was 26–72%, highest percent infection occurring with 50/tube, percent infection, although lower, was then maintained. Percent infection in Cooba oats was

2–12%, highest percent infection occurring with 50/tube, percent infection, although lower, was then maintained.

Second stage larvae kept in water at 20°C for 1–3, 4–7, 8–11 and 11–14 days successfully infected tomato seedlings but in a second experiment larvae kept for 8–11 days and longer became parasitised.

Introduction

Covercrops used in viticulture in Australia vary from crops that a highly favourable hosts of rootknot nematodes (large numbers of eggs are produced on them), to crops that are unfavourable hosts (few eggs are produced on them) (McLeod and Warren 1993, McLeod 1994). Covercrops are sown in autumn, when soil temperatures are falling. Rootknot nematode infestation is prevalent in many viticultural districts (Mcleod, Reay and Smyth 1994).

Field studies suggest that rootknot disease starts on favourable host crops more easily than on less favourable hosts (Sauer and Giles, 1957; Thomason, 1962; Vrain, Barker and Holtzman, 1978; Roberts, Van Gundy and McKinney, 1981; Pung, Barbetti and Sivasithamparam, 1992; McLeod, Somers and Gendy, 1995). Reproduction of rootknot nematodes on viticultural covercrops is of concern because infected covercrops may be a source of increased infection of vines.

Reproduction of rootknot nematode on a crop in the field is subject to influence by

a complexity of factors (Ferris and Van Gundy, 1979). These may be usefully divided into factors influencing infection – penetration into and initial occupation of the crop – and factors operating after infection. The present study focused on factors influencing infection. Factors known to be influential in relation to infection by rootknot nematode include temperature, level of infestation, age of larvae and host status (Bird and Wallace, 1965; Wallace, 1966; Anwar, Trudgill and Phillips, 1994). The purpose of Part 7 of DAN 92/2 was to assess the significance of temperature, level of infestation and age of larvae in relation to infection of viticultural covercrops of different host status.

Materials and Methods

Nematode and crops

M. javanica, originally from grapevine roots and soil from a vineyard at Pokolbin NSW, was maintained in culture on tomato or tobacco plants in 20cm pots of potting mix, 1 volume coarse sand: 1 volume peat. Infective second stage larvae were obtained for experiments by shaking chopped infected roots from three month old cultures in 0.5% NaOCl for 4 minutes (Hussey and Baker, 1973). The resulting suspension was then poured through 1000, 150 and 25 μm opening banked sieves and the root material placed on the sieve bank and thoroughly washed. Eggs, retained on the 25μm sieve, were washed onto a 25μm mesh supported just under water in a covered dish. Suspensions of hatched, second stage infective larvae, free of eggs, were then drawn from the hatch dish.

The crops included in the experiments were tomato (cv. Grosse Lisse), a standard very

favourable host; Dun field pea, a very favourable host (McLeod and Warren, 1993) viticultural covercrop; Trikkala subterranean clover, a very favourable host (McLeod, 1994) sward clover; Rangi forage rape, a moderately favourable host (McLeod and Warren, 1993) and a potential biofumigation crop in viticulture (McLeod and Da Silva, 1994), and Cooba oats, a just favourable host viticultural covercrop (McLeod and Warren, 1993).

Temperature and infection (experiment 1)

This experiment included Grosse Lisse tomato, Trikkala subterranean clover, Dun pea, Rangi rape and Cooba oats. Two germinated seeds, with radicles 10mm long, were placed in 15 Petri dishes of 1.5% water agar and seed and radicle covered with fine, moist sand. Then 600 nematode larvae (mean of four estimates), in 0.2 ml of water, were introduced per dish and 5 dishes of each crop placed in illuminated plant growth cabinets, with temperature ranges 10–15, 15–20 and 20–25 °C, lower temperature was for 16 hours (night) and higher for 8 hours (day). Seven days later the seedlings' roots were washed, stained in lactoglycerol with 0.05% methyl blue (Bridge, Page and Jordan, 1982), macerated and the number of nematodes in them counted.

Infestation level and infection (experiment 2)

Germinated seed of Grosse Lisse tomato, Trikkala clover, Rangi rape and Cooba oats was sown in 4 x 14 cm tapered plastic tubes filled with a mix of 1 volume perlite: 1 volume peat–sand mix and one seedling per tube allowed to grow. Three weeks after sowing 5 tubes of tomato and Trikkala clover were infested with 25, 50, 75, 100 and 200 second stage larvae

and 5 tubes of Rangi rape and Cooba oats infested with 50, 100, 200, 500 and 1000 larvae. Two weeks after infestation the seedlings' roots were washed, stained and the nematodes in them counted as in experiment 1.

Age of larvae and infection (experiment 3)

Suspensions of hatched larvae free of eggs were obtained as in previous experiments, from a single lot of infested tomato roots. The hatch dish was kept at 20°C for 5 days when the hatched larvae were discarded and the hatch restarted. Batches of hatched larvae were then collected after 3, 7, 11 and 14 days, each batch being stored at 20°C, providing batches of larvae kept at 20°C in the absence of a host for 1–3, 4–7, 8–11 and 12–14 days. Eight 3 week old Grosse Lisse tomato plants per batch were then inoculated with 1000 larvae in 0.5ml of water. Ten days later the roots were stained and numbers of nematodes counted. A similar experiment, with the aim of comparing infection of crops of different host status, was then started. Batches of larvae were kept for 8–11, 15–18 and 22–25 days at 20 C. The experiment did not continue because inspection showed the larvae had been severely parasitised.

Results

Within all three temperature ranges more nematodes infected the favourable hosts tomato, Dun pea and Trikkala clover than infected the moderately favourable host Rangi rape and the just favourable host Cooba oats (experiment 1, Table 1). In all hosts infection was dramatically reduced in the low, 10–15 C, range. However the experiment's results suggest

that host infection and temperature relations are also complex (significant host by temperature interaction). In the high range, 20–25°C, infection of tomato and Dun pea was reduced, infection of clover and Cooba oats increased while infection of Rangi rape was unchanged, relative to infection at 15–20°C.

In experiment 2 (Table 2), within the range 25 to 200 larvae /tube, percent infection (number in-root as a percent of number added) in tomato was 54 to 77% and percent infection was maintained when infestation level increased. In Trikkala clover percent infection varied from 18 to 45%, percent infection was maintained up to 100/tube, but a reduced percent infection occurred with 200/tube. In the range 50 to 1000/tube, percent infection of Rangi rape was 26 to 72%, highest percent infection being with 50/tube, percent infection, although lower, was then maintained. Percent infection in Cooba oats was 5 to 12%, highest percent infection occurring with 50/tube; as with Rangi rape, percent infection, although lower, was then maintained.

In experiment 3, larvae up 11–14 days old successfully infected tomato seedlings (Table 3).

Discussion

The results of experiments 1 and 2 add to existing evidence (Bird, 1972; Ferris, and Hunt 1979; Anwar, Trudgill and Phillips, 1994) that over a wide range of crops, lower numbers of larvae infect less favourable hosts than infect favourable hosts. An implication of this is that the process determining host status must operate very early in the host parasite

relationship, affecting numbers entering and/or early occupation. Anwar, Trudgill and Phillips'(1994) experiments suggest that numbers that enter is the main difference, emergence of larvae after entering was trivial roots, whether of favourable or unfavourable hosts. Lownsbery and Viglierchio (1961) observed that larvae of *M.hapla* accumulated around roots of oats and rye but few penetrated, again suggesting difficulty in entering as the reason for low infection. Balhadère and Evans (1994) discuss mechanisms that may explain reduced entry of *M. naasi* into resistant cereals and grasses.

Some infection of crops occurred in experiment 1 at 10–15°C regardless of host status, and at 15–20 C infection of tomato and Dun pea peaked. This is at variance with field observations (Roberts, Van Gundy and McKinney, 1981; Ahuja and Sing, 1990; McLeod, Somers and Gendy, 1995) that there is little or no infection of less favourable hosts at soil temperatures below 18 C. Differences in the conditions of experiment 1 and the field could account for this discrepancy. In experiment 1 larvae were placed within 5mm of a newly emerged, growing root tip in coarse textured sand, in a semi sterile situation. In the field, larvae must explore more widely to find roots with invadable root tips, have to move through soils of varied texture and are exposed to parasitism and predation. Bird and Wallace (1965) have shown that mobility of *M. javanica* is restricted at temperatures which have little effect on infection in experiments similar to experiment 1. Entry of *M. javanica* into a favourable host can happen during a short time at a favourable temperature (Dao D, 1970; Wallace and Grandison 1971). Thus reduced infection in the field at low temperatures may be related more to restriction of mobility than to reduced entry. The lower percent infection of less favourable hosts would further lower the probability of them becoming infected.

Within the range of infestation levels covered in experiment 2 there was no indication of a threshold level, a level below which there was to infection, even on Cooba oats. As with temperature, this may be different in the field, lower percent infection of less favourable hosts would result in little or no infection at levels at which there may be infection of favourable hosts. In tomato, percent infection was maintained with increased infestation, a ceiling, a level above which infection does not increase (percent infection decreases), as noted by Wallace (1966) with *M. javanica* and Hashmi *et al.* (1994) with *M. incognita*, was not evident. A more extended range may have shown a ceiling, Wallace (1966) estimated a ceiling between 400 and 500 *M. javanica* on tomato. In Trikkala clover there is a suggestion of a ceiling of 18% with 200/tube, but in experiment 1 (table 1) , 28% infection occurred with 600 larvae/dish. The results with Rangi rape perhaps suggest a ceiling which is overcome by 1000/tube. The high percent infection of Rangi rape with 50/tube could be an anomaly. In Cooba oats, as with tomato, there was no indication of a ceiling. The reasons for a ceiling percent infection are not known. Wallace's (1966) suggestions are: limited space and invasion sites at the root tip; increasing destruction of tissue and inhibition of root growth resulting in diminishing invasion opportunities; reduced attraction of invaded roots and interference between individual nematodes.

Significant infection by larvae kept for as long as 11–14 days in the absence of a host suggest longer retention of infectivity than reported by Wallace and Grandison (1971), although Wallace (1966) found some infection by *M. javanica* larvae 8 days old and Moens and Hendricks (1993) report some infection by *M. incognita* larvae after 2 weeks in water at 20°C. However the evidence and the outcome of the attempt to extend experiment 3 suggests that the infective life of larvae in the field is likely to be short term, a matter of days not

weeks. If this is so, maintenance of high numbers of infective larvae in the field would require extended production and hatching of large numbers of eggs.

Table 1: **Influence of temperature on infection by rootknot nematode *Meloidogyne javanica* of crops differing in host status (experiment 1).**

Temperature range °C	Grosse Lisse tomato[1]	Dun field pea[1]	Trikkala clover[1]	Rangi forage rape[2]	Cooba oats[3]
	larvae/2 seedlings[4]				
20–25	180 (3)[5]	90 (15)	207 (35)	85 (14)	55 (9)
15–20	246 (41)	500 (83)	169 (28)	88 (15)	23 (4)
11–15	28 (5)	73 (12)	14 (2)	9 (1.5)	4 (0.7)

1) Very favourable host.

2) Moderately favourable host.

3) Just favourable host.

4) Number of larvae in roots of 2 seedlings 7 days after infestation, mean average of 5 estimates.

5) Percent infection, number in roots as a percentage of number added at infestation (600).

Table 2: **Influence of infestation level on infection by rootknot nematode *Meloidogyne javanica* of crops differing in host status (experiment 2)**

Infestation level Larvae/tube	Grosse Lisse tomato[1] l/plant[4]	Trikkala clover[1] l/plant	Rangi forage rape[2] l/plant	Cooba oats[3] l/plant
25	16 (64)[5]	8(36)	–	–
50	27 (54)	22 (44)	36 (72)	6 (12)
75	45 (60)	34 (45)	–	–
100	77 (77)	30 (30)	32 (32)	6 (6)
200	126 (63)	35 (18)	51 (26)	10 (5)
500	–	–	128 (26)	9 (2)
1000	–	–	368 (37)	46 (5)

1) Very favourable host.

2) Moderately favourable host.

3) Just favourable host.

4) Number of nematodes in roots of a seedling after two weeks, mean of 5 estimates.

5) Percent infection, number in roots as a percentage of number added.

Table 3. **Influence of age of larvae on infection by of rootknot nematode *Meloidogyne javanica***

Time larvae at 20°C in water, without host	**Larvae/plant[1)]**
1–3 days	58
4–7 days	33
8–11 days	113
11–14 days	99

1) Number of larvae in roots of a Grosse Lisse tomato plant 10 days after infestation, average of 8 estimates.

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PART 8. INFLUENCE OF SEASON OF THE YEAR ON MATURATION OF ROOTKNOT NEMATODE IN VITICULTURAL COVERCROPS OF DIFFERENT HOST STATUS

Summary

In summer and autumn in a glasshouse on Grosse Lisse tomato and Dun field pea, favourable hosts, rootknot nematode *Meloidogyne javanica* produced eggs in 5 to 5.5 weeks while on Rangi forage rape, and Cooba oats, less favourable hosts, egg production took 1 week longer to start. In autumn–winter on tomato and Dun pea egg production started after 7 weeks, on Rangi rape after another 3 weeks and on Cooba oats small numbers of eggs were detected only after 17 weeks. In winter on tomato and Dun pea, egg laying started after 8.5 weeks, on Rangi rape after another 5 weeks while on Cooba oats no eggs were detected over a 13.5 week observation period.

Growth, as measured by nematode profile area, was faster in favourable hosts than in less favourable hosts. At 4 and 6 weeks from infestation favourable hosts contained many more maturing nematodes than unfavourable hosts. Comparison of numbers of larvae infecting with numbers maturing gave maturation percentages of 55–100%, maturation percent was not closely related to host status. Rate of egg production on tomato was severely slowed in winter. Because of slower maturation on Rangi rape, start of egg laying on it in winter was delayed until early spring when temperatures were rising so egg laying proceeded normally.

It is proposed that in a wide range of crops, reduced egg production (less favourable host status), slower growth and reduced infection in less favourable hosts result from the

same defence barrier or resistance process in the host. It is suggested that reduced infection is the main contributor to lowered egg production in many unfavourable hosts.

Introduction

Covercrops in Australian viticulture are sown in autumn, when soil temperatures are falling, crops are in the ground during the winter and are either ploughed in or slashed and mulched in spring. The crops grown range from crops that allow much rootknot nematode reproduction (favourable hosts) to crops that support little or no reproduction (moderately favourable or unfavourable hosts) (McLeod and Warren, 1993, McLeod, 1994). Reproduction on the covercrop is significant since nematode increase on the covercrop could be a source of increased infection of vines. Rate of maturation of the nematode, rate of development from the larval to the adult and egg laying stages, is also significant; if the nematode is in an early stage when the crop is turned in reproduction may be lessened (Part 6 of DAN 92/2).

The factors most likely to determine rate of maturation of endoparasitic nematodes in the field are soil temperature (Jones, 1975) and host suitability (Trudgill, 1995). The purposes of the experiments described here were to compare development of rootknot nematode *Meloidogyne javanica* in viticultural covercrops of different host status and to see how this may be influenced by season of the year.

MATERIALS AND METHODS

Crops and nematode

Crops included in experiments were tomato (cv. Grosse Lisse), a standard, very favourable host; Dun field pea, a very favourable host (McLeod and Warren, 1993) viticultural covercrop; Rangi forage rape, a moderately favourable host (McLeod and Warren, 1993) and a potential biofumigant crop (McLeod and Da Silva, 1994) and Cooba oats, a just favourable host (McLeod and Warren 1993) viticultural covercrop. Nematodes were obtained from cultures of *M. javanica*, originally from grapevine roots and soil from a Pokolbin, NSW, vineyard, maintained in pots on tomato or tobacco plants.

Experimental

Hatched infective second stage larvae were used in all experiments, to provide uniform infesting inocula. Larvae were collected free of eggs by shaking chopped, infected roots with egg masses in 0.5% NaOCl for four minutes and pouring the suspension through 1000, 150 and 25μm banked sieves. The 25μm sieve was then placed in a container with water just above the mesh for 3 to 5 days, providing a concentrated suspension of active larvae free of eggs.

Crops were sown in 15cm pots of sand–peat potting mix (1 volume coarse sand: 1 volume peat moss) in a glasshouse. Temperatures were logged by a Hobo data logger[1] with

[1] Hastings Data Loggers, Wauchope, NSW Australia.

a temperature probe at mid-depth (7.5cm deep) in a pot.

Enough pots were sown to allow uprooting of plants in four pots of each crop up to 12 times and the pots were arranged in 4 blocks with sampling time and crop randomised within blocks. Three seedling were allowed to grow in a pot and pots were infested 3 weeks after sowing with approximately 1000 larvae in 1 ml of water to a pot.

Starting 4 weeks after infestation, and then weekly, root samples from four pots of each crop was stained with 0.05% methyl blue in lactoglycerol (Bridge, Page and Jordan, 1982), to determine stage of nematode development. Measurements of nematode growth, numbers of developing nematodes and of egg production were also made in some experiments. For ease of presentation, experiments are described in the order, summer, autumn, autumn-winter and winter but the winter experiment was done in the winter of 1994, the others followed.

Experiment 1, rate of growth during summer, was started (infested) on 13 December 1994, it included the crops Grosse Lisse tomato, Dun pea and Rangi rape and it ended on 17 February 1995. At 4, 5, 6 and 7 weeks from infestation nematode growth was recorded by measuring the profile area of 20 specimens from each crop using a camera lucida and squared graph paper (Bird and Wallace, 1965). At 5 weeks (tomato and Dun pea) and 6 weeks (Rangi rape) from infestation numbers of eggs present on each crop were estimated by the NaOCl method, as described by McLeod and Warren (1993). Numbers of eggs were also estimated 1 week later.

Experiment 2, development during autumn, started (was infested) on 7 March 1995, it included Grosse Lisse tomato, Dun pea, Rangi rape and Cooba oats and it ended on 28 April 1995. Four and 6 weeks after infestation numbers of maturing nematodes, that is number of females and well developed larvae, were estimated. Washed roots were stained in methyl blue lactoglycerol, macerated and nematodes separated by straining the macerate through 1000, 300 and 150μm banked sieves.

Experiment 3, development during autumn–winter, was infested on 12 April 1995, it included Grosse Lisse tomato, Dun pea, Rangi rape and Cooba oats and it ended on 15 August 1995. At 17 weeks from infestation 4 oat pots were checked for presence of eggs by the NaOCl method used in experiment 1.

Experiment 4, development during winter, was infested on 10 June 1994, it included Grosse Lisse tomato, Rangi rape and Cooba oats and it ended on 21 September 1994. Numbers of eggs on tomato were estimated 8.5 weeks from infestation and one week later; eggs on Rangi rape were estimated at 13.5 weeks and 1 week later, using the NaOCl method.

Results

During summer, experiment 1 (Table 1), on the very favourable hosts tomato and Dun pea, female stage nematodes were first detected 4 weeks after infestation and eggs were first detected after 5 weeks. On the less favourable host Rangi rape females were first noted at 5 weeks and eggs at 6 weeks. In autumn, experiment 2, females first appeared on tomato and

Dun pea at 4.5 weeks and eggs at 5.5 weeks while on the less favourable hosts Rangi rape and Cooba oats females appeared at 5.5 and eggs at 6.5 weeks. In autumn–winter (experiment 3), on tomato and Dun pea, females took 5 weeks to mature and eggs first appeared at 7 weeks. On the moderately favourable host Rangi rape development to the female stage took 7 weeks and eggs did not appear till 10 weeks from infestation. On the just favourable host Cooba oats females or eggs were not found in the weekly examinations, however egg counts at 17 weeks showed the presence of small numbers eggs, a total of 160 from 4 pots. In winter (experiment 4), on tomato, development to females took 6.5 weeks and to egg production took 8.5 weeks to start while on Rangi rape the corresponding times were 8.5 and 13.5 weeks. No females or eggs were found on Cooba oats even after 13.5 weeks, however no egg counts were done on this crop in this experiment.

Nematode growth, as measured by profile area in experiment 1, on very favourable and moderately favourable hosts is recorded in Table 2. At 4 weeks from infestation nematodes were at the young female stage in tomato and Dun pea while in less favourable host Rangi rape most were in various larval stages; this was reflected in a marked difference in nematode size. This difference, although less marked, remained evident throughout later samplings. The results suggest that from 6 to 7 weeks, when egg laying was in progress (Table 1), size of females decreased.

The estimates of numbers of maturing nematodes established in the different crops (experiment 2, Table 3) at 4 and 6 weeks show many more in very favourable hosts than in less favourable hosts.

Table 4, based on experiments 1 and 4, presents a comparison of rates of egg production, measured near the start of egg laying, at two different times of the year. In summer (experiment 1) over January, large numbers of eggs were already present on tomato, (6×10^3, and Dun pea (23×10^3) when egg production was first detected and numbers of eggs increased to 99×10^3 and 49×10^3 times over 1 week. In winter (experiment 4), over August, on tomato egg numbers were very low, 250 in 1 of 4 pots when egg laying was first detected and the increase over 1 week was to an average of 310. In summer (experiment 1) over January, on Rangi rape, very small numbers of eggs had been produced (30 eggs on 1 of 4 pots), when egg laying was first detected but numbers increased to an average of 1.07×10^3 over 1 week. In winter egg laying began on Rangi in September, 1 month later than on tomato; as in summer egg numbers were very low, 150 in 2 of 4 pots when egg laying was first detected, egg production over 1 week then matched the summer egg production rate, egg numbers increasing to an average of 1.5×10^3.

Table 5 presents a comparison of numbers of larvae infecting 7 days after infestation (DAN 92/2 Part 7, Table 1) and numbers of maturing nematodes after 6 weeks (Table 3 experiment 2). The data from Part 7, table 1, was adjusted from actual numbers /2 seedlings to numbers/3 seedlings because 3 plants were grown in a pot in experiment 2. Results for 15–20°C were used because infection was high in all hosts in this temperature range. From this data percent maturation, number maturing as a percentage of number infecting, is on tomato 55%, on Dun pea 100%, on Rangi rape 76% and on Cooba oats 39%.

Discussion

The results clearly demonstrate that, as would be expected, rate of maturation is influenced by season of the year and also by crop host status. However there is interaction of season and crop host status. In summer and autumn sowings maturation differed little and differences between crops were minor. Maturation was slowed in winter on all crops but slowing was more marked in the moderately favourable host Rangi rape and no females or eggs were found on the just favourable host Cooba oats over a 13.5 week period. The relation between season and host status is further complicated when egg production results are considered, this is discussed later.

In winter, soil temperatures in viticultural areas would be much lower than obtaining under the conditions of experiment 4, so maturation would be slower again and differences between crops accentuated. In the field nematode development would be stopped when soil temperatures fall below the basal temperature for rootknot nematode development, which has been estimated at 8.25 to 12.9°C, depending on *Meloidogyne* species and climate adaptation (Vrain, Barker and Holtzman, 1978; Lahtinen, Trudgill and Tiilikkala, 1988; Madulu and Trudgill, 1994). In Western Australia maturation to egg laying of *M. arenaria* on subterranean clover over winter can take 15 weeks (Pung, Barbetti and Sivasithamparam, 1992). At Red Cliffs near Mildura, Victoria barley crops grown over winter are not infected by *M. javanica* (Sauer and Giles 1957), similar observations have been made with winter cereal crops and *M. incognita* in California, USA (Roberts, Van Gundy and McKinney, 1981). Slower maturation should provide more time during which an infected crop could ploughed in to reduce egg laying. However, from the previous results (DAN 92/2 Part 6) ploughing

in should be done before nematodes reach the adult stage, within 4 to 5 weeks of temperatures becoming favourable for development.

Slower rootknot nematode growth in resistant or less favourable host crops, as found here (Table 2) in Rangi rape and Cooba Oats has been noted in several other studies (Dropkin, 1963; Bird, 1972; Ferris and Hunt, 1979; Brembreg and Lung, 1989; Anwar, Trudgill and Phillips, 1994). Bird (1970) found that deficient host nutrition slows growth of *M. javanica* in a favourable host and suggested that slowed growth is a response to stress. Bird and McGuire (1996) showed that antimetabolites applied to tomato plants also slowed growth of *M. javanica*. Thus in a less favourable host slower growth could be due to the host providing suboptimal nutrition or to the presence of defence chemicals. Below optimal temperatures would compound the effect, accentuating the difference between favourable and less favourable hosts.

Comparison of rates of egg production in summer and winter crops (Table 4) showed that the egg production phase is slowed at lower temperatures. Egg production on the winter crop of Rangi rape showed a pattern which emphasises the complexity of relation between season and host status. Slower nematode maturation on the crop in winter delayed start of egg production till September when temperatures were rising so that the nematode then produced eggs quickly.

Comparison of numbers of maturing nematodes at 6 weeks with numbers infecting at 7 days, although in different experiments, suggests that a high proportion, 39–100% matured. Percent maturation was not however closely related to host status. On one good host, Dun

pea, it was 100%, on the other, tomato, it was 55%; on one less favourable host, Rangi rape it was 76% on the other, Cooba oats, it was 39%.

In the less favourable hosts studied, fewer eggs were produced, growth of the nematode within the host was slower and fewer nematodes infected. A similar situation has been reported with rootknot nematodes over a wide range of crops (Bird, 1972; Ferris and Hunt, 1979; Anwar, Trudgill and Phillips, 1994). The hypothesis is advanced that in many hosts all of these are the effects of the same defence process or barrier. If it is a chemical or physical barrier, the defence factor operates to reduce infection and to slow growth but maturation is not prevented. It is suggested that reduced egg production on less favourable hosts is then due predominantly to the reduced infection. However it should be noted that in some hosts other mechanisms operate. For example, resistance in which similar numbers infect and hypersensitive type reactions stop maturation is extensively documented (Wallace, 1963).

Table 1: **Effect of season of the year on maturation of rootknot nematode *Meloidogyne javanica* in crops differing in host status.**

Season	Stage detected	Time from infestation, weeks			
		Grosse Lisse Tomato[1]	Dun field pea[1]	Rangi forage rape[2]	Cooba oats[3]
Summer	females	4	4	5	Not grown
13.12.94 to 17.2.95	eggs	5	5	6	
Autumn	females	4.5	4.5	5.5	5.5
7.3.95 to 28.4.95	eggs	5.5	5.5	6.5	6.5
Autumn–winter	females	5	5	7	None at 10
12.4.95 to 15.8.95	eggs	7	7	10	17
Winter	females	6.5	Not grown	8.5	None at 13.5
10.6.94 to 21.9.94	eggs	8.5		13.5	

1) Very favourable host.

2) Moderately favourable host.

3) Just favourable host.

Table 2: **Growth of rootknot nematode *Meloidogyne javanica* in crops differing in host status (summer experiment 1)**

When measured (weeks after infestation)	Nematode profile area section ($\mu m^2 \times 10^4$)		
	Grosse Lisse tomato[1)]	Dun field pea[1)]	Rangi forage rape[2)]
4	25.96	23.41	0.174
5	29.16	24.90	11.70
6	30.42	26.36	18.70
7	27.15	21.40	17.00

1) Very favourable host.

2) Moderately favourable host.

Table 3: **Numbers of maturing rootknot nematode *Meloidogyne javanica* in crops differing in host status at 4 and six weeks from infestation (autumn, experiment 2)**

Crop	Number maturing	
	4 weeks	6 weeks
Grosse Lisse tomato[1)]	119	203
Dun field pea[1)]	355	778
Rangi forage rape[2)]	33	100
Cooba oats[3)]	3	14

1) Very favourable host.

2) Moderately favourable host.

3) Just favourable host.

Table 4: **Comparison of initial rate of egg production by rootknot nematode *Meloidogyne javanica* on crops of different host status in summer and in winter (experiments 1 and 4)**

Crop	Season and month when eggs layed	Increase over 1 week eggs/pot[3]
Grosse Lisse tomato[1)]	Summer, January	33,000
	Winter, August	250
Dun field pea[1)]	Summer, January	26,000
	Winter	Not grown
Rangi forage rape[2)]	Summer, January	1,060
	Winter, September	1,460

[1)] Very favourable host.

[2)] Moderately favourable host.

[3)] Mean of 4 estimates 1 week after egg laying first detected – mean of 4 estimates of number when egg laying first detected.

Table 5. **Comparison of numbers of rootknot *Meloidogyne javanica* infecting crops differing in host status shortly after infestation and numbers maturing**

Crop	Larvae infesting /2 seedlings[4]	Larvae infecting /3 seedlings[5]	Maturing at 6 weeks /3 plants[6]
Grosse Lisse tomato	246	369	203
Dun pea[1]	500	750	778
Rangi rape[2]	88	132	100
Cooba oats[3]	23	36	14

[1] Very favourable host.

[2] Moderately favourable host.

[3] Just favourable host.

[4] Data from Part 7, Table 1, numbers infesting 2 seedlings at temperature range 15–20°C.

[5] Numbers in second column adjusted to 3 seedlings.

[6] Data from Table 3, 3 plants /pot grown in experiment 2.

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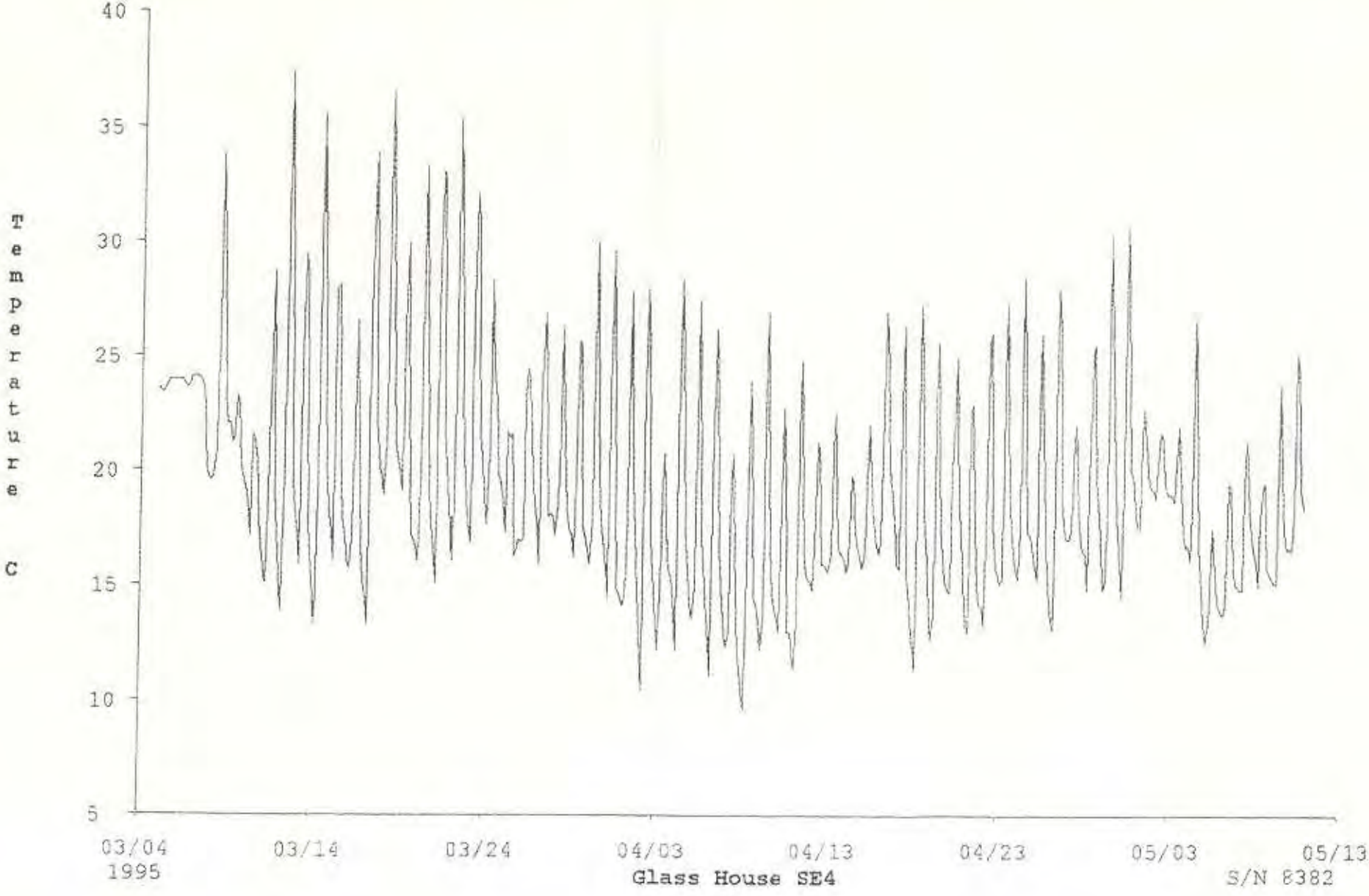
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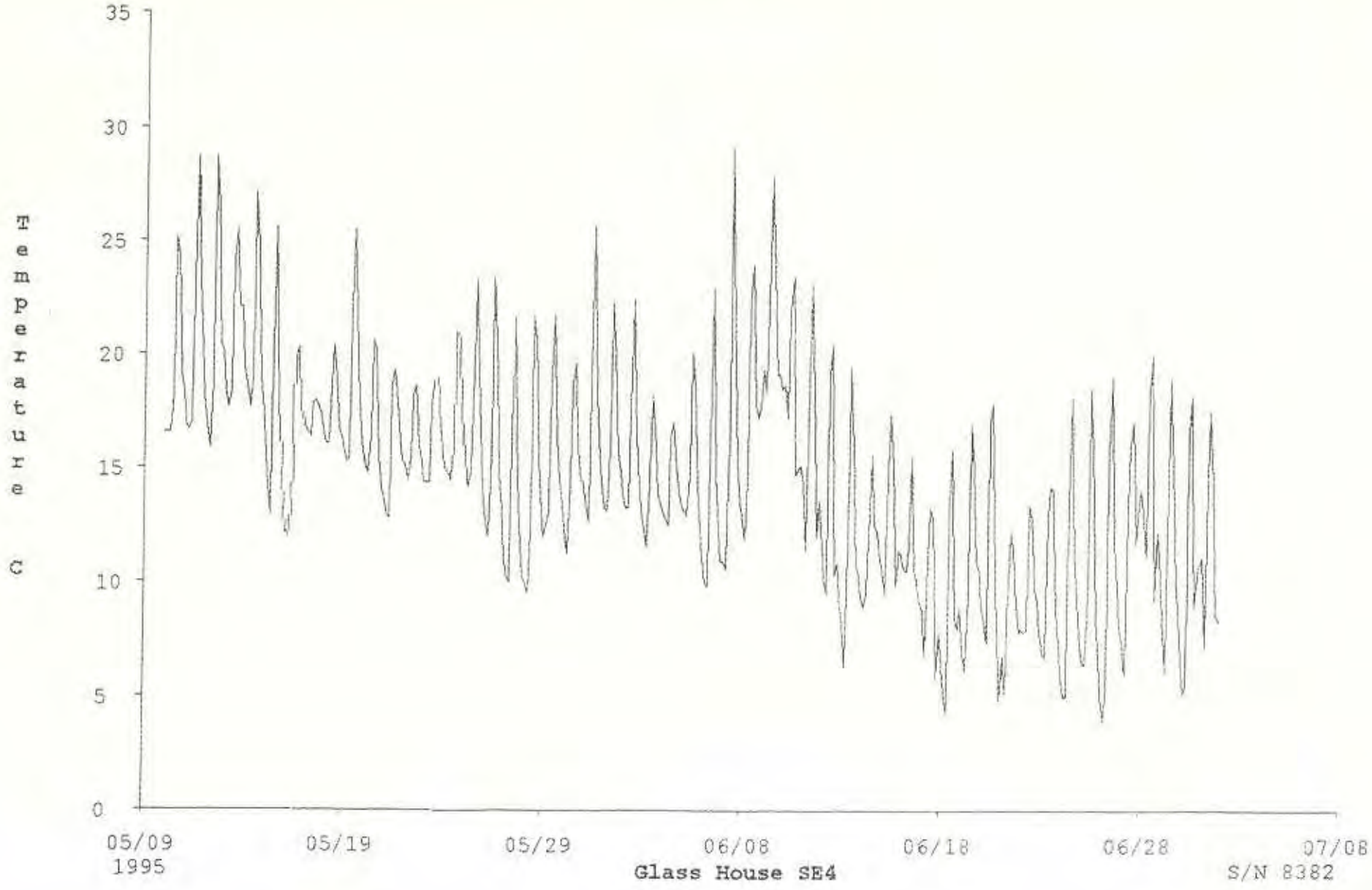
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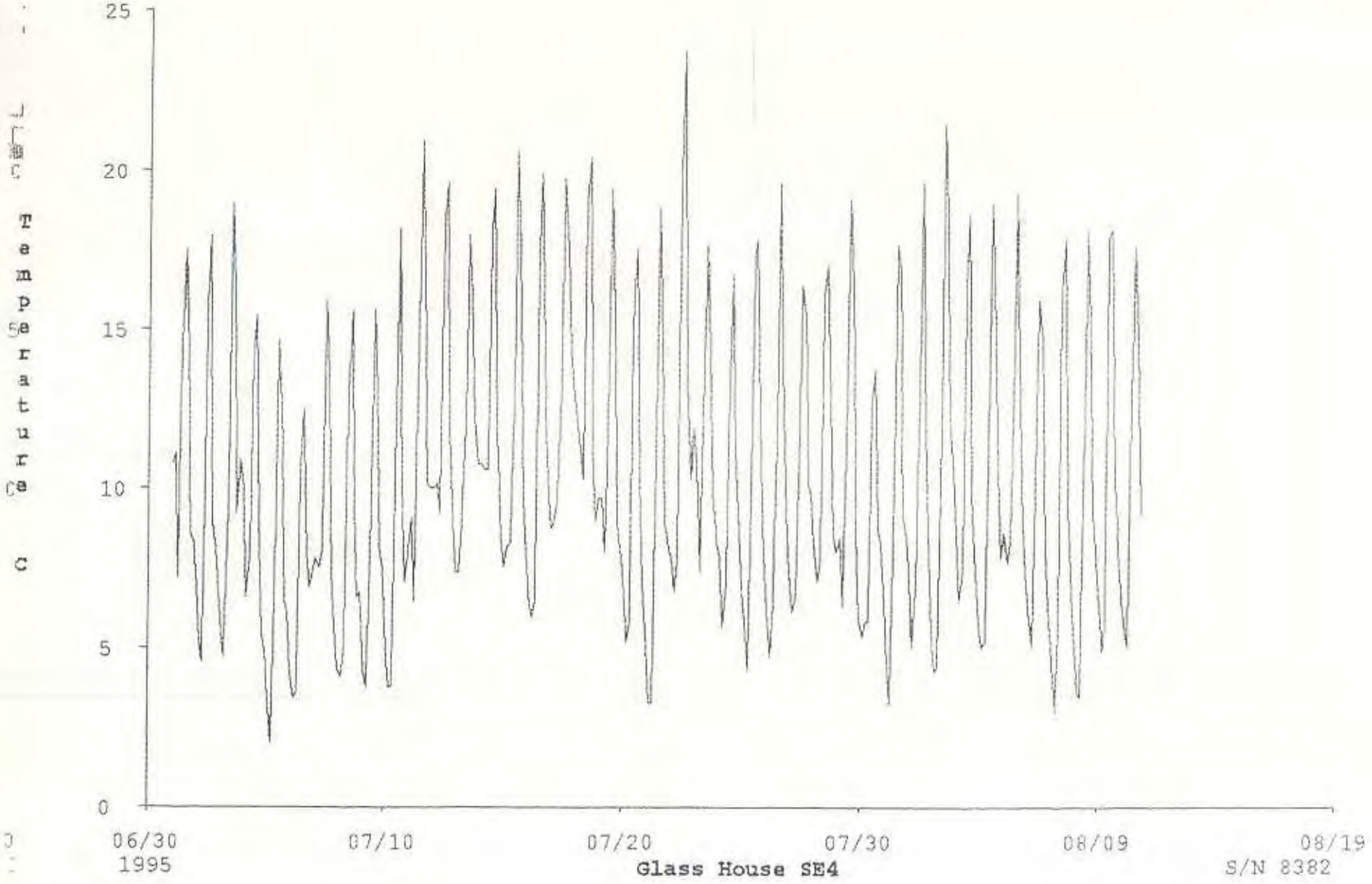
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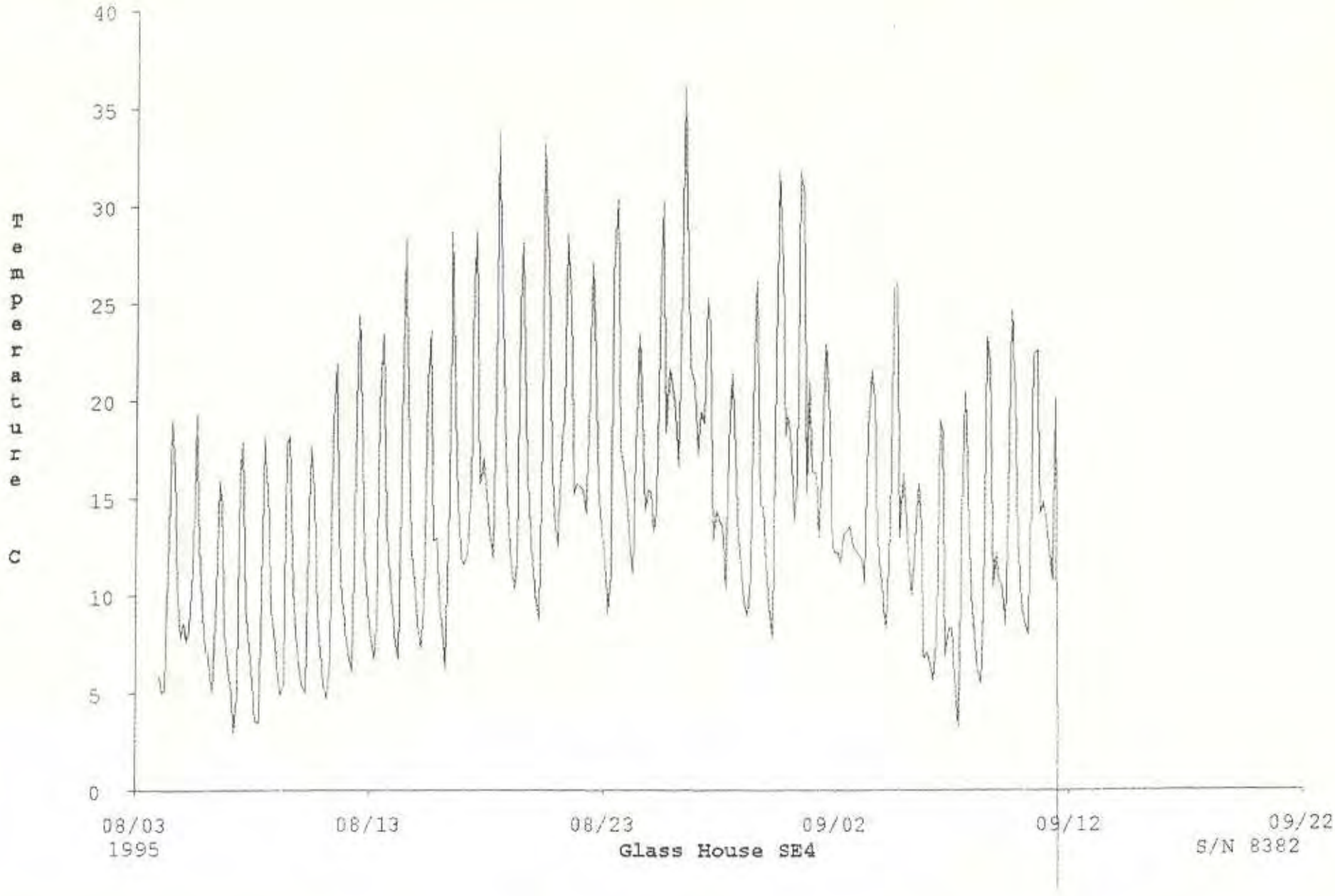
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PUBLICATIONS AND TECHNOLOGY TRANSFER

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