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Impact of citrus nematode (*Tylenchulus semipenetrans*) densities in soil on yield of grapevines (*Vitis vinifera* 'Shiraz') in south-eastern New South Wales

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Summary

'Shiraz' is a popular red wine grape variety grown in NSW, Australia, and is susceptible to citrus nematode (*Tylenchulus semipenetrans*). The extent of damage, particularly yield loss, or the damage threshold level of *T. semipenetrans* in 'Shiraz' is not known. In this study we investigated the population dynamics and the effects of a range of population densities of *T. semipenetrans* on yield of 'Shiraz' in a naturally infested vineyard across three growing seasons. Results showed that the population density of *T. semipenetrans* J_2 in soil did not increase or decrease consistently during the trial period. However, the population densities varied significantly ($P < 0.05$) between the temporal seasons in a year and were in order of summer > spring > autumn > winter. Yield from vines with *T. semipenetrans* J_2 population densities greater than 9,000·kg^{-1} dry soil (average population over 11,000·kg^{-1} dry soil) was 15 % lower compared to vines with 500-3,000 (average 932) *T. semipenetrans* J_2·kg^{-1} dry soil in 2004 but not in 2001 and 2003. Regression analysis showed a linear trend ($r = -0.36$) on yield decrease with the increase of *T. semipenetrans* densities in soil in 2004, when the 'Shiraz' vines were eight years old. Pruning weight was reduced by 18.7 to 22.9 % when nematode population densities were greater than 12,000·kg^{-1} dry soil (average population over 19,000·kg^{-1} dry soil).

K e y w o r d s : Citrus nematode, damage threshold level, initial population density (IPD), 'Shiraz' grapevines.

Introduction

Citrus nematode (*Tylenchulus semipenetrans*) causes slow decline, loss of feeder roots, poor vine vigour and yield reductions up to 30 % in grapevines (Seinhorst and Sauer 1956, Van Gundy 1961, McKenry 1992, Nicol and Van Heeswijck 1997). This nematode has been found in many vineyards in New South Wales (NSW) (McLeod 1978, Rahman *et al*. 2000), Victoria (Seinhorst and Sauer 1956, Sauer 1962, Harris 1984) and South Australia (Stirling 1976). Apart from citrus and grapevines the other host range includes olive, pear and persimmon (Siddiqi 1974). It is common in all types of soils but prefers loamy soil to sandy soil (McLeod 1978, Harris 1984, McKenry 1992).

No specific diagnostic symptoms have been defined for *T. semipenetrans* in grapevines (*Vitis vinifera*) but symptoms such as poor vine growth and vigour, and low yield in conjunction with high population densities have been taken as the basis for diagnostic and control recommendations. Yield losses depend on pest nematode population densities as well as the sensitivity of a particular crop cultivar (tolerance) and environmental conditions. Establishing a threshold density (a population density above which significant yield loss can be expected) requires information on the relation between pest population density and yield loss, which needs assessing at the cultivar and regional environment level. There is some information on the relative tolerance of our main grape varieties/cultivars to root knot nematode (Stirling and Cirami 1984) but very little to citrus nematode. Data on yield reduction of grape varieties due to *T. semipenetrans* infestation come entirely from nematicide application experiments (Sauer 1966, Van Gundy 1961, Walker 1989, McKenry 1992). For example, *T. semipenetrans*-infested Sultana vines produced 10-20 % more yield at the fourth season after being treated with Carbofuran 5 % (w/v) and Nemacur® 40 % (w/v) for three consecutive growing seasons (Edwards 1991). However, these do not separate nematode control effects from possible nematicide side effects and give no information on the relation between yield loss and nematode population density. Grafted vines on different rootstocks also produced higher yields than own-rooted vines in a *T. semipenetrans* infested vineyard (Edwards 1988, 1989). Overall, these reports suggested that citrus nematode may cause considerable loss of yield in own-rooted grapevines.

'Shiraz' is a popular red wine variety in Australia and nationally occupies 40.5 % of the area of red grapes and produces 41.1 % of total red grape production (ABS. Australian Wine and Grape Industry 1329.0.2005). The Riverina of south-eastern NSW is a major red grape producing region with 57.1 % of the area and 73.9 % of production in NSW. Many vineyards planted with own-rooted 'Shiraz' have been reported infested with *T. semipenetrans* in this region (McLeod 1978) but there are no reports available on yield loss. Therefore, this study was conducted primarily to

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investigate the population dynamics and density (as measured by population density $J_2 \cdot kg^{-1}$ dry soil) of *T. semipenetrans* over four growing seasons, and its effect on yield of 'Shiraz' with the aim of establishing an estimate of a damage threshold in Riverina conditions in south-eastern NSW.

Material and Methods

Vineyard description and selection: Five vineyards in the Riverina region planted with own-rooted 'Shiraz' with a history of *T. semipenetrans* infection were selected for pre-trial nematode assessment. Soils from 10 randomly selected vines from each of these vineyards were sampled to determine *T. semipenetrans* J_2 population densities$\cdot kg^{-1}$ dry soil for each individual vine. Then one vineyard, with *T. semipenetrans* but free from root knot nematode (*Meloidogyne* spp.) infestation, was selected for this study. The vineyard was four years old at the start of the trial in October 2000. Vine spacing was approximately 3.5 m (between rows) x 2.0 m (between vines), and vine beds were approximately 600 mm wide, with soil mounded to 350 mm and drip irrigated. The vineyard was located at 34°17'S and 146°03'E with an altitude of 128 m and had sandy to sandy loam soil with a pH of 6.8 (0.01M $CaCl_2$). Previously the vineyard was planted with citrus trees.

Estimation of initial population density (IPD) of *T. semipenetrans* in individual vine: In October 2000, five rows within the selected vineyard were chosen at random. In each row, 20 vines from 20 different panels making a total of 100 vines (5 x 20) were selected and marked for collecting soil samples. As there were no apparent above ground symptoms of nematode infection, the middle vine from each panel (five vines$\cdot$panel^{-1}) was chosen. Two soil samples, one from each side of the vine, were taken from each vine along the central line of the vine row, bulked and a sub-sample of 600 to 800 g was taken for nematode analysis. Samples were collected at a distance of 300 mm from the vine trunk and to a depth of 300 mm using a narrow end-ed shovel (90 mm). Nematodes were extracted from two 200 g soil from each sub-sample by using the tray method (Whitehead and Hemming 1965) with 5-day incubation. The resulting suspension was passed twice through a 15 µm nylon mesh sieve and nematodes were back-washed from the sieve into a 75 ml plastic container. Numbers of *T. semipenetrans* J_2 in the supernatant were counted using a Doncaster counting dish (Doncaster 1962) under a stereomicroscope at 50X. Counts from these duplicate extractions were averaged and nematode numbers$\cdot kg^{-1}$ dry soil was calculated. The moisture content of each soil sample was determined by drying 100 g soil in an oven at 100 °C for 48 h.

Selection of vines with specific initial population density (IPD) group: Based on these estimates, five vines were selected and marked in each row so as to include one vine in each of the following five IPD groups 500-3,000, 3,001-6,000, 6,001-9,000, 9,001-12,000 and over 12,000$\cdot kg^{-1}$ dry soil (Tab. 1). Thus there were five vines (one vine from each of five rows) for each of the IPD groups which were designated as 1, 2, 3, 4 and 5 respectively (Tab. 1) and the trial treated as a randomized block design with five replications of each group.

Monitor on *T. semipenetrans* population density after IPD estimation: Using the procedure as for initial population density estimation, soil samples from the selected 25 vines were collected at harvest on 6 March 2001, 5 March 2003 and 3 April 2004, and also during the four temporal seasons in 2001 and 2003. The soil samples were processed for nematode extraction and counting as described above.

In addition to *T. semipenetrans* J_2, a small number of ring (*Criconema* spp.) and spiral (*Helicotylenchus* spp.) nematodes were present in some samples but were not counted.

Population level of *T. semipenetrans* in roots: Approximately 10-15 g young roots from each vine were also collected at harvest in March 2001 and 2002. Roots from individual vine were cleaned off from adhering soil, cut into small pieces (< 10 mm long)

Table 1

Range and means (n = 5) of the initial population density (IPD) groups of *T. semipenetrans* J_2 per kg dry soil and number of female populations (n = 5) per 10 g roots

IPD group (October 2000)	Range (J_2 per kg dry soil)	Mean (J_2 per kg dry soil)[1]	Number of *T. semipentrans females* per 10 g roots	
			March 2001	March 2002
1	500-3000	934 (6.84) a	39 aA	16 aA
2	3001-6000	5064 (8.53) b	18 bA	24 aA
3	6001-9000	6836 (8.83) b	41 aA	21 aA
4	9001-12000	11614 (9.36) c	47 aA	22 aA
5	Over 12000	19341 (9.87) d	62 aA	23 aB
LSD < 0.05		0.41	2.5	2.5

[1] Back transformed means (n = 5) of *T. semipenetrans* J_2 per kg dry soil with transformed means ($\log_e x$) in parenthesis. Within columns, means with different small letters and within row, means with different capital letters differ significantly at $P < 0.05$. LSD value for mean J_2 per kg dry soil is based on $\log_e x$ transformed data.

and two grams stained in boiling 0.1 % acid fuchsin for 7 min. These stained roots were dissected under stereomicroscope at 50X and *T. semipenetrans* females counted.

Harvesting and pruning of grapevines: Grape bunches from each vine were hand picked, counted (except 2001) and weighed in 2001, 2003 and 2004. Harvest in 2002 was not possible due to short notice from the grower. Canes of each vine were cut back to 3 bud positions (excluding basal bud) in the winters of 2003 and 2004, and weights of pruning recorded.

Weather data: Data on rainfall and temperature for the trial period were collected from the CSIRO, Riverina, NSW, which is 1 km away from the vineyard.

Data analysis: Population densities of *T. semipenetrans* J_2 in soil were transformed to natural logarithm ($\log_e x$) and were analysed using linear mixed models fitted in ASREML (Gilmour *et al*. 2002). Number of grape bunches, pruning weight and female populations of *T. semipenetrans* were also analysed using the same model. In all these cases statistical significance was assessed by LSD ($P < 0.05$ or less). Regression analysis to establish relationship between nematode populations in soil at each harvest and yield was done using Genstat 9.

Results

Population densities of *T. semipenetrans* J_2 in soil: The mean values of initial population density (IPD) of *T. semipenetrans* J_2 in soil (October 2000) differed significantly ($P<0.05$) between the five IPD groups in the following order: IPD group 1<2=3<4<5 (Table 1). These differences between the IPD groups did not persist in the subsequent samples at each harvest during the trial period ($P = 0.69$). There was no consistent increasing or decreasing trends in population densities in soil for any of the 25 vines over a four year trial period (Fig. 1).

The population densities of *T. semipenetrans* varied significantly between the temporal season in 2001 but not in 2003. However, there were significant season by year effects ($P<0.017$) suggesting that the same season in different calendar years had different population levels (Fig. 2). As such the *T. semipenetrans* J_2 in soil increased from year to year: summer 2001 < summer 2003, autumn 2001 < autumn 2003, winter 2001 < winter 2003 and spring 2001 < spring 2003 (Fig. 2).

The number of females in roots differed only in that IPD group 2 had significantly ($P > 0.05$) fewer than the

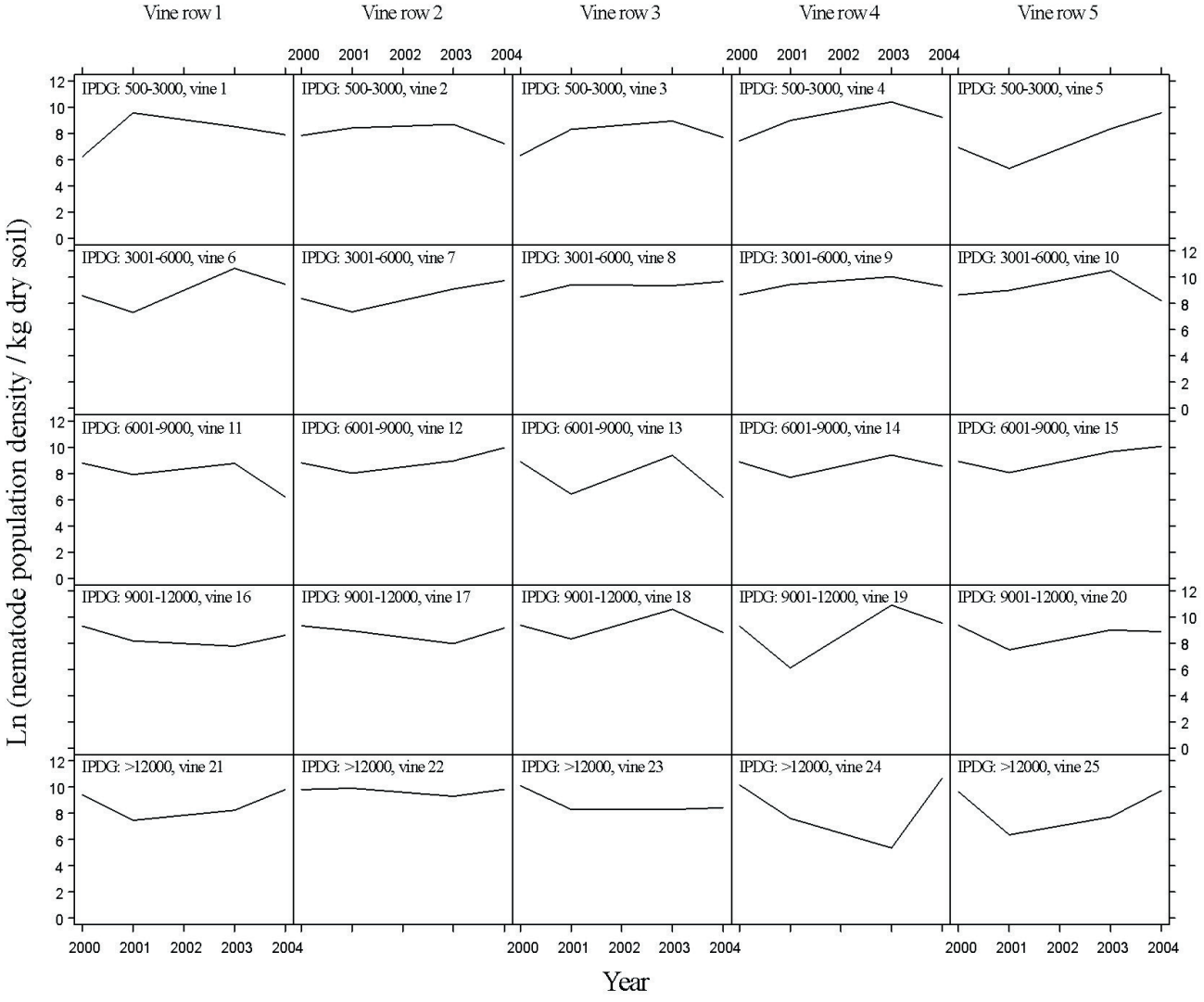

Fig. 1: Changes in population density ($\log_e x$) of *T. semipenetrans* $J_2 \cdot kg^{-1}$ dry soil during 2000-2004 in a 'Shiraz' vineyard, Riverina, NSW, Australia.

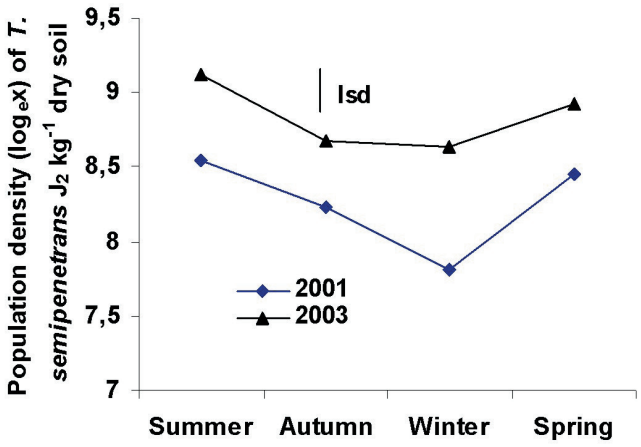

Fig. 2: Seasonal changes in population density ($\log_e x$) of *T. semipenetrans* J_2·kg^{-1} dry soil in a 'Shiraz' vineyard, Riverina, NSW, Australia, 2001 and 2003. Each value is average population density of 25 vines. Bar represents lsd value at 0.05 level of significance.

other groups in 2001 and group 5 had fewer in 2002 than in 2001 (Table 1).

Grape yield and bunches: Grape yield varied significantly ($P < 0.05$) between the IPD groups in 2004 but not in 2001 and 2003 (Table 2). In 2004, yields were significantly ($P > 0.05$) lower in vines with the two highest IPD groups 4 and 5 (10.4 kg·vine^{-1}) than the vines with the lowest IPD group 1 (12.2 kg·vine^{-1}). This was equivalent to 2.6 t·ha^{-1} (15 %) lower yields in IPD groups 4 and 5 compared to IPD group 1. Regression analysis also showed a linear trend on yield loss with the increase of *T. semipenetrans* in soil in 2004 (r = -0.36, $P = 0.04$) but not in 2001 ($P = 0.45$) and 2003 ($P = 0.32$) (Fig. 3).

The grape bunch numbers (data not shown) and pruning weight (kg·vine^{-1}) did not vary significantly between the IPD groups. However, pruning weights were reduced by 23.8 % and 20 % in 2003 and 2004 respectively in vines with IPD group 5 compared to IPD group 1 (Table 2).

Discussion

Population dynamics and reproduction ability of a particular nematode is measured by using its initial and final population densities in a crop cycle. It is very common that the final population density at the end of a crop cycle is greater than its initial population density at the beginning of the crop cycle. Results from our study indicated no consistent increase or decrease of *T. semipenetrans* J_2 population densities in soil at harvest in 2001, 2003 and 2004 from its

Table 2

Effect of the initial population density (IPD) groups of *T. semipenetrans* J_2 per kg dry soil on yield and pruning weight of Shiraz, Riverina, NSW, Australia

IPD group (*T. semipenetrans* J_2 per kg dry soil)	Yield (kg berries per vine)			Pruning weight (kg per vine)	
	2001	2003	2004	2003	2004
1 (500-3000; average 934)	9.4	10.6	12.2 a	2.1	1.5
2 (3001-6000; average 5064)	8.3	11.4	10.7 ab	1.8	1.5
3 (6001-9000; average 6836)	8.0	10.6	11.8 ab	1.8	1.4
4 (9001-12000; average 11614)	9.2	10.3	10.4 b	1.8	1.3
5 (over 12000; average 19341)	9.1	10.2	10.4 b	1.6	1.2
LSD < 0.05	ns	ns	1.7	ns	ns

Within columns, means (n = 5) with different letters differ by LSD at $P < 0.05$ level, ns = not significant.

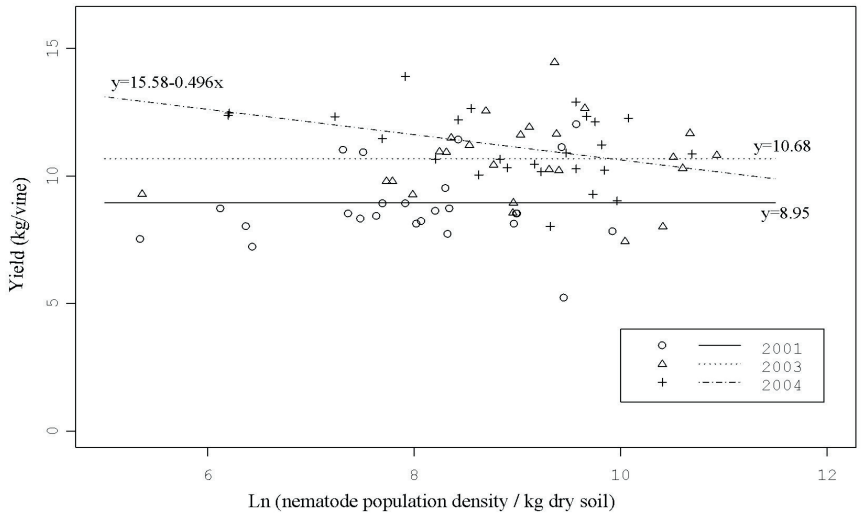

Fig. 3: Relationship between various population densities of *T. semipenetrans* J_2·kg^{-1} dry soil and yield of 'Shiraz' vines, Riverina, NSW, Australia.

initial population density in 2000. The population densities fluctuated between the sampling years. The reason is not known but may be due to variation in weather, abundance of natural enemies and competitors in soil from year to year as suggested by Jones and Kempton (1978). The total annual rainfall in 2001, 2002, 2003 and 2004 were respectively 489.0 mm, 246.1 mm, 555.4 mm and 262 mm at the site of the trial vineyard.

Results from this study also showed that the population density of *T. semipenetrans* on Shiraz vines grown in the Riverina varied significantly between different seasons within a year and between the years. Both in 2001 and 2003, the population density in soil declined gradually in order of summer to autumn to winter (except 2003) before increasing again in spring. A higher population density in summer is most likely related to vine root growth cycles because nematodes prefer to feed and reproduce on young roots. Grapevines produce new roots during November to December in Australia (Coombe 1988). Population increase of *T. semipenetrans* with a flush of new root development has also been observed in citrus trees in Florida (O'Bannon *et al*. 1972, Duncan and Noling 1987). It is also likely that more favourable environmental conditions for nematode reproduction in conjunction with new root growth in summer may also contribute for such a population increase. The daily average temperature in summer during 2001-2004 was 23.5 °C in the trial site which is within the favourable temperature range of 25-31 °C for citrus nematode development and reproduction (Siddiqi 1974). The daily average temperature in autumn, winter and spring were 16.5, 9.6 and 16.1 °C respectively during the same period. The reason for lower numbers of females in roots in 2002 compared to 2001 (Tab. 1), although not significant in most cases, is not known but may partly be associated with low rainfall throughout the year, as citrus nematode is very susceptible to drought (Siddiqi 1974). The total rainfall in 2001 and 2002 were 489.0 mm and 246.1 mm respectively at the trial site.

Knowing the damage threshold density of a particular pest is important when deciding whether to apply any control strategy. Results from this study with *T. semipenetrans* suggest that the IPD above 9,000 $J_2 \cdot kg^{-1}$ dry soil (IPD group 4 and 5) will reduce yield in Shiraz vines. Vines with population densities above this level suffered yield reductions of approximately 15 % in the 2004 harvest when 'Shiraz' vines were eight years old. Walker (1989) also recorded 26 % less yield over three growing seasons from vines infected with *T. semipenetrans* J_2 (6760·kg^{-1} soil; average of seven samples over three years) compared to Nemacar® treated vines ('Valdiguie'). A population density of 500 $J_2 \cdot kg^{-1}$ soil for *T. semipenetrans* has been adopted as a damage threshold population density in vineyards in California (McKenry 1992) but our results suggest that this level is not appropriate for grapevine grown in Australia, at least on 'Shiraz'.

The influence of nematode density in soil on yield can often be expressed as a linear regression of yield on log nematode numbers. A similar analysis of our results indicated a linear trend (r = -0.36) on yield decrease with the increase of *T. semipenetrans* densities in soil in 2004 only when 'Shiraz' vines were eight years old. These results suggest that root injury by *T. semipenetrans* may have little effects on yield of 'Shiraz' for the first few years after infection but will require several years before manifesting any vine decline with any consequent yield loss. Reynolds and O'Bannon (1963) observed 'grapefruit tree decline' between 3-5 years when population density of *T. semipenetrans* were very high in the root systems. It should also be noted that 'Shiraz' is a high vigour variety and so be able to tolerate any early damage of roots from *T. semipenetrans* infection before affecting top growth and yield, at least for the first few years during the establishment period.

Although vines with high *T. semipenetrans* J_2 population densities (mean population over 11,000·kg^{-1} dry soil; IPD groups 4 and 5) suffered 15 % yield loss, bunch number and pruning weight were not reduced. This result suggests *T. semipenetrans* may reduce yield after bunch initiation, by reducing bunch weight, and its effect does not extend to reduction of vine top growth. Our results are also in agreement with Harris (1986) who did not find any significant differences in bunch and cane weight between the vines ('Sultana') which had 450-1788 *T. semipenetrans* $J_2 \cdot 500\ g^{-1}$ soil. Variation in top growth and yield from vine to vine may also occur due to variation in vigour, thickness of vine trunks and nutrient level in a vineyard (Raski *et a*l.1981, Stirling 1982). More studies involving these factors are of future interest. The host status of own-rooted 'Shiraz' to *T. semipenetrans* is not sufficiently known. We observed a high population level in soil and endoparasitic stages including mature females in roots in this study which suggest that 'Shiraz' is a good host to *T. semipenetrans.* Furthermore a 15 % yield loss from vines with a mean population density of 11,614-19,341·kg^{-1} dry soil also indicated its susceptibility to citrus nematode. Although such a high population density may not be very common in vineyards, we suggest that a control measure (chemical or non-chemical) at this population density may prevent any further yield loss in 'Shiraz'. Application of carbofuran (Furadan 5G, 5 % w/w) or liquid phenamiphos (Nemacur® 40 % w/v) at 2.5-10 kg a.i.·ha^{-1} in inter-row areas was found to be very effective at reducing *T. semipenetrans* in established vineyards (Edwards 1991). Bayer Australia Limited recommended application of phenamiphos (Nemacur® 400 $g \cdot l^{-1}$) at 3 $ml \cdot m^{2-1}$ with irrigation water to control nematodes in established vineyards. As an alternative to chemical use, amendment of inter-row soil with brassica green manure was also found to be effective at suppressing nematodes in established vineyards (Walker and Morey 1999, Rahman and Somers 2005). For example, Walker and Morey (1999) reported up to 76 % suppression of *T. semipenetrans* by amending inter-row soil with brassica (*B. juncea* and *B. napus*) green manure in established citrus orchards. Amendment of inter-row soil with green manure of Indian mustard (*B. juncea*) cv. Nemfix also suppressed root knot nematode (*Meloidogyne javanica*) populations up to 86 % in established vineyards (Rahman and Somers 2005). However, our results on the impact of *T. semipenetrans* on yield in 'Shiraz' vines were collected from one vineyard only and therefore further observation including a standard nematicide control treatment in several vineyards with histories

of long term infection (> 8 years) with *T. semipenetrans* will be required to confirm these preliminary results.

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