VG97054
Resistance of Brussels Sprouts to Root Knot Nematodes (Meloidogyne Spp.) and Verticillium Dahliae, September 1998

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RESISTANCE OF BRUSSELS SPROUTS TO ROOT KNOT NEMATODES (*MELOIDOGYNE* SPP.) AND *VERTICILLIUM DAHLIAE*

HRDC Final Report
September 1998

Project VG97054

By G. E. Walker

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SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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INDUSTRY SUMMARY

Brussels Sprouts were susceptible to 3 commonly occurring species of Root knot Nematode (*Meloidogyne* sp.) in greenhouse tests. These nematodes caused root galling and suppressed shoot growth by up to 36%. Root galling was more severe from *M. incognita* and *M. javanica* than from *M. hapla* which indicates that growers should not rely on the presence of galling to diagnose nematode damage. A damage threshold of approximately 4,300 nematodes per 400 g of soil was suggested in the greenhouse but thresholds under field conditions need to be determined. Growers should be using diagnostic services to monitor the levels of plant parasitic nematodes in their fields. Damage from Root knot Nematodes is likely to be higher in warm, sandy soils and following highly susceptible rotation crops such as lucerne.

Both cultivars "Oliver" and "Roger" were susceptible to *M. incognita*, *M. javanica* and *M. hapla*, but whereas all 3 nematode species suppressed shoot growth of "Oliver", only *M. incognita* and *M. javanica* suppressed shoot growth of "Roger". Growers may be able to utilize the higher resistance of "Roger" to *M. hapla* in ground infested with this nematode, however, this will require development of rapid diagnostic tests to identify Root knot Nematodes to species level as conventional tests only identify nematodes to genus.

Two stains (a brassica strain and a non-brassica strain) of *Verticillium dahliae* , the soil-borne fungus which causes the disease Verticillium Wilt, were tested for pathogenicity to Brussels Sprouts in the greenhouse. Only the brassica strain was pathogenic to Brussels Sprouts and it suppressed shoot growth of "Oliver" but not "Roger". The higher resistance of "Roger" to *V. dahliae* may be useful to growers intending to plant in infested ground, however, testing demonstrated that infection with *M. incognita* could overcome this resistance so nematode control may be essential.

Other nematodes were found to be associated with commercial plantings of Brussel Sprouts; of particular concern was the finding of high levels of *Pratylenchus neglectus* (a first Australian record for this nematode on Brussels Sprouts). It is strongly recommended that further research be conducted to evaluate the pathogenicity of this nematode, particularly as it is known to exacerbate damage caused by Verticillium Wilt in other crops.

TECHNICAL SUMMARY

Inoculation with *Meloidogyne* sp. at levels of at least 100, 000 eggs per plant suppressed shoot growth of Brussels Sprouts, suggesting a damage threshold of about 4, 300 juveniles per 400 g of soil, although damage thresholds in the field could be lower than those in the greenhouse because of the more stressful growing conditions in the former. Both cultivars, "Oliver" and "Roger", were susceptible to *M. incognita, M. javanica* and *M. hapla*. All 3 species reduced shoot growth of "Oliver", but only *M. incognita* and *M. javanica* reduced shoot growth of "Roger" indicating that the latter cultivar is more tolerant to *M. hapla* than the other 2 species. Shoot dry weights were decreased by up to 36% in comparison with uninoculated plants. Shoot growth was reduced at the expense of higher root growth, indicating a diversion of resources to compensate for root damage. Shoot height of "Roger", but not "Oliver", was also suppressed.

Root galling in decreasing order of severity by nematode species was *M. incognita, M. javanica* and *M. hapla*, and both final population levels and reproduction rates of these nematodes also followed this order indicating lowest susceptibility to *M. hapla*. Nematode reproduction rates declined from initial inoculum levels of 100, 000 to 200, 000 eggs suggesting that root damage at the higher inoculum level was severe and limited reproduction.

A non-brassica strain of *V. dahliae* was apparently avirulent to Brussels Sprouts, while a brassica strain (from *B. oleracea* var. *botrytis)* suppressed shoot growth of "Oliver" but not "Roger", indicating a higher susceptibility in the former cultivar. The higher resistance of "Roger" to *V. dahliae* could be useful to growers planting in ground known to be infested with this pathogen. However, infection rate of *V. dahliae* was increased by co-infection with *M. incognita* in "Roger" but not in "Oliver". This interaction between *M. incognita* and *V. dahliae* suggests that damage could be severe where both pathogens are present, particularly with highly virulent strains of *V. dahliae*.

High and potentially damaging levels of other nematodes, particulary *Pratylenchus neglectus*, a first Australian record for this nematode on Brussels Sprouts, were found associated with Brussels Sprouts in the field. Further study to establish the pathogenicity of these nematodes is required, particularly as *Pratylenchus* spp. are known to exacerabate damage from *V. dahliae* in some other crops.

INTRODUCTION

This project arose from requests from the Brussels Sprouts Group of the S.A. Farmers Federation to investigate resistance of Brussels Sprouts (*Brassica oleracea* var. *gemmifera*) to Root knot Nematodes (*Meloidogyne* sp.) following the finding, by the SARDI Plant Research Centre Diagnostic Centre, of high levels of these nematodes in land proposed to be planted . There was concern that crops could be adversely affected by these nematodes as little information is available on their resistance and tolerance. Some plantings were occurring in sandy soils during hot, summer months when the damage potential from these nematodes would be at its highest.

In Britain, the only nematodes reported as damaging to Brussels sprouts are the Brassica cyst Nematode (*Heterodera cruciferae*) and the Beet cyst Nematode (*H. schachtii*) (MAFF, 1979). In California, it is reported (Univ. of Calif., 1985) that Brussels sprouts can "sustain significant yield reductions when heavily infested" with Root knot Nematodes, suggesting that a real risk existed. However, inquiries with vegetable extension officers and seed producers (J. Westra van Holthe, Product Manager Vegetables, S&G Seeds pers. comm.) in Australia indicated that little information on nematodes in Brussels sprouts existed locally, that growers did not usually undertake diagnostic testing for nematodes in soils before planting, and that seed companies and breeders had little or no information on cultivar resistance. This was confirmed by the total lack of records of any plant parasitic nematodes occurring on Brussels sprouts in South Australia (Cook and Dube, 1989). There is only one Australian record for a plant parasitic nematode occurring on Brussels sprouts (McLeod et al., 1994) and this is for *M. javanica* in New South Wales.

Pot experiments were conducted to evaluate the effects of varying levels of the 3 most commonly occurring *Meloidogyne* species, *M. hapla, M. incognita* and *M. javanica*, on the growth of locally used cultivars. The soil-borne fungus *Verticillium dahliae*, cause of the disease Verticillium Wilt, occurs on Brussels sprouts in California (Univ. of Calif., 1985) and is reported to be capable of limiting yields, but not killing plants. Since interactions between nematodes and this fungus have been been reported in other crops, isolates of the fungus were included in pot experiments, either alone or in combination with *M. incognita*. Different strains of this fungus have recently been identified in other countries (Chang and Eastburne, 1994; Subbarao et al., 1995). Two series of pot experiments were conducted; an initial series to rapidly acquire some information on resistance at inoculum levels expected in the field from results of diagnostic testing, and a second series at higher inoculum levels to establish limits of tolerance and to compare the resistance of 2 different cultivars. Initial information was extended to the Brussels Sprouts Group of the S.A. Farmers Federation to enable affected growers to evaluate immediate risks of planting into infested ground at inoculum levels known from testing of soil. This Group and IHD, Knoxfield also agreed to send in plant and soil specimens to enable information to be gathered on the occurrence of plant parasitic nematodes on Brussels sprouts crops.

MATERIALS AND METHODS

Pot Experiments: Series 1.

Inoculum production: DNA-tested populations of *M. javanica* and *M. incognita*, and a local population of *M. hapla* were multiplied on tomato plants and eggs were extracted from roots using 1% Na OCl. A local isolate of *V. dahliae* originally isolated from *Ixodia achillaeoides* was multiplied on Potato-dextrose agar (PDA); 2-week-old plates were macerated in deionized water (5 plates in 1 L) giving spore concentrations of 1 X 10^7 and 3.5 X 10^3 /mL conidia and microsclerotia respectively.

Brussels sprouts: seedlings cv. "Oliver" were transplanted into steam-disinfested sandy loam soil in 12.5 cm pots on a greenhouse bench (15-24°C). Three weeks later they were inoculated with nematodes and the fungus as appropriate:

a) uninoculated control (nematode-free wash water and suspension of uninoculated PDA only)
b) *M. javanica* 20, 000 eggs/pot
c) *M. hapla* 20, 000 eggs/pot
d) *M. incognita* 10, 000 eggs/pot
e) *M. incognita* 20, 000 eggs/pot
f) *M. incognita* 60, 000 eggs/pot
g) *V. dahliae* 10^8 conidia and 35, 000 microsclerotia /pot
h) *V. dahliae* 10^8 conidia and 35, 000 microsclerotia
+ 20, 000 *M. incognita* eggs/pot

Nematode eggs were added to 6 holes made in the soil near the plant stem; the *V. dahliae* suspension was similarly pipetted into 6 holes in the soil. All pots not inoculated with *V. dahliae* received 10 mL of a suspension made by blending 5 uninoculated PDA plates in 1 L of deionized water, and all pots not inoculated with nematodes received nematode-free wash water from nematode-infested tomato roots.

Plant growth: pots were arranged in a randomized block design on a greenhouse bench and were grown for 17 weeks at 15-24°C (providing approximately 27, 600 degree-hours above 9.9°C (or about 3.4-4.2 nematode generations). Plants were watered daily and fertilized with a complete liquid fertilizer weekly.

Data collected: at harvest the root systems of plants were dissected from the shoots and the fresh weights of both roots and shoots were determined. Shoots were dried (80°C for 24 hr) and reweighed (shoot dry weight). Root systems were rated for galling (0=0%, 1=trace, 2=<25%, 3=25-50%, 4=50-75%, and 5=>75% of roots galled respectively). The tap roots of uninoculated control and all *V. dahliae*-inoculated plants were dissected and the presence of internal staining was recorded (brown to black staining of vascular tissues is a common symptom of infection by *V. dahliae*); vascular tissue from all these plants was incubated at 28°C on PDA amended with 200 mg/L of streptomycin sulphate and were examined for *V. dahliae*. Root systems were coarsely chopped and nematodes were extracted in a modified Seinhorst mist apparatus for 4 d. *Meloidogyne* larvae in suspensions were then counted.

Statistical analyses: plant weights and nematode counts were analysed using the Analysis of Variance test ($P<0.05$). Nematode counts were transformed as ln (count) before analysis. Root gall ratings were analysed using the Chi-square test ($P<0.05$).

Pot Experiments: Series 2.

Inoculum production: inocula were prepared as in series 1, however, the *V. dahliae* isolate used came originally from diseased Cauliflower plants (*B. oleracea* var. *botrytis*).

Brussels sprouts: seedlings cvv. "Oliver" and "Roger" were transplanted into steam-disinfested sandy loam soil in 12.5 cm pots on a greenhouse bench (24±2°C). Three weeks later they were inoculated with nematodes and the fungus as appropriate:

a) uninoculated control (nematode-free wash water and suspension of uninoculated PDA only)
b) *M. javanica* 100, 000 eggs/pot
c) *M. javanica* 200, 000 eggs/pot
d) *M. hapla* 100, 000 eggs/pot
e) *M. hapla* 200, 000 eggs/pot
f) *M. incognita* 100, 000 eggs/pot
g) *M. incognita* 200, 000 eggs/pot
g) *V. dahliae* 44 X 10^6 conidia and 125, 000 microsclerotia /pot
h) *V. dahliae* 44 X 10^6 conidia and 125, 000 microsclerotia
+ 100, 000 *M. incognita* eggs/pot

Experimental conditions, numbers of replicates, details of harvesting, extraction and statistical analyses were as described for series 1 excepting that in analyses of variance, cultivar was used as the main plot and treatment (inoculum) as the subplot. Nematodes were extracted from the total soil from each pot over 5 d on Whitehead and Hemming trays. Nematode reproduction rates were calculated assuming a 10% viability of eggs in the initial inoculum. Greenhouse temperatures were recorded from a temperature probe inserted into potting soil and attached to a TinyTag ® datalogger (Hastings Dataloggers, Port Macquarie NSW) reading at 30 min intervals. Minimum, maximum and average temperatures recorded over the growth period following inoculation were 15.6, 24.1 and 20.3°C respectively and providing 32, 745 degree-hours above 9.9°C (approximately 4.1-5.0 nematode generations).

Nematodes in field specimens

Plant parasitic nematodes were extracted from soil and root samples from commercial plantings in Whitehead and Hemming trays (5 d) and a modified Seinhorst mist apparatus (4 d) respectively and were identified to genus.

RESULTS

Pot Experiments: Series 1.

Effects on plant growth in cv. "Oliver"

No significant differences were found between root and shoot weights of nematode-inoculated and uninoculated plants (Table 1), indicating that inoculation with *M. javanica* and *M. hapla* at 20, 000 eggs per pot, and with *M. incognita* at up to 60, 000 eggs per pot, did not adversely affect plant growth. Inoculum levels used were apparently below damage thresholds and these results indicated that this cultivar was relatively tolerant to these nematodes.

Plant growth was also not affected by inoculation with the *I. achillaeoides* strain of *V. dahliae* (Table 1) suggesting that this strain was only weakly virulent to cv. "Oliver" if at all.

Table 1. Effects of inoculation with 3 *Meloidogyne* spp. and/or an *I. achillaeoides* strain of *V. dahliae* on shoot and root weights of Brussels Sprouts cv. "Oliver" .

Inoculum	**Shoot weight (g)**		**Root fresh weight (g)**
	Fresh	**Dry**	
Uninoculated	170	30	63
M. javanica			
20,000 eggs	172	32	80
M. hapla			
20,000 eggs	173	32	87
M .incognita			
10,000 eggs	186	28	82
20,000 eggs	180	29	74
60,000 eggs	177	28	81
V. dahliae	184	32	78
V. dahliae + *M .incognita* 20,000 eggs	179	32	78
LSD ($P<0.05$)	ns	ns	ns

Disease symptoms observed in cv. "Oliver"

All 3 nematode species caused galling of cv. "Oliver" roots but *M. hapla* caused significantly less galling than the other 2 species (Table 2). Symptoms of infection by *V. dahliae* such as wilting or chlorosis were not observed.

Table 2. Effects of inoculation with 3 *Meloidogyne* spp. and/or an *I. achillaeoides* strain of *V. dahliae* on numbers of *Meloidogyne* spp. juveniles extracted from roots, nematode reproduction rate, root-galling index, and re-isolation of *V. dahliae* from taproots of Brussels Sprouts cv. "Oliver".

Inoculum	**Meloidogyne juveniles**		**Relative reproduction rate***	**Root galling index**	***V. dahliae* isolation frequency**
	Per plant	**Per g root**			
Uninoculated	-	-	-	-	-
M. javanica					
20,000 eggs	19	0.2	0.01	3.2 a	-
M. hapla					
20,000 eggs	131	1.6	0.07	1.1 b	-
M .incognita					
10,000 eggs	291	4.7	0.30	2.6 a	-
20,000 eggs	165	2.4	0.08	3.0 a	-
60,000 eggs	49	0.6	0.01	4.3 a	-
V. dahliae	-	-	-	-	0
V. dahliae + *M .incognita* 20,000 eggs	297	3.5	0.15	2.9 a	0
LSD ($P<0.05$)	ns	ns	-	-	-

* not including nematodes in soil

Nematode reproduction rates

Reproduction rates for all 3 nematode species were below 1 (Table 2) indicating that cv. "Oliver" would be classed as resistant. At an initial inoculum level of 20, 000 eggs, reproduction rates were higher for *M. incognita* and *M. hapla* than for *M. javanica* , and for *M. incognita* were increased by co-inoculation with *V. dahliae* (Table 2). Reproduction rates declined with increasing initial inoculum level of *M. incognita* (Table 2). Actual reproduction rates would be much higher than those reported in Table 2 as nematodes in soil are not included (see Series 2 results).

Re-isolation of *V. dahliae* from taproots of cv. "Oliver"

V. dahliae was not detected in taproots of cv. "Oliver" either in plants inoculated with the fungus alone or in plants inoculated with both the fungus and *M. incognita* (Table 2).

Pot Experiments: Series 2.

Effects on plant growth in cvv. "Oliver" and "Roger"

1) Shoot weights: There was no significant difference ($P<0.05$) between shoot weights, fresh or dry, between the 2 cultivars. All treatments suppressed "Oliver" shoot dry weights; all treatments except inoculation with M. hapla or V. dahliae suppressed "Roger" shoot dry weights (Table 3).

Table 3. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on shoot weight of Brussels Sprouts cvv. "Oliver" and "Roger".

Cultivar-treatment	**Shoot weight (g)**	
	Fresh	**Dry**
"Oliver"		
Uninoculated control	200.2	36.3
M .incognita 100,000 eggs	177.5	27.9
M .incognita 200,000 eggs	169.2	27.4
V. dahliae	206.7	29.5
V. dahliae + *M .incognita* 100,000 eggs	181.6	28.7
M. hapla 100,000 eggs	189.0	31.0
M. hapla 200,000 eggs	183.5	27.7
M. javanica 100,000 eggs	176.8	31.0
M. javanica 200,000 eggs	170.4	27.5
"Roger"		
Uninoculated control	220.5	36.2
M .incognita 100,000 eggs	182.3	27.9
M .incognita 200,000 eggs	138.6	23.4
V. dahliae	246.9	38.9
V. dahliae + *M .incognita* 100,000 eggs	150.0	22.3
M. hapla 100,000 eggs	199.0	32.2
M. hapla 200,000 eggs	214.8	34.5
M. javanica 100,000 eggs	171.9	25.9
M. javanica 200,000 eggs	145.1	23.3
LSD1 (P=0.05)*	26.4	4.7
LSD2 (P=0.05)	26.3	4.7

*LSD1 is for comparing means within a cultivar; LSD2 is for comparing treatments in different cultivars.

3) Root weight: Mean fresh root weight was higher for "Roger" compared with "Oliver" (60.9 and 66.9 g respectively; LSD, $P<0.05=5.8$). Root growth was stimulated by inoculation with *M. incognita*, *M. javanica* 100,000 eggs, *M. hapla* 200,000 eggs, or *V. dahliae* + *M .incognita* 100,000 eggs (Table 4).

Table 4. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on root weight of Brussels Sprouts cvv. "Oliver" and "Roger".

Treatment	**Root fresh weight (g)**
"Oliver" & "Roger" (combined data)	
Uninoculated control	55.2
M .incognita 100,000 eggs	70.4
M .incognita 200,000 eggs	73.2
V. dahliae	54.7
V. dahliae + *M .incognita* 100,000 eggs	64.6
M. hapla 100,000 eggs	56.0
M. hapla 200,000 eggs	65.6
M. javanica 100,000 eggs	71.6
M. javanica 200,000 eggs	63.9
LSD (P=0.05)	9.3

3) Plant height: The mean height of cv. "Roger" was significantly higher than cv. "Oliver" (30.2 and 28.4 cm respectively; LSD, $P<0.05 = 1.5$). Shoot height of "Roger" was suppressed in comparison with the uninoculated control by all treatments apart from inoculation with *M.hapla* 200,000 eggs or *V. dahliae*; shoot height of "Oliver" was not suppressed by any treatment but was increased by inoculation with *V. dahliae* (Table 5).

Table 5. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on shoot height of Brussels Sprouts cvv. "Oliver" and "Roger".

Cultivar-treatment	**Shoot height (cm)**
"Oliver"	
Uninoculated control	27.2
M .incognita 100,000 eggs	27.7
M .incognita 200,000 eggs	25.0
V. dahliae	36.1
V. dahliae + *M .incognita* 100,000 eggs	31.0
M. hapla 100,000 eggs	27.2
M. hapla 200,000 eggs	29.1
M. javanica 100,000 eggs	25.6
M. javanica 200,000 eggs	26.3
"Roger"	
Uninoculated control	35.6
M .incognita 100,000 eggs	27.2
M .incognita 200,000 eggs	24.3
V. dahliae	34.7
V. dahliae + *M .incognita* 100,000 eggs	28.1
M. hapla 100,000 eggs	30.4
M. hapla 200,000 eggs	35.4
M. javanica 100,000 eggs	29.7
M. javanica 200,000 eggs	26.1
LSD1 (P=0.05)*	2.6
LSD2 (P=0.05)	2.8

*LSD1 is for comparing means within a cultivar; LSD2 is for comparing treatments in different cultivars.

4) Root galling index: Roots were more severely galled by *M .incognita*, followed by *M. javanica*, and lastly by *M. hapla* (Table 5).

Table 6. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on root galling index of Brussels Sprouts cvv. "Oliver" and "Roger".

Cultivar-treatment	**Root galling index***
"Oliver"	
Uninoculated control	-
M .incognita 100,000 eggs	3.9 a
M .incognita 200,000 eggs	4.2 a
V. dahliae	-
V. dahliae + *M .incognita* 100,000 eggs	3.8 a
M. hapla 100,000 eggs	1.1 c
M. hapla 200,000 eggs	1.2 c
M. javanica 100,000 eggs	3.4 b
M. javanica 200,000 eggs	3.8 b
"Roger"	
Uninoculated control	-
M .incognita 100,000 eggs	4.6 a
M .incognita 200,000 eggs	4.6 a
V. dahliae	-
V. dahliae + *M .incognita* 100,000 eggs	4.3 a
M. hapla 100,000 eggs	1.9 c
M. hapla 200,000 eggs	1.9 c
M. javanica 100,000 eggs	4.3 a
M. javanica 200,000 eggs	4.4 a

* means followed by the same letter are not significantly different (χ^2 test, $P<0.05$).

Effects on final nematode populations in cvv. "Oliver" and "Roger"

1) Root populations: Comparatively low populations of *Meloidogyne* juveniles were found on roots. Numbers of *Meloidogyne* juveniles from root systems were significantly higher in plants inoculated with *V. dahliae* + *M .incognita* 100,000 eggs than all other treatments; numbers of *M .incognita* juveniles declined significantly at the higher inoculum level (Table 6).

Table 7. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on the final number of *Meloidogyne* juveniles per root system of Brussels Sprouts cvv. "Oliver" and "Roger".

Treatment	**Number of *Meloidogyne* juveniles per root system***
"Oliver" & "Roger" (combined data)	
Uninoculated control	-
M .incognita 100,000 eggs	62.4 b
M .incognita 200,000 eggs	35.1 d
V. dahliae	-
V. dahliae + *M .incognita* 100,000 eggs	1,018.5 a
M. hapla 100,000 eggs	105.0 bc
M. hapla 200,000 eggs	51.7 cd
M. javanica 100,000 eggs	91.0 bd
M. javanica 200,000 eggs	159.3 b

* statistical analyses conducted using ln-transformed data; back-transformed means are presented in the table; means followed by the same letter are not significantly different (LSD, $P<0.05$).

2) Soil populations: Large numbers of *Meloidogyne* juveniles were present in potting soil (Table 7); final populations were higher in "Oliver" than in "Roger" (ln-transformed means 13.7 and 13.2 respectively, LSD P,0.05=0.4). Final populations were not significantly different between the 3 treatments involving *M .incognita* (ln-transformed means 14.4, 14.2 and 14.1; LSD $P<0.05=0.6$) but were significantly higher than both *M. hapla* treatment groups (12.1 each) and the lower inoculum level of *M. javanica* (14.0) but not the higher inoculum level (14.0).

Table 8. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on final soil population of *Meloidogyne* juveniles in Brussels Sprouts cvv. "Oliver" and "Roger".

Cultivar-treatment	**Total number (millions) of *Meloidogyne* juveniles in soil per pot**
"Oliver"	
Uninoculated control	-
M .incognita 100,000 eggs	1.69 (14.24)
M .incognita 200,000 eggs	1.37 (13.98)
V. dahliae	-
V. dahliae + *M .incognita* 100,000 eggs	1.84 (14.07)
M. hapla 100,000 eggs	0.82 (12.93)
M. hapla 200,000 eggs	0.97 (13.36)
M. javanica 100,000 eggs	0.51 (12.92)
M. javanica 200,000 eggs	3.20 (14.13)
"Roger"	
Uninoculated control	-
M .incognita 100,000 eggs	3.47 (14.49)
M .incognita 200,000 eggs	2.28 (14.50)
V. dahliae	-
V. dahliae + *M .incognita* 100,000 eggs	1.93 (14.02)
M. hapla 100,000 eggs	0.18 (11.33)
M. hapla 200,000 eggs	0.09 (10.93)
M. javanica 100,000 eggs	0.60 (12.95)
M. javanica 200,000 eggs	1.41 (13.95)
LSD1 (P=0.05)*	(0.84)
LSD2 (P=0.05)	(0.46)

*LSD1 is for comparing means within a cultivar; LSD2 is for comparing treatments in different cultivars; analyses conducted on ln-transformed data in parentheses.

3) Nematode reproduction rates: Nematode reproduction rates were highest in *M .incognita*, followed by *M. javanica* and lastly by *M. hapla*; reproduction rates declined for all 3 species at the highest initial inoculum level (Table 8).

Table 9. Effects of inoculation with 3 *Meloidogyne* spp. and/or a *B. oleracea* var. *botrytis* strain of *V. dahliae* on final population of *Meloidogyne* juveniles (from soil plus roots) and nematode reproduction rate in Brussels Sprouts cvv. "Oliver" and "Roger".

Cultivar-treatment	**Total number of *Meloidogyne* juveniles (millions)**	**Reproduction rate**
"Oliver"		
Uninoculated control	-	-
M .incognita 100,000 eggs	1.69 (14.24)	169.0
M .incognita 200,000 eggs	1.37 (13.98)	68.5
V. dahliae	-	-
V. dahliae + *M .incognita* 100,000 eggs	1.84 (14.08)	184.0
M. hapla 100,000 eggs	0.82 (12.93)	82.0
M. hapla 200,000 eggs	0.97 (13.36)	48.5
M. javanica 100,000 eggs	0.51 (12.92)	51.0
M. javanica 200,000 eggs	3.20 (14.13)	16.0
"Roger"		
Uninoculated control	-	-
M .incognita 100,000 eggs	3.48 (14.49)	348.0
M .incognita 200,000 eggs	2.28 (14.50)	114.0
V. dahliae	-	-
V. dahliae + *M .incognita* 100,000 eggs	1.93 (14.03)	193.0
M. hapla 100,000 eggs	0.18 (11.33)	18.0
M. hapla 200,000 eggs	0.09 (10.93)	4.5
M. javanica 100,000 eggs	0.60 (12.95)	60.0
M. javanica 200,000 eggs	1.41 (13.95)	70.5
LSD1 (P=0.05)*	(0.84)	-
LSD2 (P=0.05)	(0.46)	-

*LSD1 is for comparing means within a cultivar; LSD2 is for comparing treatments in different cultivars; analyses conducted on ln-transformed data in parentheses.

Re-isolation of V. dahliae from infected plants

Vascular staining of stem tissue and re-isolation frequency of *V. dahliae* were significantly higher ($\chi 2$ test, $P<0.05$) for "Roger" but not for "Oliver" in plants inoculated with with both *V. dahliae* and *M. incognita* compared to *V. dahliae* alone (Table 8). This indicated that infection by *M. incognita* increased rate of infection of "Roger" by *V. dahliae*.

Table 10. Frequency of vascular staining in stem tissue and re-isolation frequency of a *B. oleracea* var. *botrytis* strain of *V. dahliae* in Brussels Sprouts cvv. "Oliver" and "Roger".

Cultivar-treatment	**Frequency of vascular staining in stem (%)***	**Isolation of V. dahliae from stem tissue (%)***
"Oliver"		
Uninoculated control	-	-
M .incognita 100,000 eggs	-	-
M .incognita 200,000 eggs	-	-
V. dahliae	70	70
V. dahliae + *M .incognita* 100,000 eggs	30	50
M. hapla 100,000 eggs	-	-
M. hapla 200,000 eggs	-	-
M. javanica 100,000 eggs	-	-
M. javanica 200,000 eggs	-	-
"Roger"		
Uninoculated control	-	-
M .incognita 100,000 eggs	-	-
M .incognita 200,000 eggs	-	-
V. dahliae	10	10
V. dahliae + *M .incognita* 100,000 eggs	80	70
M. hapla 100,000 eggs	-	-
M. hapla 200,000 eggs	-	-
M. javanica 100,000 eggs	-	-
M. javanica 200,000 eggs	-	-

* $\chi 2$ tests indicated a significant increase in frequencies of vascular staining and re-isolation of *V. dahliae* for "Roger" but not for "Oliver" in plants inoculated with with both *V. dahliae* and *M. incognita* compared to *V. dahliae* alone.

Nematodes in field specimens

High and potentially damaging levels of *Meloidogyne*, *Pratylenchus*, *Paratrichodorus* and *Criconemella* spp. nematodes were detected in soil associated with cultivation of Brussels Sprouts and rotation crops including lucerne (Table 10). *Pratylenchus* and *Criconemella* sp. were detected in Brussels Sprouts roots, and because of the high levels of the former nematodes found, specimens were forwarded to a taxonomist for identification to species. This nematode, from Brussels Sprouts in the Adelaide Hills, was identified *as P. neglectus* (Rensch 1924) Filipjev 1936, **a first Australian record for Brussels Sprouts**.

Table 10. Maximum numbers of plant parasitic nematodes detected in soil associated with Brussels Sprouts (and rotation crop) cultivation, and in Brussels Sprouts roots from commercial crops in the Adelaide Hills.

Nematode	**Maximum numbers detected**	
	Per 400 g of soil	**Per g fresh weight of roots**
Meloidogyne	1, 026	-
Pratylenchus	1, 136	14*
Paratrichodorus	256	-
Criconemella	48	3
Paratylenchus	16	-
Tylenchorhynchus	< 10	-

* identified as *P. neglectus*

DISCUSSION

Inoculation with *Meloidogyne* sp. at levels of at least 100, 000 eggs per plant suppressed shoot growth of Brussels Sprouts, suggesting a damage threshold of about 4, 280 juveniles per 400 g of soil. These levels although high can be encountered in the field and damage in warm, sandy soils is therefore considered to pose a possible threat to production, particularly where highly susceptible rotation crops such as lucerne are grown. It is also possible that damage thresholds in the field could be lower than those in the greenhouse because of the more stressful growing conditions in the former. Growers can avoid damage by sampling soil before planting and at or near harvest to monitor nematode levels. Both culivars, "Oliver" and "Roger", were susceptible to all 3 *Meloidogyne* spp. All 3 species reduced shoot growth of "Oliver", but only *M. incognita* and *M. javanica* reduced shoot growth of "Roger" indicating that the latter cultivar is more tolerant to *M. hapla* than the other 2 species. Shoot dry weights were decreased by up to 36% in comparison with uninoculated plants. Early shoot growth is considered to be a reliable indicator of likely effects on production. Where growers intend to plant infested ground and are able to have nematodes identified to species, they could potentially reduce damage in ground with high levels of *M. hapla* by using the cultivar "Roger" in preference to "Oliver". Shoot growth was reduced at the expense of higher root growth, indicating a diversion of resources to compensate for root damage. Shoot height of "Roger", but not "Oliver", was also suppressed by *M. incognita* and *M. javanica* (but effects of *M. hapla* on shoot elongation need further investigation).

Galling of roots in decreasing order of severity by nematode species was *M. incognita, M. javanica* and *M. hapla*, and both final population levels and reproduction rates of these nematodes also followed this order indicating lowest susceptibility to *M. hapla*. Nematode reproduction rates declined from initial inoculum levels of 100, 000 to 200, 000 eggs suggesting that root damage at the higher inoculum level was severe and limited reproduction.

The *V. dahliae* strain from *Ixodia achillaeoides* was apparently avirulent to Brussels Sprouts, while the *B. oleracea* var. *botrytis* strain of *V. dahliae* suppressed shoot growth of "Oliver" but not "Roger", indicating a higher susceptibility in the former cultivar. In fact, evidence was found of stimulation to "Roger" shoot elongation by the *B. oleracea* var. *botrytis* strain of *V. dahliae*. The higher resistance of "Roger" to *V. dahliae* could be useful to growers planting in ground known to be infested with this pathogen. However, infection rate of *V. dahliae* was increased by co-infection with *M. incognita* in "Roger" but not in "Oliver". So the higher resistance of "Roger" to *V. dahliae* may not be useful to growers in ground also infected with *M. incognita*. This interaction between *M. incognita* and *V. dahliae* suggests that damage could be severe where both pathogens are present, particularly with more virulent strains of *V. dahliae* than were used in this study.

High and potentially damaging levels of other nematodes, particulary *P. neglectus*, a first Australian record for this nematode on Brussels Sprouts, were found associated with Brussels Sprouts in the field. Further study to establish the pathogenicity of these nematodes is required, particularly as *Pratylenchus* sp. are known to exacerabate damage from *V. dahliae* in some other crops.

TECHNOLOGY TRANSFER

Detailed preliminary and final reports were sent to the Brussels Sprouts Section of the S.A. Farmers Federation for circulation to growers. This group requested that this research be conducted and is regarded as the main client group. These reports were previously sent to HRDC. A scientific paper detailing research findings is in preparation and will be circulated to relevant industry extension officers.

RECOMMENDATIONS

1) Scientific

Field evaluations of resistance and tolerance of Brussels Sprouts cultivars to Meloidogyne spp. and *V. dahliae* need to be conducted to allow correlation with greenhouse results. Soil treatments such as fumigation should be evaluated to determine actual yield losses in the field. Further study is required using more highly virulent strains of *V. dahliae*. The finding of high population levels of other nematodes including *Praylenchus*, particularly P. neglectus, on Brussels Sprouts in the field suggests the need for further work to establish the pathogenicity of these nematodes. Interactions between *V. dahliae* and *Pratylenchus* in Brussels Sprouts also need to be studied. The differences observed between cultivars in resistance and tolerance indicate that rapid diagnostic tests for *Meloidogyne* spp. and *V. dahliae* may be useful to allow growers to exploit differential levels of resistance.

2) Industry

Industry needs to encourage growers to use diagnostic services to monitor levels of plant parasitic nematodes and to foster R&D for rapid diagnostic tests for nematodes and *V. dahliae* to allow utilization of identified sources of resistance. It is strongly recommended that industry support further research to establish field resistance and tolerance of different cultivars to these pathogens, and to define the pathogenicity of *Pratylenchus* sp. and other nematodes identified in this project.

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