RESEARCH NOTE

Dual Cultures of *Meloidogyne javanica* and Grapevine Rootstocks on Artificial Media

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Grapevine rootstock cultivars were cultured on more than one artificial medium. *Meloidogyne javanica* was inoculated on the roots of different cultivars on seven media and penetration and development were recorded. Plate cultures of roots allowed microscopic examination of nematode development. Nematode reproduction occurred on susceptible rootstocks only, suggesting similar results for field and *in vitro* screening. However, root and nematode development was low and inconsistent, indicating problems which will have to be resolved before general application of the technique.

The use of *in vitro* techniques for culturing nematodes has been reviewed by Mountain (1960) and Zuckerman (1969; 1971). Byars (1914) was the first to culture *Meloidogyne* spp. in an artificial medium and Palys & Meredith (1984) were probably the first to test the reaction of *Vitis* spp. against a plant-parasitic nematode using *in vitro* techniques. Van Mieghem & Goussard (1987) recently published results on the reproduction of *Meloidogyne javanica* on grapevine cultured in artificial media.

Aseptic cultures of host and parasite provide a useful tool for studying host-parasite interactions under controlled conditions. This was shown recently for grapevine and *Pratylenchus vulnus* (Palys & Meredith, 1984) and grapevine and *Plasmopara viticola* (Barlass, Miller & Antcliff, 1986). According to these workers *in vitro* observations agreed with those made under natural conditions. This needs to be verified, however, especially since several artificial media are available which may influence the response of host or parasite. Orion, Wergin & Endo (1979) suggested inter alia that the medium of Murashige & Skoog (1962) (MS-medium) was not suitable for culturing *Meloidogyne incognita* in tomato roots, but Van Mieghem & Goussard (1987) succeeded in propagating *M. javanica* in grapevine roots on this medium. Van Mieghem (1985) also compared the development of *M. javanica* in cucumber roots on seven different media and found definite differences in the suitability of media. This suggests that *in vitro* results should be considered with care.

In vitro propagation of grapevine is mostly done on MS-medium, using either a full or half strength formulation (Palys & Meredith, 1984; Barlass *et al.*, 1986; Van Mieghem & Goussard, 1987). Furthermore, Goussard (1981, 1982) added different growth hormones to this medium to enhance either shoot or root initiation. Both these practices may give rise to varying host or nematode reactions.

The present study was done to compare the growth of different grapevine rootstocks on a number of artificial media and to assess nematode reaction and development on these media.

MATERIALS AND METHODS

Grapevine rootstock cultures: Shoot tips 4-5mm long were removed from the rootstocks Jacquez, 140 Ruggeri, 99 Richter, 143B Mgt and Ramsey grown in pots in a growth chamber at 25°C. The tips were surface - sterilised and prepared as described by Barlass *et al.* (1986) before they were placed in McCartney bottles on half-strength MS-medium to which the cytokinin 6-benzyl-amino-purine had been added at 1 mg/l medium. Shoot growth was initiated in a growth chamber at 25°C and a 16-hour photoperiod.

Once shoots had developed to 25-40mm length, they were removed aseptically, cut into single nodes and placed in petri dishes containing 20ml of medium. The following media, to which the auxin α-naphthaleneacetic acid had been added at 0,1 mg/l to promote root initiation, were included:

1	Gamborg (Gamborg, 1970)	Gb medium
2	Gautheret (McClure & Viglierchio, 1966)	G medium
3	Murashige & Skoog (Murashige & Skoog, 1962)	MS medium
4	Nitsch (Nitsch & Nitsch, 1969)	N medium
5	Skoog, Tsui & White (Koenning & Barker, 1985)	STW medium

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6	White (White, 1954)	W medium
7	Control medium	C medium

The C medium had the following ingredients:

Chemicult hydroponic nutrient salt*	1g
Sucrose	20g
Agar (Bacto)	8g
Distilled water	1 liter

The pH of the media were adjusted to 5,7 and 30 replicate cultures were incubated for each medium in darkness for 10 days at 25°C, whereafter a 16-hour photoperiod was maintained. After 52 days, root growth was assessed by determining the number of roots, root length and number of secondary roots.

Nematode reaction: *Meloidogyne javanica* was reared on tomato seedlings grown in a sterilised sand-peat potting mixture at 25°C in a greenhouse. Egg sacs were removed and surface-sterilised using 0,1% $HgCl_2$ and 5% Hibitane as described by Van Mieghem & Meyer (1986). After rinsing twice in sterile distilled water, one egg sac was placed on each root culture. The reaction of root-knot larvae and their development were studied as described in the following experiments:

Attraction of larvae to different rootstock cultivars: Plate cultures containing both the susceptible (Jacquez) and resistant (Ramsey) cultivars (Loubser & Meyer, 1987) were established on MS medium. Inoculation was done as described above and the number of larvae hatched and attracted to the cultivars was determined after one, two and five days. This experiment was done at 25°C and 33°C and 20 replicates were included. Similar observations were made in a second experiment with single cultures of the five rootstocks on G medium. In both experiments the number of larvae surrounding the roots on each culture was expressed as a percentage of the total number of larvae hatched. Analysis of variance was used to distinguish differences between means.

Development of larvae in different cultivars and media: One-month-old root cultures of 140 Ruggeri on the seven media mentioned in A, as well as 99 Richter and Ramsey on G and C medium, were included. Inoculation with *M. javanica* was done as described above and replicated ten times for each rootstock-medium combination. the hatching

TABLE 1
Comparison of artificial media for *in vitro* rooting of grapevine rootstock cultivars.

Medium	Measurement	Rootstock					Average for media
		Jacquez	140 Ruggeri	99 Richter	143B Mgt	Ramsey	
Gb	R	2,2	2,8	2,5	3,2	3,7	2,9
	RL	6,0	5,8	5,5	6,7	3,3	6,5
	SR	0	1	1	2	2	1,2
G	R	4,4	4,7	6,4	7,8	4,2	5,5
	RL	6,6	12,1	7,6	14,2	15,0	11,1
	SR	1	3	2	3	2	2,2
MS	R	1,5	1,5	3,5	3,0	3,0	2,5
	RL	5,1	9,0	4,0	8,8	5,0	6,4
	SR	0	2	0	1	0	0,6
N	R	2,4	3,4	3,5	3,8	4,0	3,4
	RL	6,2	11,0	6,0	12,6	7,0	8,6
	SR	1	2	1	2	0	1,2
STW	R	4,0	7,5	5,5	5,0	3,0	5,0
	RL	3,8	5,8	4,8	6,8	8,3	7,2
	SR	1	2	2	2	2	1,8
W	R	1,4	4,2	1,7	3,8	4,0	3,0
	RL	2,8	6,0	3,7	8,4	3,3	4,8
	SR	1	1	0	2	0	0,8
C	R	4,0	5,6	6,2	7,2	4,5	5,5
	RL	5,8	10,8	6,7	14,4	14,9	10,5
	SR	1	3	3	3	2	2,4
Average for rootstocks	R	2,9	4,2	4,2	4,8	3,8	
	RL	5,2	8,7	5,5	10,3	8,1	
	SR	0,7	2,0	1,3	2,1	1,1	

Gb: Gamborg; G: Gautheret; MS: Murashige & Skoog; N: Nitsch; STW: Skoog, Tsui & White; W: White; C: Control medium. R: Average number of roots; RL: Average root length (mm); SR: Degree of secondary root formation, with 0 as no and 3 as abundant secondary roots.

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and development of larvae at 25°C were examined regularly for three months.

RESULTS AND DISCUSSION

Grapevine rootstock cultures: The effect of media on the rooting of rootstocks is shown in Table 1. Because of fungal and bacterial contamination and the great variation between replicates, statistically significant differences could not be obtained, but definite tendencies were observed. Root initiation was best overall on media G, N, STW and C. Secondary root formation tended to be better on media G and C. Roots appeared normal on these two media compared to a soft appearance on STW, Gb and N media. The latter also showed excessive callousing. On media STW and W, roots soon became black. The best root cultures were obtained with 140 Ruggeri and 143 B Mgt, whereas Jacquez showed the poorest rooting, in contrast to nursery experience regarding rooting of the latter cultivar.

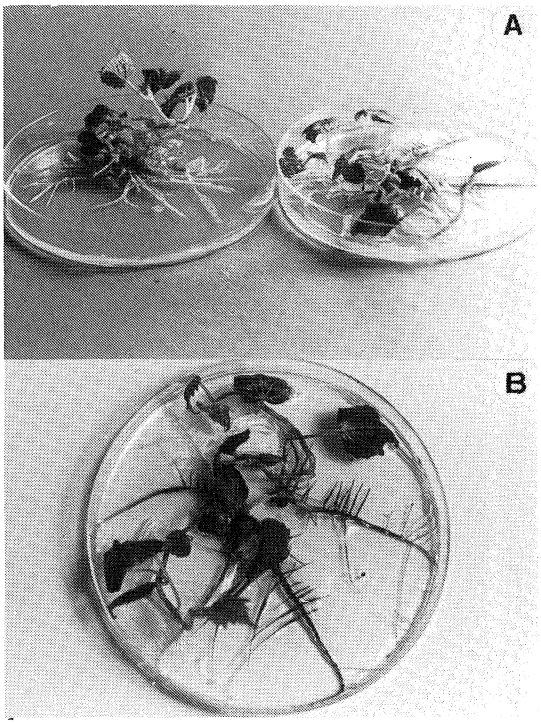

FIGURE 1
Successful rooting of 143B Mgt and 140 Ruggeri on artificial media (A: Gautheret, B: control medium).

Root development and growth of 143 B Mgt and 140 Ruggeri on G medium are shown in Fig. 1A. Comparable rooting was achieved with 143 B Mgt on the control medium (Fig. 1B). Rooting was enhanced if the shoot tip transferred to the rooting medium contained one or more leaves.

Nematode reaction

Attraction of larvae to different rootstock cultivars: The hatching and movement of larvae did not differ for susceptible and resistant cultivars in either experiment (Table 2). This is in agreement with the results of Shepherd & Clarke (1971), who considered *Heterodera* the only genus in which egg hatch is affected by root exudates. The unbiased movement of the larvae to both susceptible and resistant roots is, however, contrary to the findings of Viglierchio (1961) regarding the attraction of nematodes to hosts and non-hosts.

The two media used did not differ regarding the egg hatching of *M. javanica*. Temperature apparently had little effect on hatching or movement; after five days the number of larvae hatched at 33°C was only slightly lower than at 25°C. The presence of roots also seemed not to influence hatching at all; hatching was similar on both root cultures and medium plates without roots (data not shown). The presence of roots, however, influenced nematode movement, and 72-88% of the larvae were found associated with them. The larvae did not seem to prefer a specific root zone and often congregated at certain points along the roots, indicating a possible mutual attracting stimulus. In a subsequent experiment, however, mechanically injured roots did not increase larval attraction.

Development of larvae in different cultivars and media: The rate of infestation and development of *M. javanica* in the roots of different cultivars was low. On most media no development occurred, but best results were recorded on G

TABLE 2
Number of *M. javanica* larvae hatched and gathered around the roots of different grapevine rootstock cultivars on artificial media.

Rootstock	Number of larvae hatched			Larvae around roots (% of total)
	Day 1	Day 2	Day 3	
A. *Dual cultures on MS-medium*				
Ramsey }	1,7	18,7	32,6	41 } 80
Jacquez }				39 }
D value ($p \leq 0,05$)				9
B. *Single cultures on G-medium*				
Jacquez	2,6	22,2	36,5	77
140 Ruggeri	1,7	14,7	23,4	81
99 Richter	5,6	17,7	24,2	75
143 B Mgt	1,2	13,6	17,3	86
Ramsey	1,3	10,5	23,7	77
D value ($p \leq 0,05$)	4,8	15,4	21,3	16

and C media (data not shown). Even on these two media, however, nematode development was slow and inconsistent. A possible explanation is the sterilising process to which egg sacs were subjected. Van Mieghem & Goussard (1987) recorded egg sacs after 25 days on Chenin blanc when using egg sacs from sterile cucumber cultures. On the other hand, the media used, or the growth hormone added, may also have influenced penetration and development. Studies regarding the latter did not, however, indicate that growth hormones impede infestation (Dropkin, Helgeson & Upper, 1969; Huettel & Hammerschlag, 1986). A positive aspect of our study was that infection occurred on the susceptible rootstock 140 Ruggeri only. Galling was observed 10-14 days after inoculation (Fig. 2A), mature females after 20-50 days (Fig. 2B), and egg sacs after 50-60 days (Fig. 2C & D). Females without egg sacs were found to be incompletely developed. Galling did not occur in all cases (Fig. 2C) and no secondary infections occurred, although larvae continued to hatch. On the other hand, root cultures which were 3-4 months old at this stage showed little new root growth and were probably not susceptible to infection. Follow-up experiments showed that larvae from these cultures were able to infest new root cultures.

CONCLUSIONS

Grapevine rootstock cultivars can be cultured on more than one artificial medium, but problems with contamination, variation between replicates, and possible discrepancies between *in vitro* and field performance of a cultivar may prove a big disadvantage of this technique. The MS medium was suitable for initiating shoots from growth tips but root growth was more readily initiated on G medium. It was also possible to use a simple medium for establishing root cultures. The low success rate of the *in vitro* culturing of grapevine rootstocks makes a comparison of media difficult. Media G an C initiated the best overall root growth and it was, therefore, not surprising that most successful infections were recorded on them. None of the resistant or moderately resistant rootstocks became infested with *M. javanica in vitro*. This is a positive aspect of the use of *in vitro* techniques for studying grapevine-nematode interactions.

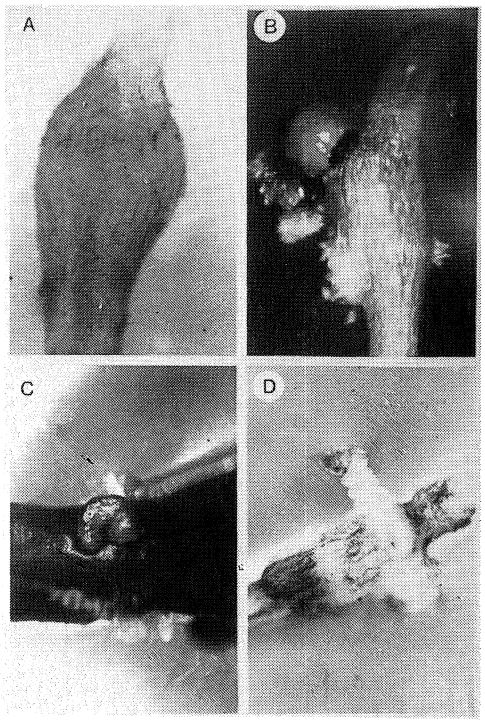

FIGURE 2
Galling of 140 Ruggeri roots and development of *M. javanica* in artificial medium. Galling was observed after 10-14 days (A), mature females after 20-50 days (B) and egg sacs after 50-60 days (C&D).

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