Dual Cultures of Grape (*Vitis* spp.) and the Lesion Nematode (*Pratylenchus vulnus*) for Studies of Nematode Tolerance

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ABSTRACT

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A method for establishing aseptic dual cultures of rooted shoot tips and excised roots of grape (*Vitis* spp.) and the lesion nematode *Pratylenchus vulnus* is reported. Cultures established by this method were maintained for more than 3 mo. Both root lesion formation and nematode reproduction were observed. In preliminary experiments, differential nematode reproduction and lesion formation on nematode-susceptible vs. nematode-tolerant grape genotypes were observed, indicating that differential plant nematode tolerance may be expressed in this system.

Aseptic cultures of plants and nematodes provide a useful system for the study of nematode-plant interactions. Aseptic cultural conditions, by excluding organisms other than the nematodes and the plant tissues, create a controlled environment in which single variables may be manipulated and nematode-plant responses observed directly. Additionally, the inherent small size of in vitro cultures compared with greenhouse- or field-grown plants allows greater numbers of plants to be tested using fewer resources. This is particularly advantageous in a woody perennial such as grape.

Dual cultures of many plant and nematode species have been reported (4,9,13). Various plant tissues, including seedlings, excised roots, and callus tissue, have been used to investigate nematode biology and host-pathogen interactions. In grape, Weischer and Wyss (12) used a dual culture of nonaseptic excised grape roots and *Xiphinema index* to study nematode feeding behavior. Rumpenhorst and Weischer (10) studied histopathological and histochemical changes caused by *X. index* using a similar dual culture system. We report a method for establishing aseptic dual cultures of the lesion nematode *Pratylenchus vulnus* on rooted shoot tips and excised roots of several grape genotypes and some preliminary results on the differential expression of nematode tolerance in vitro.

MATERIALS AND METHODS

Rooted shoot-tip cultures (Fig. 1) were established from shoot tips of 2- to 5-mo-old grapevines (*Vitis* spp.) generated from two-node hardwood cuttings grown in sterilized sandy loam soil in a growth chamber at 28 C with a 16-hr photoperiod. Shoot tips 4–5 cm long were harvested periodically and immersed immediately in distilled water before surface-sterilization. All leaves below the apical region were removed. Shoot tips were surface-sterilized by immersion for 12 min in a 20% (v/v) commercial bleach solution (1.05% NaOCl) containing several drops of liquid detergent as a wetting agent. Shoots were then rinsed in sterile water plus wetting agent followed by two additional rinses in sterile water alone. The basal 10 mm was aseptically excised and discarded. The shoot tips were then inserted in culture tubes (25 × 100 mm) containing 30 ml of a medium consisting of Murashige and Skoog's (6) major and minor salts, B5 vitamins (3), 30 g/L of sucrose, and 8 g/L of agar. Excised root cultures (Fig. 2) were established by aseptically excising roots about 1 cm long from 1- to 2-mo-old shoot-tip cultures and placing them in petri plates (25 × 100 mm) containing 30 ml of the same medium.

P. vulnus used for establishing the dual cultures were reared on carrot pieces (5) and collected from the carrots via mist-extraction (1). The nematodes were suspended in 6 ml of sterile water in culture tubes (13 × 100 mm) and concentrated by centrifugation in a clinical centrifuge at 2,750 *g* for 2 min. The concentrated volume of nematodes was 0.5 ml or less. For surface-sterilization, they were then aseptically pipetted from the bottom of the culture tube to 6 ml of a sterile 133-ppm solution of MEMC (Aretan) and centrifuged again as before. The treatment was repeated five times, using a fresh MEMC solution each time. Nematodes were then incubated in 6 ml of 133 ppm of MEMC for 12 hr followed by an 8-hr incubation in 6 ml of 4,000 ppm of streptomycin sulfate. Five rinse treatments followed, in each of which the nematodes were aseptically pipetted into 6 ml of fresh sterile water in culture tubes (13 × 100 mm) and centrifuged as before.

Nematodes were resuspended in about 3 ml of sterile water and stirred constantly with a magnetic stirring bar. Samples of 0.01–0.02 ml containing 200–300 nematodes were removed with a sterile micropipette and inoculated onto the agar surface of aseptic rooted shoot-tip cultures or onto the root surface of

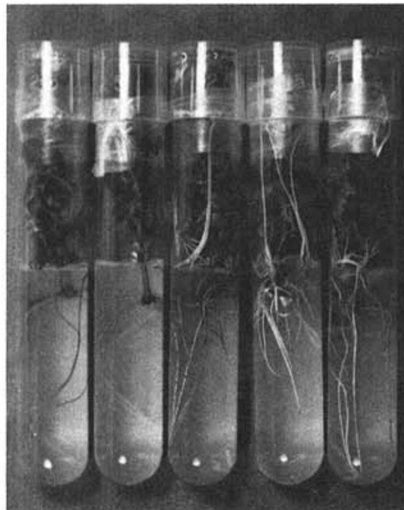

Fig. 1. One-month-old rooted shoot-tip cultures from 2- to 5-mo-old grapevines.

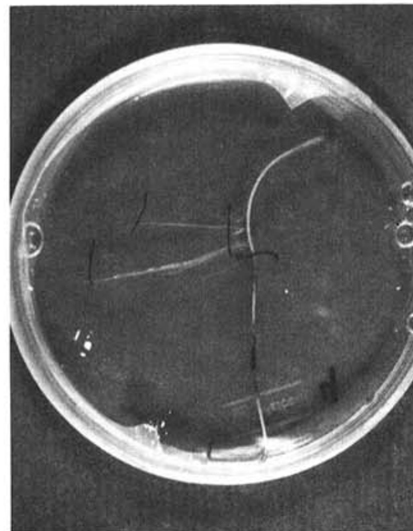

Fig. 2. Excised root culture of Thompson Seedless with lesion caused by *Pratylenchus vulnus*.

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aseptic excised root cultures of the nematode-tolerant cultivars Salt Creek and Dogridge and the nematode-susceptible cultivars Thompson Seedless, Cabernet Sauvignon, and A × R (Ganzin 1). Qualitative observations of nematode behavior and plant responses were made.

In a separate quantitative experiment, similarly treated nematodes were pipetted onto 10 excised root cultures each of the same grape cultivars. After 21 days, linear growth in each cultivar was compared with that of 10 uninoculated controls to which 0.01 ml of nematode suspension supernatant had been added.

RESULTS

In the rooted shoot-tip cultures, nematodes generally migrated through the agar to the root tips within 24–48 hr of inoculation (Fig. 3). In the Salt Creek and Thompson Seedless cultures, many nematodes were observed probing the root tips. This was also observed in the Cabernet Sauvignon and Dogridge cultures, but to a lesser extent. Nematode survival varied among the cultures from several days to several weeks, independent of the cultivar. In one exceptional Thompson Seedless culture, nematodes remained active for more than 3 mo. Root lesions appeared after 1 mo and were associated with areas of nematode accumulation. Many eggs and juveniles were observed 2 mo after inoculation. At about 3 mo, a large increase in the nematode population was noted. Numbers were not recorded. Shortly thereafter, all nematode activity ceased.

In the excised root cultures, motile nematodes were observed along the roots in all cultivars after 1 mo. The greatest numbers were present in the Thompson Seedless cultures where a considerable population increase had occurred; many eggs and juveniles were evident. Numerous root lesions were observed in areas associated with nematode probing (Fig. 2). Eggs, motile adults, and juveniles were also present on Cabernet Sauvignon and A × R roots, with some lesion formation observed. No eggs or juveniles were observed in the Salt Creek cultures.

In the quantitative root growth experiment, there were no significant differences in linear root growth after 21 days between the nematode treatments and the controls in the Salt Creek, Dogridge, or Cabernet Sauvignon root cultures (Table 1). In the Thompson Seedless cultures, however, linear root growth was reduced ($P < 0.2$) in the nematode treatment. Small lesions were observed in association with probing nematodes on the Thompson Seedless and Cabernet Sauvignon cultures. No data were obtained from the A × R nematode treatment because of contamination.

DISCUSSION

The excised root culture environment was more favorable for nematode survival than that of the rooted shoot cultures. The reason for this is unclear, but the higher oxygen availability resulting from the greater surface-to-volume ratio of the medium in the petri dishes used for the excised root cultures may have been partly responsible.

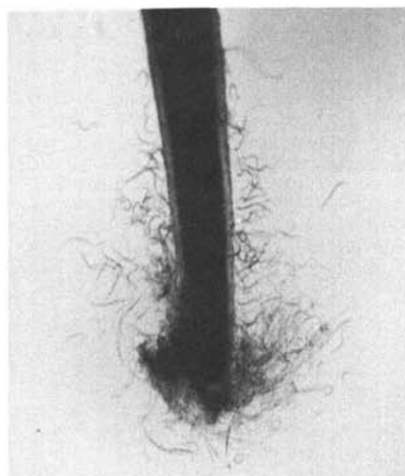

Fig. 3. *Pratylenchus vulnus* on a Cabernet Sauvignon root tip in a rooted shoot-tip culture.

In these experiments, nematode-plant interactions were characterized by nematode reproduction, root growth retardation, and root lesion formation. Comparison of these observations with available whole plant data indicates a correlation between in vitro and whole-plant tolerance/susceptibility expression. The in vitro data are supported by the results of Chitambar (2), who reported that greenhouse-grown A × R supported a *P. vulnus* population increase, whereas Salt Creek and Dogridge did not. He also reported that root weights of nematode-treated Salt Creek and Dogridge were not significantly reduced relative to untreated controls. Also consistent with our results are the findings of Pinochet et al (8), in which *P. vulnus* showed a population increase and suppressed root growth in greenhouse-grown Thompson Seedless.

Lesion formation, which is characteristic of the root damage caused by *P. vulnus* feeding activity on grape (7), was greater in the susceptible varieties Thompson Seedless, A × R, and Cabernet Sauvignon. Although this may be caused, at least in part, by increased nematode feeding, such results could also be explained by differential root sensitivities to nematode attack. At this time, no whole-plant data on differential lesion formation in tolerant and susceptible grape cultivars are available for comparison.

Whole-plant nematode tolerance is a complex phenomenon, and its mechanisms are still not completely understood (11). The dual cultures provide a unique system for studying the nature of tolerance mechanisms. For example, use of plant material representing a gradient of tissue development and organization, such as callus tissue, excised roots, and rooted shoots, would permit evaluation of host-pathogen interactions at various levels of tissue complexity and might elucidate the organizational levels at which tolerance mechanisms operate. The capacity for complete control of the culture environment also renders the dual culture system ideal for manipulating cultural variables (eg, hormone levels, nutrients, light, and temperature) and observing their effects on the expression of tolerance.

Development of an in vitro screening system for tolerance depends on the identification of measurable in vitro parameters that address fundamental aspects of nematode tolerance such that they reflect tolerance characteristics in the complex field environment. In the cultivars we tested for which whole-plant tolerance and susceptibility data are available, nematode reproduction and root growth retardation in the in vitro dual cultures paralleled the occurrence of these phenomena in the whole plants. These parameters, as well as lesion formation and root attractiveness to nematodes (currently under investigation in our laboratory), may provide the basis for an effective in vitro screen for whole-plant nematode tolerance.

Table 1. Effect of *Pratylenchus vulnus* on mean linear growth of excised root cultures of several grape cultivars[a]

	Mean linear root growth[b] (mm)	
Cultivar	**Inoculated with *P. vulnus***	**Control**
Salt Creek	23.0 ± 8.4	38.1 ± 14.3
Dogridge	22.6 ± 2.3	19.3 ± 5.4
Cabernet Sauvignon	31.4 ± 6.9	38.3 ± 8.8
Thompson Seedless	16.0 ± 3.6	42.0 ± 13.5

[a] Differences between treatment and controls were significant only for Thompson Seedless ($0.1 < P < 0.2$).

[b] Each figure represents the mean of 10 replicates ± standard deviation.

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