Distribution of a Parasite of Root-Knot Nematodes in South Australian Vineyards

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ABSTRACT

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Bacillus penetrans was widespread in medium-aged and old vineyards but was difficult to detect in vineyards that were less than 10 yr old. In some old vineyards, enough *Meloidogyne* females were parasitized to implicate the parasite in the natural biological control of the nematode.

Additional key words: *Vitis vinifera*

Bacillus penetrans (Thorne) Mankau is an obligate parasite of plant-parasitic nematodes with potential as a biological control agent against root-knot nematodes (1,2). The parasite has been recorded in many countries (4), and we have often observed it in routine samples from vineyards in the Riverland region of South Australia. Because little is known of the distribution and importance of *B. penetrans* in the field, this project aimed to determine how frequently the parasite occurred in vineyards, establish whether its distribution was related to vineyard age, and examine the role of *B. penetrans* in the natural biological control of root-knot nematodes on grape (*Vitis vinifera*).

MATERIALS AND METHODS

Young (5–10 yr), medium-aged (20–40 yr), and old (50–70 yr) vineyards were selected in the Riverland area of South Australia. All vineyards were on light-textured soils not previously used for agriculture. Soil cores were taken with a 2-cm-diameter Oakfield tube from depths of 15–45 cm in 20 vineyards of each age group. Roots were also collected from several vines.

Three methods were used to assess root-knot nematode populations in each vineyard. i) *Meloidogyne* juveniles were counted in two 100-g soil samples that had been processed by decanting and sieving, the sievings being placed on a Baermann funnel. ii) The *Meloidogyne* population was bioassayed by planting tomato seedlings (variety Q3) in two 10-cm pots, maintaining them in a glasshouse at 27 C for 5 wk, and then indexing the roots for root-knot nematode as follows: 0, no galls; 1, 1–24% of the roots galled; 2, 25–49%; 3, 50–74%; 4, 75–99%; 5, all the roots galled. iii) The number of *Meloidogyne* females was counted in a 5-g sample of grape roots.

The incidence of *B. penetrans* was assessed using the following methods: i) *Meloidogyne* juveniles, recovered from soil using the method described above, were examined for the presence of *B. penetrans* spores. ii) Approximately 2,000 *M. javanica* juveniles in 20 ml of water were added to two 25-g soil samples. The nematodes were obtained from cultures that were maintained in the glasshouse and screened frequently to ensure that they were free of *B. penetrans*. After Erlenmeyer flasks containing each soil-water suspension were shaken for 24 hr at 100 rpm on a rotary laboratory shaker, nematodes were extracted by decanting and sieving followed by centrifugation in sugar solution (484 g/L). The number of *Meloidogyne* juveniles with and without *B. penetrans* spores attached was counted in samples of 60–100 nematodes. iii) An estimate of the proportion of healthy and infected *Meloidogyne* females in grape roots was obtained by squashing females without egg masses on a microscope slide and checking for *B. penetrans*.

RESULTS

Root-knot nematodes were present in almost every vineyard, but some methods of estimating populations suggested that there were more nematodes in young vineyards than in old and medium-aged vineyards (Table 1). *B. penetrans* occurred most frequently in old and medium-aged vineyards (Table 2), sometimes being present in relatively high concentrations. For example, in seven vineyards, 100% of the *Meloidogyne* juveniles that had been agitated in a soil-water suspension for 24 hr were infested with spores; in three vineyards, 33–40% of the *Meloidogyne* females present in roots were parasitized. *B. penetrans* was rare in young vineyards and could only be detected when *Meloidogyne* juveniles were agitated in soil-water suspensions (Table 2).

DISCUSSION

Although root-knot nematodes are common in vineyards in South Australia (3), the widespread incidence of *B. penetrans* was previously unrecognized. The nematode was probably introduced into vineyards on planting material or farm implements because it has not been found in virgin soil in South Australia (Stirling, *unpublished data*). *B. penetrans* may have occurred in virgin soil on nematodes other than *Meloidogyne*, but it was probably introduced with the nematode.

The survey results suggest that *B. penetrans* took many years to reach concentrations that could be detected readily. The parasite was rare in vineyards less than 10 yr old, and vineyards with relatively high concentrations of *B. penetrans* were all more than 25 yr old. This slow increase was not unexpected; *B. penetrans* spores are not mobile, and infection depends on the chance contact between *Meloidogyne* juveniles and spores. The probability of spores adhering to a nematode initially would have been low and would have increased only slowly as successive generations of infected females ruptured and released spores into soil.

Nematode populations tended to be lower in old vineyards (where *B. penetrans* usually occurred) than in young vineyards (where *B. penetrans* was absent). Although differences in the nematode population may have been caused by differences in factors such as root distribution or root density, *B. penetrans* may also have reduced nematode populations in old vineyards. The parasite certainly prevented the reproduction of substantial numbers of *Meloidogyne* females in some old and

Table 1. *Meloidogyne* populations in vineyards of various ages[y]

Method	Age of vineyard		
	Young	Medium	Old
Meloidogyne juveniles per 100 g of soil	444 a[z]	99 b	75 b
Root-knot index	2.4 a	1.9 a	2.3 a
Meloidogyne females per gram of root	16 a	7 a	9 a

[y] Data are means from 20 vineyards.
[z] In each horizontal row, means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

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Table 2. Incidence of *Bacillus penetrans* in young, medium-aged, and old vineyards

Method	Incidence[y]			% Infested[z]		
	Young	Medium	Old	Young	Medium	Old
Juveniles from field soil (direct assessment)	0	5	6	0 a	13 a	7 a
Juveniles from field soil (after shaking for 24 hr)	8	14	17	3 a	28 b	29 b
Females in roots	0	5	7	0 a	6 a	3 a

[y] Number of vineyards in 20 with *B. penetrans*.
[z] Mean percentage of infested nematodes in 20 vineyards. In each horizontal row, means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

medium-aged vineyards. The level of parasitism in vineyards was similar to that observed in some sugarcane fields in Mauritius (4).

Further studies are required to ascertain the role of *B. penetrans* in the field. If such studies confirm the potential of *B. penetrans* as a biological control agent, we may be able to augment its natural increase by introducing the parasite into young vineyards and so reduce the time taken for it to reach a concentration sufficient to control root-knot nematodes.

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