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Studies on spatio-temporal distribution of *Pasteuria penetrans* (ex Thorne, 1940) Sayre and Starr, 1985, the bacterial parasite of root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood in grape vineyards

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ABSTRACT: **Studies on the spatial distribution and seasonal fluctuation of the bacterial parasite, *Pasteuria penetrans* infecting root-knot nematode, *Meloidogyne javanica*, were conducted in naturally infested grape vineyards. Within a vineyard, a negative correlation was evident between incidence of *P. penetrans* and population of *M. javanica*. The incidence of *P. penetrans* was maximum during the month of October. However, Nematode populations were more influenced by the ambient temperature. The vertical distribution of *P. penetrans* was similar in 0-45cm-soil profile, while it was significantly less at 45-60cm depths.**

KEY WORDS: *Pasteuria penetrans*, *Meloidogyne javanica*, grapes, spatio-temporal distribution

INTRODUCTION

The mycelial and endospore-forming bacterium, *Pasteuria penetrans* (ex Thorne, 1940) Sayre and Starr, 1985 is a promising parasite of root-knot nematodes, *Meloidogyne* spp. (Chen and Dickson, 1998; Walia *et al.*, 2000). In earlier study, a high incidence of *P. penetrans* was recorded in *M. javanica*-infested grape vineyards in Hisar district, Haryana. Apparently, *P. penetrans* played an important role in the natural regulation of *M. javanica* populations on grapes. To understand this phenomenon in more detail, studies were undertaken on the spatio-temporal distribution of *P. penetrans vis-à-vis* the host nematode population dynamics in a vineyard. The results obtained on these ecological parameters are reported in this paper.

MATERIALS AND METHODS

Two grape vineyards (var. Perlette) in village Dobhi (District Hisar, Haryana), previously infested with *M. javanica* and *P. penetrans*, were selected for this study.

Selection of sampling sites

Soil samples were collected during December, 2000 from three different spots (0-30cm depth) from each of the two vineyards, and 200cc soil was processed by wet screening and sugar centrifugal floatation technique (SCFT) for the recovery of

M. javanica J_2 (Schindler, 1961). After estimating the total second-stage juvenile (J_2) population, 10 juveniles were picked randomly and observed at 400x under a compound microscope for *P. penetrans* spore adherance to the nematodes.

Seasonal fluctuation

From each vineyard, two spots were selected–one having maximum incidence of *P. penetrans* and/or least J_2 population; and the second exhibiting nil incidence of *P. penetrans* and/or maximum population of nematode. Samples were drawn every month for one year from these fixed spots to record observations on nematode populations, per cent J_2 encumbered with *P. penetrans* spores and number of spores per J_2.

Distribution at different depths

One spot from a vineyard was further selected to study the prevalence of *P. penetrans* spores at different depths. Samples were drawn from 0-15, 15-30, 30-45 and 45-60cm depths and at a horizontal distance of 60cm from the main stem during June, 2002. Soil mechanical analysis was performed separately for all the four depths; and it was found to be sandy loam with pH between 8.0-8.4. Samples were processed by SCFT for nematode extraction and observations were recorded similarly. The remaining soil was air-dried for 40 days and filled in 5cm Petri-plates @ 20g per plate after thorough mixing to conduct bioassay. Freshly hatched healthy *M. javanica* J_2 were poured @ 1000 per plate in a 10ml water suspension. The plates were kept at 28°C for 48 hours after which J_2 were extracted by SCFT. Observations were recorded on *P. penetrans* spore encumbrance on 10 J_2 picked randomly.

RESULTS AND DISCUSSION

Selection of sampling sites

In one vineyard (Dobhi I), out of the three spots sampled, the one with highest nematode population (2160) and no bacterium, and the second with least nematode population (560) and maximum incidence of bacterium (20%) were selected for further investigations. A significant negative correlation between incidence of *P. penetrans* and populations of *M. javanica* was discernible (r =-0.974). Similar trend was evident at the second vineyard (Dobhi II) also (Table 1).

Table 1. Incidence of *P. penetrans* infecting *M. javanica* at different spots in two grape vineyards of village Dobhi

Site/Spot	# J_2/200 cc soil	% J_2 encumbered with endospores	# Endospores/J_2
Dobhi I			
1*	2160	0.0	-
2	1680	10.0	1-2
3*	560	20.0	2-8
Coefficient of correlation (r) between J_2/200 cc soil and % J_2 with endospores = -0.974			
Dobhi II			
1	5500	0.0	-
2	3780	10.0	1-2
3	900	30.0	1-2
Coefficient of correlation (r) between J_2/200 cc soil and % J_2 with endospores = -0.999			

* Spots selected for further study

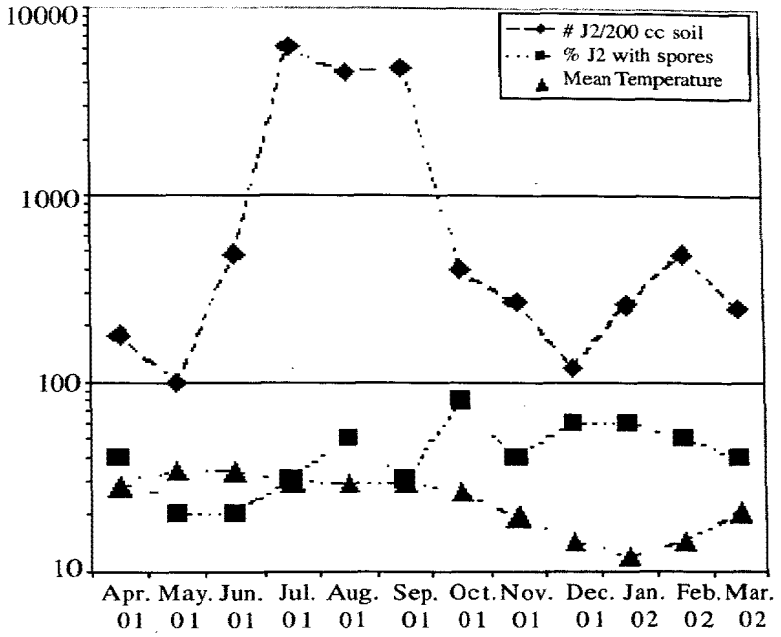

Fig.1. Seasonal fluctuation of *M. javanica* and *P. penetrans* in a grape vineyard

Seasonal fluctuation

Studies on the seasonal fluctuation of J_2 population of *M. javanica* in soil and incidence of *P. penetrans* were commenced in April, 2001 in one vineyard (Dobhi I). The two spots selected for this study had revealed up to 90 per cent incidence of *P. penetrans* at one spot in a pilot survey (October, 2000), and nil incidence at the other. However, during the course of study, the second site (without *P. penetrans*) also revealed the presence of the bacterium, hence it was abandoned henceforth.

The J_2 soil population was markedly influenced by ambient temperature conditions. It remained low (100-480 per 200 cc soil) during April to June (mean 32.2° C), then exploded to 4560-6180 during July to September (29.2° C), and again lowered to 120-480 between October to March (17.87° C). Maximum incidence (80%) of *P. penetrans* was recorded during October, and remained relatively high (40-60%) during November to March (Fig. 1).

The heavy built up of *M. javanica* population during July to September with 30-50 per cent J_2 spore encumbrance might have resulted in considerable female infection, resulting in higher incidence of *P. penetrans* in the following months.

Distribution of *P. penetrans* at different depths in a vineyard

Total number of J_2 recovered was significantly higher (313.5 per 200cc soil) at 30-45cm depths, followed by 45-60cm (246). The number of J_2 in the upper two levels i.e., 0-15 and 15-30cm was statistically on par. Conversely, the incidence of *P. penetrans* was maximum (12.5%) at 0-15cm, while it was nil at 15-30cm. The average numbers of endospores per J_2 at this site varied from 0 (at 15-30cm) to 0.13 (at 0-15cm) and were statistically non-significant (Table 2).

A bioassay of the samples collected from different depths of both the sites was also conducted and the results are presented in Table 2. Maximum incidence (23.3%) of *P. penetrans* was recorded at 15-30cm depths and it was on par with 0-15 and 30-45cm depths. The incidence was significantly less (3.3%) at 45-60cm. Similar trend was observed with regard to average number of endospores per J_2.

Table 2. Prevalence of *P. penetrans* at different soil depths in a grape vineyard infested with *M. javanica*

Soil depth (cm)	Total # J_2/ 200cc soil	% J_2 encumbered with Pp		# endospores per J_2	
		No bioassay	Bioassay	No bioassay	Bioassay
00-15	137.0	12.5 (21.70)*	16.8 (24.55)*	0.13 (1.13)**	0.20 (1.20)**
15-30	173.5	00.0 (01.00)	23.3 (29.82)	0.00 (1.00)	0.30 (1.30)
30-45	313.5	07.5 (16.89)	16.8 (24.55)	0.10 (1.10)	0.20 (1.20)
45-60	246.0	07.5 (16.89)	03.3 (08.23)	0.10 (1.10)	0.03 (1.03)
CD (P = 0.05)	59.8	(10.50)	(10.60)	(NS)	(0.146)

Data in parentheses of columns marked * Angular transformed values; ** n+1 values;
Pp = *Pasteuria penetrans*

Without bioassay the incidence of *P. penetrans* was found to be nil at 15-30cm depths, while bioassay revealed maximum incidence and average number of endospores at the same depth. It will be worthwhile to point out that nil incidence of *P. penetrans* at 15-30cm may be because of 'escape'. This also indicates the merit of results obtained through bioassay. It is inferred that the incidence of *P. penetrans* endospores was statistically on par from 0-45cm depths and it was significantly less at 45-60 cm.

Kamra and Dhawan (1998) made certain observations on the vertical movement of endospores of *P. penetrans* (infecting *Heterodera cajani*) using different soil types in a laboratory study using polythene pipes. Their studies revealed that the movement and distribution of endospores increased with greater pore size and decreased with increasing silt and clay content of the soil. Mateille *et al.* (1996) have also reported considerably reduced percolation of *P. penetrans* endospores in clay soil under a water drip supply.

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