MASS PRODUCTION OF *PASTEURIA PENETRANS* TO CONTROL ROOT-KNOT NEMATODES

R. D. Sharma, Embrapa Cerrados, CP 08223, CEP 73310-970, Planaltina, DF,
sharma@cpac.embrapa.br

INTRODUCTION

In the absence of suitable methods for the *in vitro* culture of *Pasteuria penetrans* Thorne (Sayre & Starr), the mass production system developed by Stirling & Wachtel (1980) is widely used to produce inoculum for experimental purposes. Sharma & Stirling (1991) made a modest attempt to improve the existing mass production system.

The present study provides additional information on the optimization of spores production *in vivo* culture systems using higher temperature range, increased plant density per recipient, much older plants, higher inoculum levels and multiple nematode inoculations.

MATERIALS & METHODS

Experiment 1. Effect of temperature on the production of *Pasteuria penetrans* spores in pots

Two weeks old tomato seedlings cv. Floradade were transplated into 800g pasteurised sand in 15cm-diameter pots. Twenty days later, *M. javanica* juveniles were added to a sonicated suspension of *P. penetrans* in water at 30 ^{0}C. When most nematodes had 10-12 spores/juvenile attached; 10,000 juveniles were inoculated into each pot. Plants were grown at two different temperatures (20-25 ^{0}C and 26-28 ^{0}C) and roots were harvested after 82 days of inoculation.

Experiment 2. Effect of different *Meloidogyne javanica* inoculum levels on the production of *Pasteuria penetrans* spores in trays

Three levels (25,000; 50,000 and 100,000 J2) of *M. javanica* inoculum with 10-12 spores/J2 attached were inoculated in 1/3 rd of the trays (28x11.33x4.5cm) grown with hundred 12- day-old seedlings of tomato cv. Floradade at 28 ^{0}C and roots were harvested after 52 days. In these trays, fine stones were used up to 2-cm depth and above the stone layer a 2cm layer of pasteurised and fertilized sand was used where tomato seedling were grown.

Experiment 3. Effect of plant densities on the multiplication of *Pasteuria penetrans* spores in pots

Two plant densities (1 and 4 plant/pot) with nematode inoculum density/plant being 20,000 J2 with 10-15 spores/J2 adhered were inoculated to one month old tomato cv. Tiny Tim (dwarf) seedlings at 28 ^{0}C. Roots were harvested after 54 days.

Experiment 4. Effect of multiple *Meloidogyne javanica* inoculations on the production of *Pasteuria penetrans* spores in trays

Effect of multiple (1. - Pi 150,000 J2 at 0-day; 2. - Pi 100,000 J2 at 0-day + 50,000 at 7-day and 3 - Pi 50,000 J2 at 0, 7 and 13-day interval) nematode inoculation with *M. javanica* juveniles encumbered with 10-15 spores/J2 inoculated in trays grown with 15-day old tomato seedling (50 seedlings/tray) at 30 ^{0}C during 54 days.

RESULTS & DISCUSSION

Experiment 1

The number of spores per g root and per pot at 26-28^{0}C was 2.5 times more in relation 20-25 ^{0}C and 12.67 and 13.07 times more in relation to Stirling and Wachtel (1980) method (Table 1).

Experiment 2

With increasing nematode inoculum level and high plant density per unit area in a tray system at 28 ^{0}C resulted in very high spore production than in pot systems used in conventional method developed by Stirling and Wachtel (1985). The number of spores per female and per g of root decreased with increasing nematode inoculum levels but the total spore production per recipient increased linearly (Table 2). In this system the incubation period was 30 days shorter in relation to experiment 1.

Experiment 3

The number of spores per female/plant/pot was 1.39 times higher than in four plants per pot whereas the number of spores/g root and per pot was 1.28 and 2.21 times more, respectively in pot with four plants. The number of spores per plant/pot produced in this experiment were lower in relation to experiment 1 which may be due to lower varietal susceptibility and shorter incubation period (Table 3).

Experiment 4

In experiment 4, the number of spores per female increased linearly with increasing nematode inoculation interval. The number of spores/g root and total spores produced per recipient also increased in a similar way as mentioned above except in treatment B where the initial nematode inoculation was 100,000 J2 at o-day and the remaining 50,000 was made on 7th day. This may be due to the reduced final plant stand per recipient.

The main factors influencing the number of spores produced were nematode inoculum density, host plant susceptibility, the optimum temperature and time of harvest. The results of these experiments with different nematode inoculum densities suggests that for each method, root systems had a maximum carrying capacity and that increasing the number of juveniles inoculated above the levels used in these studies did not increase the number of infected females and hence the spore concentration in roots.

The use of tray method proved to be more efficient and economical for spore production. In this method the quantity of substrate and physical space used is extremely low in comparison to conventional pots used for normal spore production.

CONCLUSION

Table 1. Effect of temperature on the production of *Pasteuria penetrans* spores in *Meloidogyne javanica*-infested roots of tomato cv. "Floradade" grown in pots (800 g sand) after 82 days. (Pi.-10, 000 J2/pot; Plant age - 35 days).

Temperature (^{0}C)	Dry weight roots/plant (g)	Spores/female	Spores/g root (x 10^8)	Spores /pot (x 10^8)
26-28	1.65	607,931	12.67	20.91
20-25	1.60	896,650	5.13	8.21
Stirling & Wachtel (1980)	1.60	-	1	1.60

Pi - 5,000 J2 with 5-20 spores attached; Plant age-14-21- day old; Mean of 5 replications.

Table 2. Effect of different *Meloidogyne javanica* inoculum levels on the production of *Pasteuria penetrans* spores in tomato roots in trays at 28 ^{0}C after 52 days of nematode inoculations. (Plant age - 12 days; Initial plant density-100 per 1/3 portion of the tray; J2 with 10-12 spores attached). Mean of 3 replications.

Nematode inoculum level (Pi)	Final plant stand per tray (1/3)	Total dry wt of roots/ portion (g)	Dry wt of roots/plant (g)	No. of spores per female (x 10^3)	No. of spores/g dry roots (x 10^8)	No. of spores per portion (x 10^8)
25, 000	29	2.80	0.096	714	0.276	2.318
50, 000	20	3.30	0.165	706	2.070	20.493
100, 000	26	4.53	0.174	296	4.040	54.904

* A – 150,000 J2 at 0-day; B – 100,000 at 0-day + 50,000 at 7-day; C – 50,000 at 0-day + 50,000 at 7-day + 50,000 at 13 day.

Table 3. Effect of plant densities on the multiplication of *Pasteuria penetrans* spores in pots at 28 ^{0}C after 54 days of inoculations.

Plant density per Pot	Inoculum level (J2)	Dry wt roots/plant (g)	No. of spores per female (x10^3)	No. of spores/g roots (x 10^8)	Number of spores/pot (x 10^8)
1	20,000	4.38	1.239.2	0.89	3.898
4	80,000	7.56	893.6	1.14	8.618

Pi - 20.000 J2/plant; Mean of 5 replications.

Table 4. Effect of multiple *Meloidogyne javanica* inoculations on the production of *Pasteuria penetrans* spores in trays after 54 days at 30 ^{0}C. Tomato cv. "Floradade" 15-day- old; 10-15 spores/J2 and 50 plants/recipient; Mean of 3 replications.

Time and inoculum level	Final plant stand per recipient	Total dry wt of roots/ recipient	Dry wt of roots/plant (g)	No. of spores per female (x 10^3)	No. of spores/g dry roots (x 10^8)	No. of spores per recipient (x 10^8)
A – 150,000	36	5.00	0.286	298	1.281	19.215
B – 100,000 + 50,000	24	7.35	0.653	350	0.710	15.655
C – 50,000 + 50,000 + 50,000	28	7.04	0.502	813	1.700	35.904

* A - 150,000 J2 at 0-day; B - 100,000 at 0-day + 50,000 at 7-day; C - 50,000 at 0-day + 50,000 at 7-day + 50,000 at 13 day.

REFERENCES

- Sharma, R.D. & G.R. Stirling . 1991. *In vivo* mass production systems for *Pasteuria penetrans*. Nematologica, 37:483-484.
- G.R. Stirling & M.F. Wachtel. 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 26: 308-312.

Ministério da Agricultura, Pecuária e Abastecimento

Arte: Leila Sandra
SIN - Embrapa Cerrados