CONTROL OF ROOT-KNOT NEMATODES AT THE EPCOT CENTER USING *IN VIVO* PRODUCED *PASTEURIA PENETRANS*.

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The efficacy of *Pasteuria penetrans*, an obligate bacterial parasite of root-knot nematodes, has been demonstrated in greenhouse and microplot experiments. A greenhouse complex used as an agricultural display at the Land Pavilion in EPCOT Center at Orlando, Florida has successfully used *P. penetrans* for the past six years to control root-knot nematodes. An update on the background, progress and techniques at the Land provides insight into the use of *Pasteuria penetrans* for nematode control.

The greenhouse complex at the Land became infested with *Meloidogyne incognita* three years after its initial opening. The nematode infection spread through the greenhouse on a variety of vegetable crops plants including spinach, several varieties of beans and squash, sunflower, lettuce, and kale that caused above ground disease symptoms that were not acceptable. Tourists, approximately 14,000 per day, travel through this complex by boat or on walking tours and the EPA does not allow the use of nematicides in this facility. Portable steam machines were initially used to control the nematode problem but the reoccurrence of root-knot into steamed-treated areas was rapid and population densities returned to damaging levels quickly. Another more costly attempt to contain the nematode problem involved removing all the soil from the greenhouse and replacing it with steam-sterilized soil and a new plastic barrier. Within nine months another nematode population, *M. arenaria* race 2 was found infecting a site of scarlet bean and citrus.

A cooperative project was started with Dr. Don Dickson at the University of Florida Department of Entomology and Nematology in 1996 to use the biological control organism *Pasteuria penetrans.* Nematode juveniles (J2) were collected from the infected area to identify the specific *P. penetrans* isolate that would attach and infect *M. arenaria* race 2. Endospore production was accomplished by attaching *P. penetrans* endospores to *M. arenaria* (J2) and inoculating them onto tomato plants grown in clay pots in a greenhouse. After 45 days the root systems containing spore-filled females were harvested, dried, ground to a fine powder and stored in plastic containers until needed.

Scarlet bean in the experimental site was harvested and replanted every 90 days. At harvest all roots were examined for root-knot galls and all infected areas were located. Soil in the infected areas was removed by pushing a 10-inch diameter PVC pipe to a depth of six inches. The soil inside the pipe was removed and replaced with steam-sterilized soil mixed with endospore-laden root powder (approximately 100,000 endospores/g of soil). The tube was removed from the soil and Scarlet bean seedlings

were placed in the center of each of the treated areas. Every 90 days Scarlet bean plants were removed and replanted. At this time the incidence and number of galls were recorded. Females from infected root samples were collected and assayed for the percentage infected with *P. penetrans.* Soil samples from the row were collected and J2 were extracted from the soil. The incidence and numbers of J2/100cc of soil attached with endospores and average number of endospores/J2 was determined. The results of this experiment after five 90-day cycles (Figure 1) demonstrated that *P. penetrans* reduced root-knot populations lowering the amount of galling and number of plants infected.

Researchers at the Land began using *P. penetrans* for root-knot control and continually monitor root systems for root-knot galls when plants displays are pulled and changed. When root-knot infections are detected a mixture of two isolates of *P. penetrans* is applied as a seedling treatment or as row application. The seedling treatment is similar to the one described above. Seedlings are planted into holes with approximately 500cc of clean sandy soil mixed with endospores in root-powder at the rate of 100,000 endospores per cc of soil. The in-row treatments are accomplished by spreading the root-powder down the length of the drip irrigation line.

Nematode and *P. penetrans* populations were surveyed in the Land greenhouse on December 20, 2001. Nematodes were extracted from soil samples and number of J2/100cc of soil and the number of J2 infected with *P. penetrans* were recorded. Soil samples were also analyzed for the presence of *P. penetrans* using a PCR technique (Table 1). Root-knot populations were detected at 4 sites in relatively low numbers. The percentage of J2 attached with endospores was high in the sunflower display. J2 detected in a bean and pumpkin display were not infected with endospores but J2 populations were low and *P. penetrans* was detected in these soil samples.

Nematode populations remain at low levels in all sites in the Land greenhouse. The cost of production of *in vivo P. penetrans* for the Land greenhouse has been estimated to be approximately $15,000 per year. Recent research at Entomos, Inc. will allow the low-cost mass-production and formulation of *in vitro*-produced endospores of *P. penetrans.*

Figure 1. Root percentage of roots galled and percentage of plants galled at sites inoculated with *Pasteuria penetrans.*

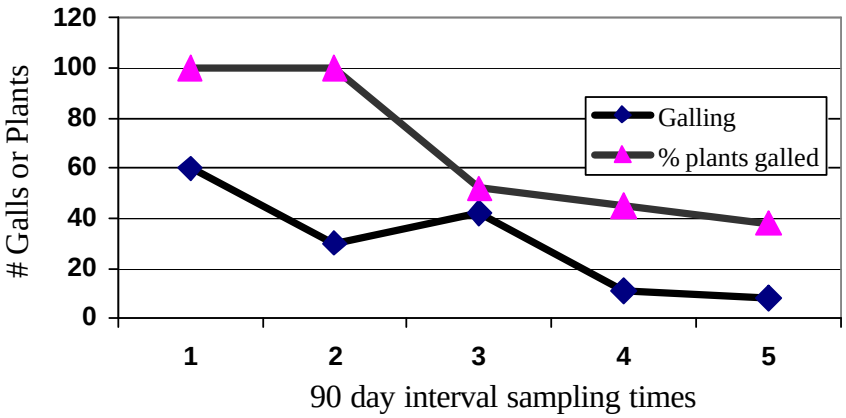

Table 1. Detection of *Pasteuria penetrans* on root-knot juveniles and in the soil in the Land greenhouse soil collected on December 20, 2001.

Sample Site	Location	Current Crop	#RKN 100cc soil	% J2 w/Spores	Ave # Spores Attached	Pasteuria Added	PCR Results
DA-4	Temp isle	Sunflower	28	85	47.6	P100,20	+++++
DB-2	Temp isle	Sunflower	0	0	0	P100, 20	+++++
DB-3 B	Temp isle	Sunflower	28	71	2.8	P100,20	+++++
DC-2	Temp isle	Vegetable	0	0	0	P100,20	neg
Cot-1	Temp	Cotton	0	0	0	no	++
C-1	Temp	Corn	0	0	0	P100	+++
B-1	Temp	Beans	4	0	0	P100	neg
DA-1	Temp	Pumpkin	0	0	0	P100,20	++