PRODUCTION OF THE NEMATODE BIOLOGICAL CONTROL AGENT *PASTEURIA SPP*. THE HARD WAY. Thomas Hewlett, Susan Griswold and Kelly Smith: Pasteuria Bioscience LLC, Alachua, Florida

Introduction *Pasteuria* spp. are obligate parasites of plant parasitic nematodes. Researchers have shown that they are capable of reducing nematode populations below economic thresholds necessary for profitable yield increases (1). This bacterium produces spores that attach to specific species of nematode as they move through the soil. Once attached to the nematode cuticle they germinate, penetrate inside and begin growing vegetatively. During this growth phase the bacteria either compete with or attack the reproductive system of the nematode and greatly reduce or stop egg production. Near the end of the nematode life cycle the bacteria sporulate and, upon degradation of the nematode body, spores are released back into the soil.

Control of nematode populations with *Pasteuria* spores in mircoplots and field studies begins to occur when the density of spores in the soil reaches 10,000 spore/g of soil. Applications of spores at 100,000 spores/g have produced nematode control and yield increases similar to other nematicides. To achieve nematode control in large acreage therefore involves production of large amounts of these spores.

Pasteuria is presently used for control of root-knot nematodes in greenhouse vegetable production in Japan and Kenya. EPCOT center, part of Disney World in Orlando, is the only location in the U.S.A. where *Pasteuria* is used as a control agent for root-knot nematodes. The production method used by these groups, the hard way (3), is by growing the *Pasteuria* on the nematode host and harvesting spore filled cadavers of the nematode. Production of enough spores to apply at the 100,000 spore/g rate is costly. Depending upon facilities and labor available it can cost from $5,000-10,000 per acre to produce enough spores. However, as in the case of the groups listed above, it may be the only possible alternative for nematode control.

P. penetrans is the only *Pasteuria* species presently being produced in large quantities. This species attacks root-knot nematodes, *Meloidogyne* spp. Root-knot nematodes are endoparasites of plant root systems. The infected females can produce as many as 2 million spores and because they are embedded in the roots, the roots can be harvested and manipulated with the spore filled females inside. In contrast, ectoparasitic nematodes are much more difficult to work with as their spore filled bodies must be extracted from the soil and the number of spore produced/cadaver is several fold less then in root-knot species.

Pasteuria Production, The Hard Way

Identification of correct bacteria isolate: Successful control of root-knot nematode populations depends upon identifying *Pasteuria* isolates that will attach and reproduce in the target nematode pest. *Pasteuria* species are ubiquitous and it is often possible to collect spores from nematode infested fields for use in spore production. Pasteuria Bioscience presently works with two isolates of *Pasteuria*. *Pasteuria* is relatively host specific. Different isolates can usually attach to and infect one or more species of root - knot.

To determine if *Pasteuria* isolates can parasitize the target nematode populations, root-knot nematodes must be collected from the site that is to be treated. Attachment efficacy

of spores on root-knot juveniles is done using a centrifugation technique (2). If spores attach to the nematode juveniles the infected nematodes are placed on a tomato plant or suitable host. Females are extracted from roots at 20 and 45 days and observed for vegetative growth and spore production, respectively. Bacteria isolates that produce at least 80% attachment to juveniles and produce spores in females are selected for large-scale production.

Production of initial inoculum, root powder Production of large amounts of spores necessitates the production of large amount of root–knot juveniles. Root-knot populations collected from treatment site must be cultured on suitable host plants in a green house in pots. Eggs, 5-10 thousand, are inoculated into a 6- inch pot. Eggs collected from these cultures are hatched and juveniles collected for spore attachment.

Spores for initial attachment are collected from spore filled females extracted from root systems using a cellulytic enzyme. Females are collected and ruptured with a tissue grinder to form a water suspension and spores densities are determined with a hemacytometer.

Juveniles are attached in centrifugation technique at approximately 100 spore/juvenile in the tube. Juveniles are then inoculated onto host plant in 6-inch pots, using 10-20 thousand juveniles/pot. After 60 days the root system of these plants is collected, air dried and ground into a fine powder. The number of spores produced is highly variable ranging from 10 –500 million per root system. The number of spores/g of root-knot powder must be determined before the product can be used for field application.

To further amplify the amount of *Pasteuria* spores produced, the inoculum produced can be used to attach more healthy juveniles for inoculation into larger numbers of potted host plants.

Application techniques: *Pasteuria* in root powder can be applied pre-plant or at-plant. The rate of application will depend upon the density of spores in the root powder. Due to the high cost of production, *Pasteuria* should be applied at the exact site of nematode infestation. The powder can be applied to the surface of the area to be treated and thoroughly mixed into the soil to a depth of 3- 6-inches. In the Land Pavilion at EPCOT a technique is used where root-knot infected sites are replanted by digging out the soil at the planting site and replacing it with clean soil mixed with *Pasteuria* root-powder. It is also applied in-furrow for some of their display crops.

Literature

1. Chen, Z.X. and D.W. Dickson. 1998. Review of *Pasteuria penetrans*. Biology, Ecology and Biological Control Poetential. Journal of Nematology 30, 313-340.

2. Hewlett, T. E. and D. W. Dickson. 1993. A centrifugation method for attaching endospores of Pasteuria spp. to nematodes. Journal of Nematology 25: 785-788.

3. Stirling, G. R. and M. F. Wacthtel. 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 26:308-312.