**PREPARATION FOR COMMERCIAL PRODUCTION OF PASTEURIA SPP. TO CONTROL ROOT-KNOT NEAMTODE.** **T.E. Hewlett, L.M. Schmidt, J. Waters C. Barmore, and C. Marshall*. Pasteuria Bioscience, Inc. 12085 Research Drive, Suite 185, Alachua, FL. 32615**

Pasteuria Bioscience, Inc. recently completed the first commercial launch of its biological nematode control product Econem®, which employs *Pasteuria* spp. for control of Sting nematodes on turf. Econem® consists of a clay carrier coated with *Pasteuria* spp. spores which specifically parasitize Sting nematodes. In the winter of 2011 we expect to launch our second *Pasteuria* spp. product for control of Lance nematode on turf. In 2012 we anticipate launching *Pasteuria* spp. products for control of Reniform nematode on cotton, and Soybean cyst nematode on soybean. The next product in the pipeline is *Pasteuria penetrans*, which parasitizes Root-knot nematodes. Large scale fermentation of an isolate that infects *M. incognita* has been subjected to testing in Cotton field trials.

However, development of *Pasteuria penetrans* for control Root-knot nematodes presents a greater challenge, with the presence of numerous biotypes that do not infect all species of Root-knot nematodes found on agronomic, vegetable and ornamental crops. *Pasteuria penetrans* are observed to be specific for a particular species/race(s) of Root-knot nematode, therefore, it is necessary to identify *Pasteuria penetrans* spp. isolates that parasitize a range of the economically important Root-knot nematode species and/or choose several isolates that when blended, can control all economically important Root-knot nematodes.

Attachment/infection studies are required to identify the most efficacious *P. penetrans* isolates for control of Root-knot nematodes. Pasteuria Bioscience, Inc. maintains a substantial library of Root-knot nematode species and *Pasteuria penetrans* isolates in green-house cultivation on Rutgers tomato. *Meloidogyne* are speciated using a molecular taxonomical approach by PCR.

Host preference and compatibility is determined by performing host-parasite attachment tests. *P. penetrans* endospores and 2-5 day-old *Meloidogyne* sp. J2 are comingled at a rate of 100,000 endospores: 200 nematodes in a 300 µl conical microfuge tube and centrifuged for 4 minutes at 10,000 rpm. The presence or absence of endospores on the cuticle and the number of spores attached per J2 for a 20 nematode set is assayed by microscopic observation. The host-parasite association is considered compatible when 100% of the assayed J2 harbor endospores, numbering an average of 2 or more endospores per nematode. Pasteuria Bioscience, Inc. currently has a library of some 25 *P. penetrans* isolates available for assay. The results of recent attachment tests are shown in Table 1.

To test host infection, nematode specimens from the attachment test are placed in a water bubbler for three days, at room temperature (23 $^{o}$C), to allow time for endospores to

germinate and infect the host. Nematodes are then collected and surface sterilized using a 3% solution of hydrogen peroxide. Ten nematodes are transferred into individual tissue

| Root-knot nematode | *P. penetrans* isolates in the Pasteuria Bioscience, Inc. culture collection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pp1 | Pp2 | Pp6 | Pp7 | Pp8 | Pp9 | Pp10 | Pp13 | Pp21 | Pp30 |
| *M. incognita* | X | X | O | X | X | O | X | X | X | O |
| *M. arenaria* | X | X | O | X | X | X | X | | | O |
| *M. javanica* | X | O | O | | | | | | | |
| *M. mayaguensis* | X | O | O | | | | | | | |

X= 100% of J2 nematodes with endospores attached

O= Endospore attachment less than 100%

Blank= Awaiting testing

culture in a 9 well plate containing Pasteuria Bioscience, Inc. *Pasteuria* growth media. The nematodes are aseptically crushed in the media and incubated at 30 $^{o}$C. After 24 hrs the culture media is evaluated under 600X magnification for the presence of *Pasteuria* spp. vegetative cell development.

Through this approach we have identified and will continue to screen for *P. penetrans* candidate isolates that have multiple species host affinity and show promise for development in a blended product to control the majority of the important Root-knot species. Once indentified, candidates for scale-up production will be cultured in a 60 liter capacity fermenter providing inoculum for conducting pot tests and field trials on a variety of *Meloidogyne* spp.. Initial field testing is scheduled for 2012.