

## Germination and Infection Rate of *In vitro* Produced *Pasteuria* spp. Endospores on Sting Nematode.

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The majority of earlier studies of rate of germination and infection of *Pasteuria* spp. on nematodes involve *Pastueria penetrans* parasitizing root-knot nematodes. Spore-filled root-knot females produce relatively large numbers of endospores. These spore filled females can easily be harvested from root systems. *Pasteuria* spp. of ectoparasitic nematodes are much more difficult to study since fewer endospores are produced and the spores must be extracted from the soil and hand picked under a dissecting scope. The development of an artificial growth media for *Pasteuria* spp. allows for detection of *Pasteuria* cells and other growth structures present in the nematode body. *Belonolaimus longicaudatus* and the *Pasteuria* spp. that parasitizes it, *P. usagee*, were used in a study to determine the rate of germination and infection of *in vitro*- grown *Pasteuria* spores. *In vitro* produced *P. usagee* endospores were attached with a centrifuge technique. Approximately 200 spore-encumbered nematodes were placed in clean moist sand, in small petri plates. Nematodes were extracted from plates after 1, 2, 3 and 7 days and crushed in six well plates with growth medium (1 infected nematode in each of 12 wells). Plates were observed immediately and after 24 hrs and presence of cells, mycelial balls, thalli and spores were recorded and the experiment was repeated a second time.

Endospore germination and cell growth of *P. usgae* had begun by 24 hrs. Cells, mycelial balls and thalli were present by 48 hrs and spores were present at the 7-day sample. Spore germination is much more rapid than reported for *P. penetrans* parasitizing root-knot nematodes, but germination was determined by the presence of mycelial balls. Sporulation also occurred at a much higher rate, within 7 days, as compared to about 20 days for root-knot nematode.

Some of the differences in growth rate between these two *Pasteuria* species could be due to the nematode culture techniques used in the different studies. The *Pasteuria* infected nematode used for root-knot studies were extracted from root tissue and therefore were actively feeding. Infected sting were placed in sand with no food source during the experiments. The earlier studies of endospore germination and infection of *P. penetrans* on root-knot was done when mycelial balls were thought to be the vegetative growth phase. Therefore the estimate of germination time is probably earlier

in root-knot than was reported as germination and cell growth occurs first and the presence of mycelial balls indicates the beginning of sporulation.