PLANT AND SOIL NEMATODES

OPPORTUNITIES FOR RESEARCH ON *PASTEURIA*, A POTENTIALLY USEFUL BIOCONTROL AGENT OF PLANT-PARASITIC NEMATODES

The genus *Pasteuria* is closely related to *Bacillus*, one of the most widely studied genera of bacteria. Currently, seven nematode-attacking species of *Pasteuria* are recognised but the genus is likely to contain many taxa and exhibit considerable diversity at an intra-species level. Hundreds of nematode species are known hosts, including many *Meloidogyne* and *Pratylenchus* species, the most widespread and important nematode pests in Australia. Since *Pasteuria* is host-specific, prevents its host from reproducing, and produces endospores that survive in soil for many years due to their tolerance of heat and dryness, it has attributes which suggest it is an excellent biocontrol agent of plant-parasitic nematodes. This fact sheet provides an overview of the research that could be done on this potentially useful biocontrol agent.

Molecular assays for detection and quantification of *Pasteuria*

Pasteuria is usually detected by extracting nematodes from soil and checking whether they are encumbered with endospores. However, examining nematodes at a relatively high magnification under a microscope is a time-consuming process and this limits the number of samples which can be processed.

Current methods of quantifying populations of *Pasteuria* also have their limitations. Endospores can be extracted from roots and soil but this is a tedious task, as samples must be dried, ground, and sieved. Also, endospores are difficult to count, as they are only 2-4 µm in diameter and are usually mixed with soil and root debris. Indirect measurement such as counting the number of spores attached to assay nematodes is possible, but results depend on the extent to which nematodes move in the soil and come into contact with endospores, and this is affected by temperature and the moisture content of the soil.

A molecular-based method of assessing *Pasteuria* would avoid these issues and provide opportunities to study host-parasite interactions in detail. Any molecular assay that is developed must be able to detect and quantify *Pasteuria* endospores in soil and also differentiate the various host-specific species and strains that are likely to occur in agricultural soils.

Occurrence of *Pasteuria*

Most of the information on the occurrence of *Pasteuria* in Australia has come from occasional observations that are made when diagnostic samples are being examined. Better information on the distribution and diversity of *Pasteuria* is required and it can be obtained by undertaking formal surveys focused on specific nematodes in particular crops or regions. The objectives of those surveys must be to identify and quantify the nematodes encumbered with *Pasteuria* endospores; assess the proportion of the nematode population that is parasitised; determine whether *Pasteuria* is likely to be having an impact on nematode populations; and collect isolates for taxonomic, molecular, and host-specificity studies.

Once a molecular assay for *Pasteuria* is developed, it should be possible to determine whether the bacterium is providing some degree of nematode control. For example, data for a particular crop and nematode would be analysed to determine whether there was a negative relationship between the amount of *Pasteuria* DNA in soil and the population density of the host nematode. Additionally, sites with and without *Pasteuria* could be re-surveyed every few years to see whether nematode populations responded differently over time in situations where *Pasteuria* was present or absent.

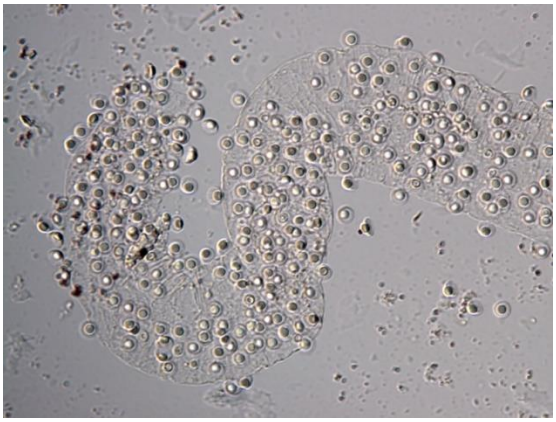
Examples of survey work on *Pasteuria* that could be undertaken on various crops in Australia

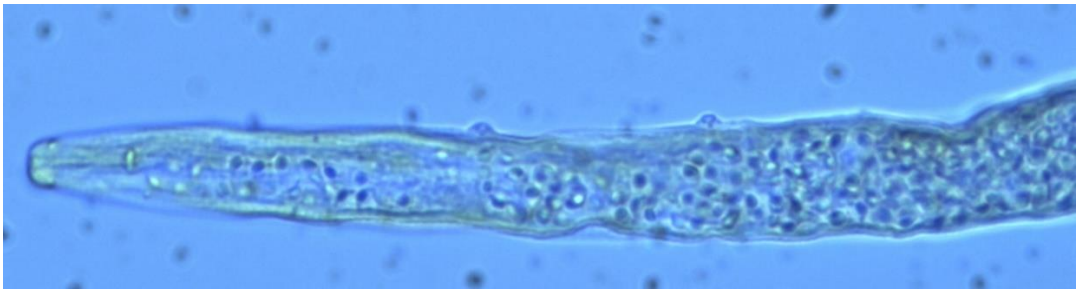
Pastures. Since *Pasteuria* is an obligate parasite of nematodes, it requires a constant supply of host nematodes to multiply. Thus, undisturbed environments such as pastures are likely to have relatively high populations of *Pasteuria*. Recent work at two grass pasture sites in north Queensland indicated that about 50% of the root-lesion nematodes were either parasitised by *Pasteuria* or had spores attached (Stirling et al. 2017). As *Pratylenchus* is one of the most common plant-parasitic nematodes on grass pastures, surveys should be undertaken to determine whether *Pasteuria* is providing some control of this pest. It would also be worthwhile checking whether *Pasteuria* is having an impact on root-knot nematode in environments where the pest is likely to reach high population densities (e.g. the medic and clover pastures growing in light-textured soils in Victoria, South Australia and Western Australia).

Turfgrass. A wide range of plant-parasitic nematodes occur on turfgrasses, and so turfgrass sites provide opportunities to obtain a range of *Pasteuria* isolates and study the way *Pasteuria* interacts with various plant-parasitic nematodes. Research on isolates capable of attacking southern sting nematode (*Ibipora lolii*) is particularly important as it is the most damaging nematode pest of turfgrass in Australia. In a limited survey of 12 locations in NSW and Western Australia, *Pasteuria* was found on two adjacent golf courses on Sydney's south-eastern coast (Stirling et al., 2013).

Grain crops. *Pratylenchus thornei* and *P. neglectus* are important pathogens of grain crops and plant breeders are currently developing varieties with resistance and tolerance to these nematodes. However, there has only been one attempt to determine whether *Pasteuria* is already providing some control of these nematodes. *Pratylenchus thornei* encumbered with *Pasteuria* endospores were found in 25% of surveyed fields in the northern grains region but infestation levels were relatively low. However, this pest was only introduced to most fields in the last 20-30 years, and so it is possible that *Pasteuria* is still in its multiplication phase in this region. What is really needed is a thorough survey of areas in southern NSW, Victoria, and South Australia where *Pratylenchus neglectus* has probably been present for at least 100 years. Since there is some evidence to indicate that tillage is detrimental to *Pasteuria*, the focus of that survey should be fields that have been under minimum till for the last 30-40 years.

Trees and vines. Research done in the 1980s showed that *P. penetrans* reached high population densities and provided some control of root-knot nematode in old vineyards in the Riverland region of South Australia. *P. penetrans* is also relatively common on bananas and is likely to be found on any perennial host of root-knot nematode, and so these crops should be surveyed to check whether the parasite is providing some control. However, future work should also focus on *Pratylenchus*, as it is likely to be the dominant plant-parasitic nematode in apple-growing areas, and in many vineyard soils.





Most *Pasteuria* research in Australia and elsewhere in the world has focussed on *Pasteuria penetrans*, the species which parasitises root-knot nematode. These two photographs show that *Pasteuria* also parasitises other plant parasitic nematodes. The top photograph shows endospores in the cadaver of a root-lesion nematode (*Pratylenchus zeae*) from a sugarcane field in north Queensland (Stirling et al. 2017). The bottom photograph shows endospores attached to the cuticle and within the body of stunt nematode (*Merlinius brevidens*) from a wheat field at Gatton, Queensland.

Pasteuria thornei*: a biocontrol agent of *Pratylenchus

Other than taxonomic studies undertaken in the 1980s, very little has been published on *Pasteuria thornei,* the *Pasteuria* species that attacks *Pratylenchus*. Consequently, very little is known about the many factors likely to affect its biocontrol capacity. Some of the key questions to be answered are: how long does it take for an

endospore to germinate after it adheres to a nematode; does the nematode have to enter a root before endospores will germinate; are endospores equally likely to adhere to adults and juvenile stages of the nematode; how many endospores are produced in an infected nematode and does this vary between adults and juveniles; how many endospores must adhere to a nematode to ensure that infection occurs; once a nematode is infected, how long does it take to for the parasite to produce endospores; what is the effect of temperature on the length of the life cycle; and is pathogenicity favoured at certain temperatures?

There is also a lot to learn about the distribution of *Pasteuria thornei* endospores in the roots and rhizosphere, and the interactions that occur between endospores and their host as root-lesion nematodes move through the soil and attempt to invade the root system. For example, we need to know whether endospore-filled cadavers occur in roots or whether parasitised nematodes only die in soil. We also need some understanding of how long it takes for endospores to be released from parasitised nematodes; where those endospores finish up at a microsite level; and what endospore concentrations are achieved in the roots and rhizosphere.

If the biocontrol capacity of *Pasteuria thornei* is to be understood, the latter issue is particularly important. Studies with *Pasteuria penetrans* have shown that it provides some control of root-knot nematode at concentrations of 10^4 endospores/g soil and even better control at higher endospore concentrations. Similar data are required for *Pasteuria thornei*.

In vivo* culture methods for *Pasteuria thornei

Before any work can be done with an isolate of *Pasteuria thornei*, a culture of its host nematode must be available. This should not be difficult, as all *Pratylenchus* species can be multiplied on plants in the greenhouse and most of them can be cultured in the laboratory on sterilised carrot tissue. Obtaining supplies of endospores may be more problematic, but the best option is likely to involve a modification of the dried root method that is widely used for *P. penetrans*. This would involve adding nematodes to an endospore suspension prepared from spore-filled cadavers, encumbering them with spores and then inoculating the nematodes onto a host plant. Roots would be harvested 6-8 weeks later, air-dried, and then ground into a powder. If it was found that parasitised nematodes did not die in roots, it would be necessary to use air-dried soil rather than roots as an inoculum source.

Extraction of vermiform nematodes infected by *Pasteuria* from soil

Almost all previous work on *Pasteuria* has been done with species/strains that attack root-knot and cyst nematodes, largely because infected females are large and immobile, and can be readily retrieved from roots. Retrieval of infected vermiform nematodes is much more difficult because tray-based extraction methods rely on nematode motility and *Pasteuria* either kills its host or reduces its capacity to move. Another problem is that infected *Pratylenchus* have a higher specific gravity than healthy nematodes and this means they are not readily extracted by standard sugar centrifugation techniques. Nematologists in Florida overcame this problem by increasing the specific gravity of the sugar extraction solution to 1.26 and when this was done, they were able to extract *Pasteuria*-infected *Pratylenchus* from very sandy soils. Infected root-lesion, stunt and spiral nematodes have been retrieved from Australian sugarcane soils using this method but one limitation is that only very small samples can be processed. Also, a considerable amount of organic material is retrieved with the nematodes and so the infected nematodes are mixed with debris and are hard to find. Thus, research is required to improve this method.

Impact of tillage on *Pasteuria*

The first evidence that tillage may disturb the interaction between *Pasteuria* and its nematode host was obtained from a tillage trial that was established in a Spanish greenhouse following a crop of French beans. More than 75% of the *Pratylenchus neglectus* recovered from the soil were encumbered with *Pasteuria* endospores but the percentage of nematodes with spores attached was significantly less in tilled than untilled plots. Stirling et al. (2017) also obtained some evidence to suggest that *Pasteuria* is more likely to increase to high levels when soil is not tilled, as the parasite was more common on sugarcane farms where growers had moved to a minimum till farming system 10-15 years ago. Also, root-lesion nematode was more likely to be parasitised in undisturbed grass pastures and fields where sugarcane had recently been planted following a grass pasture, than in fields where sugarcane had been grown for many years.

Given the widespread adoption of minimum tillage in many cropping industries over the last 30 years, it is important to determine whether it is having a positive impact on the occurrence of *Pasteuria* and levels of parasitism. Thus, *Pasteuria* levels in tilled and non-tilled soils should be compared.

It is also important to understand the interactions between *Pasteuria* and its nematode host at a microsite level and determine whether these interactions are affected by tillage. Spore-filled nematodes are

most likely to be found within or near the roots of the nematode host, and because plant roots form channels in the soil as they elongate and those roots later decay to form open biopores, concentrations of *Pasteuria* spores are likely to be highest at microsites within root channels that were utilised by previous crops. If this is confirmed, the next step is to determine whether these spore-filled root channels are important from a biocontrol perspective. When a root from the next crop grows into such a channel, is there is a reasonable chance that an endospore will adhere to a nematode as it attempts to invade the root? If the endospores in that root channel are dispersed by tillage, does the endospore concentration at a microsite level decline to the point where nematodes are much less likely to contact an endospore?

Inundative biocontrol. When *P. penetrans* has been applied in an inundative manner to control root-knot nematode, inoculum of the biocontrol agent has usually been obtained by inoculating a host plant with spore-encumbered juveniles, allowing the female nematodes to develop to maturity and become filled with endospores, and then drying the root system and grinding it into a fine powder. Field experiments with this endospore-rich material were undertaken in Australia and elsewhere during the 1980s and 1990s and the results showed that root-knot nematodes could be controlled with spore concentrations of about 10^5 endospores/g of soil. More recently, Bhuiyan et al. (2018) found that when sugarcane was grown in soil containing half that spore concentration, root-knot nematode populations were reduced by more than 80%.

In 2004, an *in vitro* method of culturing *Pasteuria* was patented by the University of Florida. A company named Pasteuria Bioscience was then established to develop *Pasteuria*-based bionematicides. The initial target was sting nematode on turfgrass and in 2009 a commercial product for control of this nematode was registered in the USA. Syngenta purchased the company in 2012 and since then has focused on marketing a product in the USA (Clariva™) that targets soybean cyst nematode (*Heterodera glycines*). *Pasteuria* is applied to soybean seed and is being promoted as the biological component of a seed-treatment package that also includes a chemical fungicide, insecticide and nematicide.

Since Syngenta currently holds the patent for *in vitro* culture of *Pasteuria*, any research on inundative control may require collaboration with that company. However, the patent will presumably lapse in the next few years and so anyone interested in developing *Pasteuria* products should check whether the patent situation restricts the *in vitro* mass production methods that can be used.

Literature cited and further reading

Anyone interested in commencing a research project on *Pasteuria* is advised to read chapters 7 and 12 of Stirling (2014), as those chapters provide a reasonably detailed review of what is known about *Pasteuria*. References to all previous work discussed above can be found in that book.

Stirling GR (2014). *Biological Control of Plant-parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture*. CAB International, Wallingford, 536 pp.

The references cited below are not included in that book, or relate to material published in the last few years.

Bhuiyan S, Garlick K, Anderson J, Wickramasinghe P, Stirling GR (2018). Control of root-knot nematode on sugarcane in soil naturally-infested with *Pasteuria penetrans* or inoculated with the parasite. *Australasian Plant Pathology* 47, 45-52.

Stirling GR, Stirling AM, Giblin-Davis RM, Ye W, Porazinska DL, Nobbs JM, Johnston KJ (2013). Distribution of southern sting nematode, *Ibipora lolii* (Nematoda: Belonolaimidae), on turfgrass in Australia and its taxonomic relationship to other belonolaimids. *Nematology* 15, 401-415.

Stirling GR, Wong E, Bhuiyan S (2017) *Pasteuria*, a bacterial parasite of plant-parasitic nematodes: its occurrence in Australian sugarcane soils and its role as a biological control agent in naturally-infested soil. *Australasian Plant Pathology* 46, 563-569.

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