THE BACTERIAL BIOCONTROL AGENT *PASTEURIA PENETRANS* CAN HELP CONTROL ROOT-KNOT NEMATODE ON SUGARCANE

By

SA BHUIYAN[1], GR STIRLING[2], K GARLICK[1], JM ANDERSON[3], P WICKRAMASINGHE[1], E WONG[1]

[1]SRA Woodford, [2]Biological Crop Protection, Brisban,. [3]UQ St Lucia

sbhuiyan@sugarresearch.com.au

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Abstract

ROOT-KNOT NEMATODE (*Meloidogyne javanica*) is one of the most damaging pests of sugarcane, often causing heavy losses in coarse-textured sandy soils. The bacterial parasite *Pasteuria penetrans* is a potentially useful biocontrol agent and in a 2015–16 survey it was found at relatively high levels in three of the 126 sugarcane fields surveyed. Soil was collected from one of the heavily-infested fields and a pot experiment established to compare root-knot nematode multiplication in naturally-infested soil and in soil where the endospores of *P. penetrans* had been killed by autoclaving. After 37 weeks, the root-knot nematode population was very high in the autoclaved soil but numbers of root-knot nematode eggs and second-stage juveniles were 99% lower in the soil that was naturally-infested with *P. penetrans*. A subsequent pot experiment with mass-produced endospores showed that when soil contained more than 6 000 endospores/g soil, root galling was not as severe as in non-infested soil and the number of root-knot nematode eggs was reduced by 71–82%. These results indicate that when high endospore concentrations are continually maintained in the root zone, *P. penetrans* will markedly reduce populations of one of the most important nematode pests of sugarcane.

Introduction

Plant-parasitic nematodes are important pests of sugarcane, costing the Australian sugar industry in excess of $80 million annually (Blair and Stirling, 2007). Root-knot nematode (*Meloidogyne javanica*) is perhaps the most damaging of these pests, often causing heavy losses on farms with coarse-textured sandy soils.

When potentially useful methods of controlling root-knot nematode are being considered, *Pasteuria penetrans* stands out as being worthy of investigation. This host-specific bacterium prevents its parasitised host from reproducing and, because it produces endospores that are resistant to environmental stresses such as heat and dryness, it can survive for long periods in soil.

Research in Australia and the USA has shown that it can reduce root-knot nematode populations on many different crops (Chen and Dickson, 1998; 2004; Stirling, 2014).

The objectives of this research were to assess the distribution of *P. penetrans* in Australian sugarcane fields; determine whether the parasite can increase naturally to levels capable of suppressing root-knot nematode; and determine the endospore concentrations required to control the nematode on sugarcane. More detailed reports of this work have been published elsewhere (Stirling *et al*., 2017; Bhuiyan *et al*., 2017).

Materials and methods

Survey

In 2015 and 2016, soil samples were collected from 126 fields in all cane-growing districts of Queensland and New South Wales. Nematodes were retrieved on extraction trays (Whitehead

and Hemming, 1965) and second-stage juveniles of root-knot nematode were checked under a microscope for the presence of *P. penetrans* endospores.

Effect of *P. penetrans* in naturally-infested soil

P. penetrans-infested soil was collected from a field near Port Bundaberg and a sub-sample was autoclaved to kill endospores of the bacterium. Pots 190 mm high and 200 mm in diameter were filled with approximately 5 kg of autoclaved or untreated soil, pre-germinated single-eye setts of Q208[ф] were planted, and each pot was then inoculated with 5 000 eggs of *M. javanica*. Plants were cut back after 19 weeks and the first ratoon plants were harvested 37 weeks after inoculation. Root and shoot biomass was measured and roots were visually rated for galling on a 0 to 5 scale where, 0= no galls and 5 = >75% of roots galled. Root-knot nematode eggs were removed from roots by submerging them in 1% bleach for 5 min and retrieved on a 38 μm sieve while second-stage juveniles were extracted from 500 g of soil using the aforementioned tray method.

Effect of different concentrations of *P. penetrans* endospores

Endospores of *P. penetrans* were produced in tomato roots using the mass production method of Stirling and Wachtel (1980). Second-stage juveniles of *M. javanica* were added to soil from the previously-mentioned farm at Port Bundaberg and incubated for 3–4 days to ensure that all the nematodes had at least some endospores attached. The nematodes were then inoculated onto tomato seedlings in pots. After 8–10 weeks, roots containing parasitised females were air-dried and ground into a fine powder. The endospore concentration in a sample of dried root powder was then determined by mixing 0.1 g of powder in 1 mL water and counting the endospores in a haemocytometer. The required amount of dried root material was then added to batches of pasteurised sand to achieve a range of endospore concentrations (0, 6 000, 12 000, 24 000 and 50 000 endospores/g soil). The sand was then added to pots, planted to sugarcane and inoculated with *M. javanica* as described earlier. Twenty months later, shoot biomass, the level of root galling and the nematode population in soil and roots were assessed.

Results

Survey

P. penetrans was found in all regions where sugarcane is grown and endospore-encumbered juveniles of root-knot nematode were found in 30% of the fields where the nematode was detected. In most cases less than 5% of the nematodes were infested and most nematodes only had one endospore attached. However, there were three fields where more than a third of the root-knot nematodes were encumbered with endospores of *P. penetrans*, with some juveniles having more than ten endospores attached.

Effect of *P. penetrans* in naturally-infested soil

The two treatments (untreated and autoclaved soil) did not affect shoot biomass but root biomass was higher in the autoclaved soil (Table 1). Numbers of root-knot nematode eggs and juveniles in the untreated soil were almost 99% lower than in the autoclaved soil and the roots also had a much lower gall rating (Table 1).

Table 1—Effects of autoclaving of soil naturally infested with *Pasteuria penetrans* on growth of sugarcane, nematode multiplication and root galling caused by *Meloidogyne javanica* 37 weeks after inoculation.

Treatment	Shoot biomass[1]	Root biomass	Eggs/plant	Second-stage juveniles/kg soil	Gall rating
Autoclaved	14.2 a	16.4 a	287 911 a	1 142 a	3.4 a
Untreated	13.2 a	8.9 b	1 516 b	7 b	0.4 b

[1] Data are the mean of five replications. Means followed by same letter in column are not significantly different according to Fisher's protected LSD test (P=0.05). Data for no. of eggs or second-stage juveniles were log-transformed before analysis.

Effect of different concentrations of *P. penetrans* endospores

Twenty months after the experiment was established, the presence of *P. penetrans* sometimes increased shoot biomass, always increased root biomass and usually reduced the number of nematode eggs, second-stage juveniles and gall ratings relative to the untreated control (Table 2).

Effects on root growth were readily apparent, as root biomass increased by 38%–104% in soils to which *P. penetrans* was added compared with the untreated control. Major differences in the level of galling in soils with and without *P. penetrans* were also observed.

Table 2—Effects of various concentration of *Pasteuria penetrans* endospores on growth of sugarcane, root galling caused by *Meloidogyne javanica* and numbers of root-knot nematodes recovered 20 months after nematodes were added to pots.

Treatment (spores/g soil)	Shoot biomass (g)[1]	Root biomass (g)	Eggs/plant	Second-stage juveniles/kg soil	Gall rating
0	34.4 b	141.3 c	93 293 a	5 195 a	3.6 a
6 000	50.5 a	288.7 a	23 856 b	452 ab	2.6 b
12 000	51.1 a	230.1 ab	16 493 b	113 b	2.4 b
24 000	39.2 ab	209.9 bc	26 646 b	377 ab	2.2 b
50 000	36.2 b	195.7 bc	17 496 b	142 b	1.4 c

[1]Values are the means of five replications. Values followed by the same letter(s) in a column are not significantly different according to Fisher's protected least significant difference (LSD) test (P=0.05). Data for no. of eggs or second-stage juveniles per plant were log-transformed before analysis.

Discussion

Our survey results revealed that *P. penetrans* is relatively common in Australian sugarcane soils but infestation levels are relatively low. However, the occurrence of the bacterium at high levels in a field near Bundaberg provided us with the opportunity to determine whether it is a useful biocontrol agent.

The results of those experiments clearly showed that *P. penetrans* has the capacity to markedly reduce populations of root-knot nematode on sugarcane. The endospore concentration in one naturally-infested soil was high enough to reduce nematode populations by 99% while the results of an experiment with mass-produced spores showed that nematode numbers were reduced by more than 80% when 50 000 endospores of *P. penetrans*/g soil were present.

Relatively high levels of nematode control were also achieved at spore concentrations of 6 000 endospores/g soil, a much lower concentration than was required for crops such as tomato and peanut (Stirling *et al*., 1990; Chen *et al*., 1996).

Although these results are encouraging, they raise questions about how sugarcane soils should be managed so that this naturally-occurring biocontrol agent will multiply to levels capable of suppressing this important pest. Since current sugarcane varieties are susceptible to root-knot nematode and *P. penetrans* is a specialised parasite of this nematode, the endospore-multiplication process will always be occurring to some extent when host nematodes are present.

However, root-knot nematode population densities will often be relatively high when *P. penetrans* is multiplying and so growers must be prepared to adopt management practices that minimise crop losses from the pest. The best way of doing this is to optimise water and nutrient inputs, as this reduces stress on the plant and should allow the crop to produce acceptable yields, despite the damage being caused by the nematode. Our survey data showed that *P. penetrans* was more common on farms with a controlled traffic/minimum till farming system than on farms where traffic was not controlled and the soil was cultivated conventionally. Thus, the other important step that must be taken to increase levels of *P. penetrans* is to minimise tillage.

Conventional tillage is detrimental to *P. penetrans* because when the soil is disturbed, endospores in root-associated microsites are moved to positions where they are less likely to encounter a host nematode, thereby limiting the parasite's capacity to multiply.

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