General nematology methods

PW17001 Final report Appendix 2 Integrated pest management of nematodes in sweetpotato

Jennifer Cobon, August 2023

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Hort Innovation SWEETPOTATO FUND

This project has been funded by Hort Innovation using the sweetpotato research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Queensland Government

This publication has been compiled by Jennifer Cobon of Agri-science Queensland, DAF.

The Department of Agriculture and Fisheries proudly acknowledges all First Nations peoples (Aboriginal peoples and Torres Strait Islanders) and the Traditional Owners and Custodians of the country on which we live and work. We acknowledge their continuing connection to land, waters and culture and commit to ongoing reconciliation. We pay our respect to their Elders past, present and emerging.

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Soil sampling

Field sampling

A composite soil sample was taken from a block/field to a depth of 10-15 cm using a clean probe, corer or auger. At each individual soil sampling site, any surface organic matter or dry soil was pushed away. Approximately 40 samples, taken randomly across the block, were emptied into a clean bucket and mixed thoroughly. Approximately 600 mL of soil was divided off and placed in a well labelled sealed plastic bag for immediate transport to the nematology laboratory.

Field trials and in crop sampling

Soil sampled randomly along the row with a clean probe as above every two metres, was then emptied into a clean bucket and mixed thoroughly. Approximately 600 mL of soil was then divided off and placed in a well labelled sealed plastic bag for immediate transport to the nematology laboratory.

Nematode extraction and identification

Plant-parasitic nematodes were extracted, identified and quantified from all soil samples over four days using a Whitehead tray (Whitehead AG *et al.,* 1965) - a modified Baermann funnel technique - after which the solution was poured over a 38 µm sieve.

Two trays with approximately 230 grams of soil in each were set up for each sample. The results were standardised per 200 grams of dry weight equivalent soil. The major plant-parasitic nematodes recovered were identified using light microscopy and morphological characteristic according to the Commonwealth Institute of Parasitology (1972-1977) descriptions.

From this survey, soils were submitted to SARDI (South Australian Research and Development Institute) for molecular identification of the root-knot nematodes species present.

Nematode cultures used for resistance screening experiments

Meloidogyne spp.

Pure cultures of each root-knot nematode species (*M. incognita, M. javanica*) were maintained in a glasshouse on tomato (cv. Tiny Tim) plants. These were originally cultured from a field sourced single eggs mass, with the species identification confirmed by PCR (Stanton *et al*.,). Nematode eggs were obtained for use as inoculum by soaking roots in NaOCl (0.5% available chlorine) for five minutes, and then retrieving eggs on a 38 µm sieve by washing thoroughly with water. Nematode egg numbers were adjusted to achieve the required inoculum density.

Rotylenchulus reniformis

Pure cultures of *R. reniformis* were maintained in the glasshouse on tomato (cv. Tiny Tim) plants grown in an 80/20 mix of pasteurised nematology sand mix and a pasteurised red ferrosol soil. This was originally cultured from a field sourced single eggs mass, with the species identification confirmed by morphological identification. Nematode eggs were obtained for use as inoculum by soaking roots in NaOCl (0.5% available chlorine) for five

minutes and retrieving eggs on a 38 µm sieve by washing thoroughly with water. Nematode egg numbers were adjusted to achieve the required inoculum density.

Pratylenchus zeae

Pure cultures of *P. zeae* were maintained as sterile monoxenic carrot cultures (Moody *et al*., 1973) which allows the *in vitro* rearing of large numbers of these nematodes as a pure source of inoculum. This was originally cultured from a field sourced single adult female nematode, with the species identification confirmed by morphological identification. To inoculate experiments, *P. zeae* was obtained by washing the nematodes from carrot cultures and retrieving the nematodes on a 38 µm sieve. Live nematode numbers were adjusted by dilution to achieve the required inoculum density.

Soil mixes for glasshouse experiments

Pasteurised sand mix for experiments with *Meloidogyne* spp. and *P. zeae*

This pasteurised sand mix consists of 150 L pit sand, 150 L bedding sand, 250 g superphosphate, 250 g blood and bone, 250 g Gypsum, 150 g Dolomite, 100 g Micromax, 50 g Potassium nitrate, 25 g Potassium sulphate.

Soil mix for experiments with R. reniformis

This mix was 80/20 mix of pasteurised sand mix and a pasteurised red ferrosol field soil obtained from Redlands Research Station. Previous work had shown R. reniformis reproduces well on plants grown in this mix and it was also easy to wash and strip the eggs from these roots for quantification.

Pot experiments

Resistance experiments

Seeds, runners or sweetpotato vines of each plant cultivar were sown directly into 1.3 -1.5 L pots of suitable soil mix. After germination, the plants were thinned so that several healthy plants remained in each pot. Plants were grown for two to four weeks before inoculation so that a healthy root system was available for the nematodes to infect.

Pots of each cultivar tested were inoculated with a known number of eggs for *Meloidogyne* spp. and *R. reniformis* or live nematodes for *P. zeae.* The nematode treatments were replicated five times for each species and maintained in a glasshouse with plants fertilised fortnightly with a liquid fertiliser (Aquasol®).

Tomato cv. Tiny Tim was grown and inoculated as the susceptible control for *Meloidogyne* spp. and *R. reniformis* experiments with maize cv. Messenger used as the susceptible control for the *P. zeae* experiments.

Harvest

Meloidogyne spp. experiments

Data obtained by monitoring temperatures with a data logger allowed the calculation of heat units for the *Meloidogyne* spp. experiments which were harvested approximately nine weeks after inoculation when it was calculated that at least 14,000 heat units (°C-hours) had been reached (sufficient for maximum egg production after inoculation). Heat hours accumulated

during the experiments were calculated assuming minimum and maximum temperatures for *M. incognita* development of 10 °C and 28 °C respectively and for *M. javanica* development 13 °C and 32 °C respectively (Trudgill 1995). At harvest, the plant tops were cut at soil level and roots washed free of soil and the fresh root weights were recorded.

Pratylenchus zeae and *R. reniformis* experiments

At harvest, (13 weeks post inoculation for *P. zeae* and 16 - 23 weeks post inoculation for *R. reniformis* experiments), the plant tops were cut at soil level and roots washed free of soil and the fresh root weights were recorded.

Nematode extraction from glasshouse experiments

Meloidogyne spp. and *R. reniformis* experiments

Nematode eggs were recovered from the roots by soaking the roots system in NaOCl (1% available chlorine) for five minutes and then pouring the suspension over a 38 µm sieve. The egg/nematode suspension was diluted appropriately and counted at a magnification of 50X.

Pratylenchus zeae experiments

To extract nematodes, the roots were sliced lengthwise and placed in a misting chamber for seven days (Hooper 1986). Nematodes were then recovered from the filtrate on a 38 µm sieve. The nematode suspension was diluted appropriately and counted at a magnification of 50X.

Resistance levels

Levels of resistance or susceptibility were determined by inoculating plants with a known number of nematode eggs/live nematodes (initial population density Pi), measuring final population density (Pf) and then making the following calculation:

Reproduction Factor (RF) = Pf/Pi.

Meloidogyne spp.

Since not all eggs in *Meloidogyne* inoculum are capable of hatching and invading roots, a conservative figure of 1/10 of the Pi was used as Pi, i.e., 1,000 for both *M. incognita* and *M. javanica* if inoculated with 10,000 eggs.

For *Meloidogyne* experiments, susceptible crop varieties were further categorised as highly, moderately or slightly susceptible according to the reproduction factor (Table 1).

Table 1 Resistance categories

Reproduction Factor	Resistance Rating
> 100	Highly Susceptible (HS)
10 - 100	Moderately Susceptible (MS)
1 - < 10	Slightly Susceptible (SS)
0.1 - < 1	Resistant (R)
< 0.1	Highly Resistant (HR)

Rotylenchulus reniformis

Since not all eggs in inoculum are capable of hatching and invading roots, a conservative figure of 1/10 of the Pi was used.

Possible rotation crops can be distinguished into two groups. Susceptible crops that are capable of supporting the development of *R. reniformis* populations, with a reproduction factor greater than 1.

The reproductive factors of *R. reniformis* in roots of resistant crops were less than 1 indicating that the final populations densities of *R. reniformis* decreased (Marwoto, B. 2010).

Pratylenchus zeae

Live nematodes were used in inoculum, so no hatching was involved, and live nematodes are capable of invading roots. Pi was the actual number of live nematodes prepared in the inoculum.

Possible rotation crops could be distinguished into two groups. Susceptible crops that can support the development of *P. zeae* populations, with a reproduction factor greater than 1.

Resistant crops have significantly less *Pratylenchus* juveniles recovered from the roots compared with the susceptible control.

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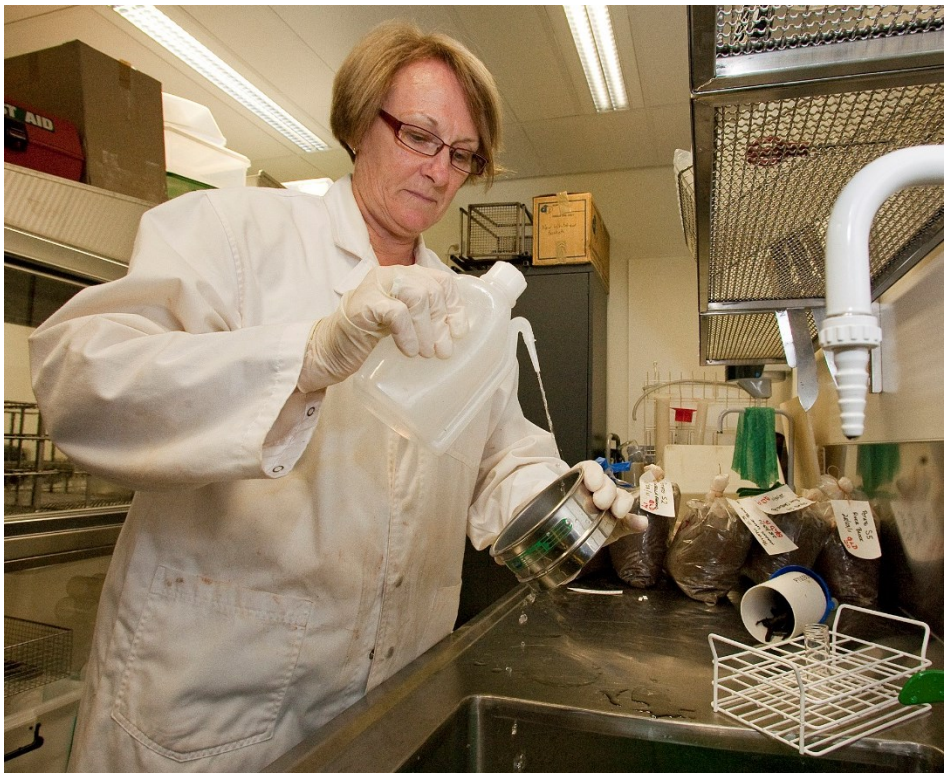

Image 1 Jennifer Cobon extracting nematode eggs on a 38 µm sieve.

Image 2 Left, Tim Shuey checking Whitehead trays used to extract nematodes from soil samples. Right, a juvenile root-knot nematode at 50X magnification.

Image 3 Wayne O'Neill identifying nematode species from collected soil samples.