



Initial survey of plant-parasitic nematodes in sweetpotato

PW17001 Final report Appendix 3 Integrated pest management of nematodes in sweetpotato

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Initial survey of plant-parasitic nematodes in sweetpotato production

Summary

Field surveys to understand region specific nematode species occurrences and identify any potential biosecurity issues were undertaken throughout the major cropping regions. Initial surveys were conducted in the sweetpotato productions areas of Wide Bay, northern NSW, Central Queensland, southern Queensland, North Queensland. A total of 85 fields were sampled across these regions: 45 in Wide Bay, 17 in northern NSW, 12 in Central Qld, 6 in southern Qld, and 5 in North Qld. Plant-parasitic nematodes were identified and quantified from a soil sample taken at a depth of 10-15 cm.

From this survey, 81 soils (43 from Wide Bay, 16 from northern NSW, 12 from Central Qld, 6 from southern Qld and 4 from North Qld) were submitted to SARDI (South Australian Research and Development Institute) for molecular identification of the root-knot nematodes species present.

Outcomes

A new detection of *R. reniformis* during the survey in southern Qld extends the known geographic range of this nematode species. Growers have a greater understanding of the plant-parasitic nematodes in each of the growing regions and of the ones causing the impacts to crop yield and damage. Individual growers have been informed which nematode species are present on their farms.

Initial survey

Initial surveys were conducted in the sweetpotato production areas of Wide Bay, northern NSW, Central Qld, southern Qld, North Qld. A total of 85 fields were sampled across these regions: 45 in Wide Bay, 17 in northern NSW, 12 in Central Qld, 6 in southern Qld, and 5 in North Qld. Plant-parasitic nematodes were identified and quantified from a soil sample taken at a depth of the 10-15 cm (Table 1).

From this survey, 81 soils (43 from Wide Bay Qld, 16 in northern NSW, 12 in Central Qld, 6 in southern Qld and 4 in North Qld) were submitted to SARDI (South Australian Research and Development Institute) for molecular identification of the root-knot nematodes species present (Table 2).

Materials and methods

General nematology methods are described in detail in Appendix 2.

Plans for intensive surveys were developed to sample a representative group of fields, both pre-plant and post-harvest. Information on each field's soil type and previous cropping history has been collected and will be used to assess the impact of these factors on nematode occurrence and population density. Drought conditions especially during the latter half of 2019 and throughout 2020 prevented further surveys as dry soil does not give a true representation of nematode numbers. Recent rainfall events in Queensland and northern New South Wales and the relaxation of Covid restrictions allowed surveys to recommence in October 2020.

Representative soil samples were taken from a block/field to a depth of 10-15 cm using a clean probe, corer or auger. Nematodes were extracted from the soil samples in a Whitehead tray over four days. Nematodes were retrieved on a 38 µm sieve and then examined under a compound microscope for identification and quantification of all plant-parasitic nematode species.

The major plant-parasitic nematodes recovered were identified using light microscopy and morphological characteristic according to the Commonwealth Institute of Parasitology (1972-1977) descriptions.

Split soil samples were received by SARDI (South Australian Research and Development Institute) for molecular identification of the root-knot nematodes species present.

Results and discussion

Eighty-five survey samples were collected and processed by the DAF nematology team. Eighty-one soil samples were sent to SARDI for molecular identification of root-knot nematode.

Initial results show that root-knot nematode (RKN), primarily *Meloidogyne incognita* and *M. javanica*, is widespread across the industry and reniform nematode appears to be extending its geographical range. A detection in the Lockyer valley is believed to be the most southerly recording of this species in Australia to date.

Morphological identification

The results of the initial survey (Table 1) determined that *Meloidogyne* spp., (root-knot nematode) was the most common nematode pest in sweetpotato. Root-knot nematode was present in 55 of 85 sites (65%) across all regions. In Wide Bay, 27 of 45 sites (60%) were found to have *Meloidogyne* spp., with 15 of 17 sites (88%) in northern NSW, 7 of 12 sites (58%) in Central Qld, 2 of 6 sites (33%) in southern Qld and 4 of 5 sites (80%) in North Qld all similarly infested with *Meloidogyne* spp.

Rotylenchulus reniformis (reniform nematode) which is a major pest in the USA was present at some sites, mainly in warmer areas with 3 of 45 sites (7%) in Wide Bay, 4 of 12 sites (33%) in Central Qld and 1 of 6 sites (25%) in southern Qld having reniform nematode present. This new detection of *R. reniformis* in southern Qld extends the known geographic range of this nematode species previously known to occur in Queensland from Bundaberg north (Table 1).

Pratylenchus zae was found from 24 sites (53%) in Wide Bay, 5 sites (29%) in northern NSW, 6 sites (50%) in Central Qld, 4 sites (67%) in southern Qld, 3 sites (60%) in North Qld (Table 1).

Spiral nematodes and *Rotylenchulus parvus* (another reniform nematode) were common, but in low numbers suggesting sweetpotato is not a good host to these species. Also in low populations were stubby, stunt, ring and dagger nematodes.

Molecular identification

From this survey, 81 soils (43 from Wide Bay, 16 from northern NSW, 12 from Central Qld, 6 from southeast Qld and 4 from North Qld) were split so that paired samples could be sent to SARDI (South Australian Research and Development Institute) for molecular identification of the root-knot nematodes species present (Table 2, Appendix 3). and also analysed by DAF using traditional techniques.

Total production area

In total from sweetpotato soil samples (n=81), SARDI identified root-knot nematode DNA using their non-specific *M. javanica/incognita/arenaria* primers from 54 sites (67%) with no DNA identified from 27 sites (33%). In 33 of these 54 samples (61%), SARDI were further able identify the root-knot nematode to species level (using a specific assay), but in 21 samples the RKN species present was not able to be determined (Table 1.2).

Using specific primers

Twenty-three of 54 sites (43%) were identified with *M. javanica* as either single or mixed populations making it the most commonly identified species of RKN throughout the sweetpotato production area (Table 1.2). *Meloidogyne incognita* was identified in 10 of 54 sites (19%) as either single or mixed populations. *Meloidogyne arenaria* was identified at 1 of 54 sites (2%) as a mixed population with *M. javanica*. *M. hapla* was identified at 7 of 54 sites (13%) as either single or mixed populations. *Meloidogyne hapla*, a cooler climate RKN, was only found at sites in southern Qld and northern NSW, while *M. javanica*, a very tropical RKN, was most predominant in the Wide Bay region and in Central Qld. *Meloidogyne incognita* is a subtropical species and was identified mostly in northern NSW and Wide Bay.

Regional results

Regionally, with the Wide Bay samples (n=43) SARDI identified root-knot nematode DNA using their non-specific *M. javanica/incognita/arenaria* primers from 28 sites (65%) with no DNA from 15 sites (35%) (Table 2, 3)

Using specific primers

- 13 sites were identified with a single population of *M. javanica* (46%)
- 3 sites were identified as *M. incognita* (11%)
- 2 sites were mixed populations of *M. javanica* and *M. incognita* (7%)
- 1 site was a mixed populations of *M. javanica* and *M. arenaria* (4%)
- species identification of *Meloidogyne* was not successful from 9 sites (32%)

From the northern NSW samples (n=16) SARDI identified root-knot nematode DNA using their non-specific *M. javanica/incognita/arenaria* primers from 14 sites (88%).

Using specific primers

- 1 site was identified as *M. javanica* (7%)
- 3 sites were identified as *M. hapla* (22%)
- 1 site was identified as *M. incognita* (7%)
- 1 site was a mixed populations of *M. javanica* and *M. incognita* (7%)
- 1 site was a mixed populations of *M. javanica* and *M. hapla* (7%)
- 2 sites were mixed populations of *M. incognita* and *M. hapla* (14%)
- species identification of *Meloidogyne* was not successful for 5 sites (36%)

From the Central Qld samples (n=12) SARDI identified root-knot nematode DNA using their non-specific *M. javanica/incognita/arenaria* primers from 7 sites (58%).

Using specific primers

- 2 sites were identified as *M. javanica* (29%)
- species identification of *Meloidogyne* was not successful for 5 sites (71%)

From the southern Qld samples (n=6) SARDI identified root-knot nematode DNA using their non-specific *M. javanica/incognita/arenaria* primers from 4 sites (67%).

Using specific primers

- 1 site was identified as *M. javanica* (25%)
- 1 site was identified as *M. hapla* (25%)
- species identification of *Meloidogyne* was not successful for 2 sites (50%).

From the North Qld samples (n=4) SARDI identified root-knot nematode DNA using their non-specific *M. javanica/incognita/arenaria* primers from 2 sites (50%)

Using specific primers

- 1 site was identified as *M. javanica* (50%)
- 1 site was identified as *M. incognita* (50%)

Development of PreDicta SP, a nematode/soil biology diagnostic service

SARDI identified eight sites with their non-specific assay from which DAF were unable to extract and identify root-knot nematode, however, DAF identified seven different sites with root-knot nematode where SARDI was unable to identify DNA using their non-specific assay.

Comparison of these results showed a very poor correlation between manual counts and the molecular results for samples with relatively low root-knot populations (manual count range 0 – 115 RKN/200mL dry soil). As a consequence of these results and consideration of costs and benefits, a decision was made to utilize manual counts for the duration of the project and not to pursue a PreDicta system for the sweetpotato industry at this point.

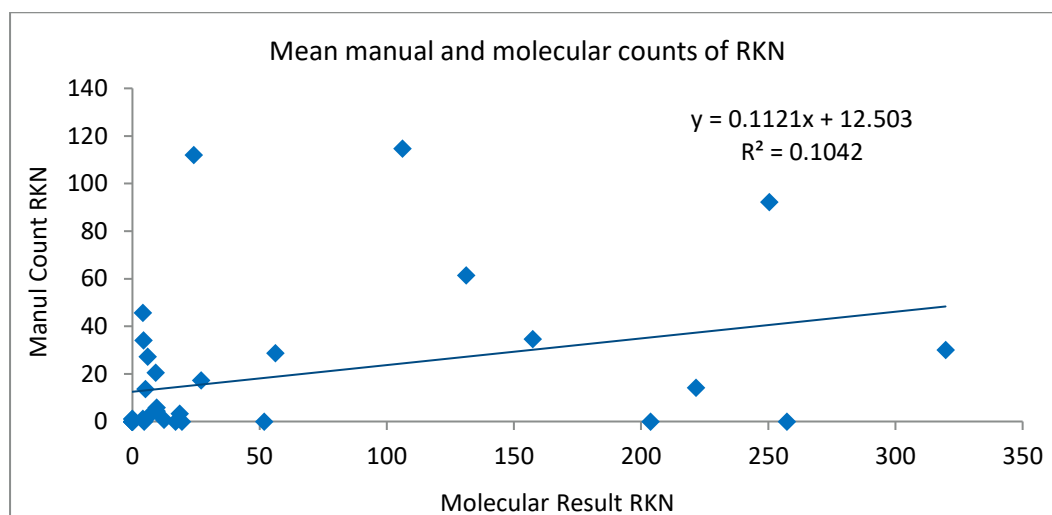


Figure 1 Mean manual and molecular counts of RKN from the initial surveys.

Conclusion

The regions with the most mixed populations were Wide Bay and northern NSW with mixes of *M. javanica/M. incognita* and *M. javanica/M. arenaria* in Wide Bay and *M. javanica/M. incognita*, *M. javanica/M. hapla* and *M. incognita/M. hapla* in northern NSW.

Meloidogyne javanica, *M. incognita* and *M. hapla* were all found as single species populations in the different regions, but *M. arenaria* was only found in a mixed population (with *M. javanica*) during this initial survey.

As a consequence of poor correlation between manual counts and the molecular results and consideration of costs and benefits, a decision was made to utilize manual counts for the duration of the project and not to pursue a PreDicta system for the sweetpotato industry at this point.

Table 1 Morphological identification of plant-parasitic nematodes/200 g dry soil weight extracted over four days using the Whitehead tray method from soils surveyed during surveys conducted 2017.

	Positive sites	Mean nematode numbers	Range of nematode numbers
Wide Bay Queensland (n=45)			
<i>Meloidogyne</i> spp. (root-knot nematode)	27 (60%)	255	(1-3413)
<i>Rotylenchulus reniformis</i> (reniform nematode)	3 (7%)	21	(5-35)
<i>Pratylenchus zaeae</i> (lesion nematode)	24 (53%)	61	(1-220)
<i>Helicotylenchus dihystrera</i> (spiral nematode)	12 (27%)	503	(1-1611)
<i>Rotylenchus brevicaudatus</i> (spiral nematode)	12 (27%)	207	(3-985)
<i>Paratrichodorus</i> sp. (stubby root nematode)	16 (36%)	16	(1-94)
<i>Rotylenchulus parvus</i> (reniform nematode)	17 (38%)	61	(1-638)
<i>Tylenchorhynchus</i> sp. (stunt nematode)	8 (18%)	6	(2-14)
<i>Xiphinema</i> sp. (dagger nematode)	5 (11%)	48	(1-187)
<i>Criconemella</i> sp. (ring nematode)	7 (16%)	22	(2-53)
Total Free-living Nematodes	45	1607	(79-2495)
northern NSW (n=17)			
<i>Meloidogyne</i> spp. (root-knot nematode)	15 (88%)	297	(1-1611)
<i>Pratylenchus zaeae</i> (lesion nematode)	5 (29%)	26	(1-88)
<i>Helicotylenchus dihystrera</i> (spiral nematode)	16 (94%)	127	(8-591)
<i>Paratrichodorus</i> sp. (stubby root nematode)	5 (29%)	3	(1-6)
<i>Rotylenchulus parvus</i> (reniform nematode)	2 (12%)	47	(12-18)
<i>Xiphinema</i> sp. (dagger nematode)	2 (12%)	55	(5-104)
<i>Criconemella</i> sp. (ring nematode)	4 (24%)	4	(1-9)
Total Free-living Nematodes	17	1577	(275-2870)
Central Queensland (n=12)			
<i>Meloidogyne</i> spp. (root-knot nematode)	7 (58%)	98	(1-587)
<i>Rotylenchulus reniformis</i> (reniform nematode)	4 (33%)	265	(15-799)
<i>Pratylenchus zaeae</i> (lesion nematode)	6 (50%)	22	(1-57)
<i>Helicotylenchus dihystrera</i> (spiral nematode)	6 (50%)	26	(1-123)
<i>Rotylenchus brevicaudatus</i> (spiral nematode)	2 (17%)	21	(18-24)
<i>Paratrichodorus</i> sp. (stubby root nematode)	5 (42%)	4	(1-7)
<i>Rotylenchulus parvus</i> (reniform nematode)	1 (8%)	130	
<i>Tylenchorhynchus</i> sp. (stunt nematode)	1 (8%)	27	
<i>Criconemella</i> sp. (ring nematode)	2 (17%)	201	(31-371)
Unknown	1 (8%)	14	
Total Free-living Nematodes	12	7552	(61-1129)

southern Queensland (n=6)			
<i>Meloidogyne</i> spp. (root-knot nematode)	2 (33%)	8	(3-13)
<i>Rotylenchulus reniformis</i> (reniform nematode)	1 (2%)	53	
<i>Pratylenchus zae</i> (lesion nematode)	4 (67%)	28	(1-106)
<i>Helicotylenchus dihystra</i> (spiral nematode)	5 (83%)	16	(1-34)
<i>Rotylenchus brevicaudatus</i> (spiral nematode)	2 (33%)	26	(17-35)
<i>Paratrichodorus</i> sp. (stubby root nematode)	4 (67%)	3	((1-4)
<i>Rotylenchulus parvus</i> (reniform nematode)	4 (67%)	60	(1-210)
Total Free-living Nematodes	6	913	(371-1431)
North Queensland (n=5)			
<i>Meloidogyne</i> spp. (root-knot nematode)	4 (80%)	1098	(1-2620)
<i>Pratylenchus zae</i> (lesion nematode)	3 (60%)	13	(1-25)
<i>Rotylenchulus parvus</i> (reniform nematode)	2 (40%)	3	(3-7)
<i>Criconemella</i> sp. (ring nematode)	1 (20%)	1	
Total Free-living Nematodes	5	3128	(1187-4051)

Table 2 Results of the molecular identification of *Meloidogyne* spp. (root-knot nematode) by SARDI from 81 sites using non-specific and specific primers from soils surveyed during the initial surveys conducted in 2018.

DAF code	Region	Using non-specific primers	Further identification using specific primers			
		<i>M. javanica/ incognita/ arenaria</i> (pgDNA/g Sample)	<i>M.hapla</i> (pgDNA/g Sample)	<i>M.javanica</i> (pgDNA/g Sample)	<i>M.incognita</i> (pgDNA/g Sample)	<i>M.arenaria</i> (pgDNA/g Sample)
RL01	Wide Bay Qld	5	0	0	0	0
RL02	Wide Bay Qld	241	0	372	0	0
RL03	Wide Bay Qld	512	0	558	0	0
RL04	Wide Bay Qld	175	0	60	0	0
RL05	Wide Bay Qld	56	0	58	309	0
RL06	Wide Bay Qld	0	0	0	0	0
RL07	Wide Bay Qld	222	0	259	0	0
RL08	Wide Bay Qld	27	0	0	0	0
RL09	Wide Bay Qld	346	0	407	0	0
RL10	Wide Bay Qld	106	0	41	0	410
RL26	Wide Bay Qld	0	0	0	0	0
RL28	Wide Bay Qld	157	0	142	0	0

RL29	Wide Bay Qld	320	0	224	0	0
RL30	Wide Bay Qld	0	0	0	0	0
RL31	Wide Bay Qld	4	0	0	0	0
RL32	Wide Bay Qld	0	0	0	0	0
RL33	Wide Bay Qld	16	0	0	244	0
RL34	Wide Bay Qld	32	0	39	0	0
RL35	Wide Bay Qld	0	0	0	0	0
RL36	Wide Bay Qld	0	0	0	0	0
RL37	Wide Bay Qld	261	0	234	0	0
RL38	Wide Bay Qld	0	0	0	0	0
RL39	Wide Bay Qld	64	0	0	0	0
RL40	Wide Bay Qld	4	0	0	0	0
RL41	Wide Bay Qld	32	0	49	0	0
RL48	Wide Bay Qld	0	0	0	0	0
RL49	Wide Bay Qld	51	0	26	0	0
RL50	Wide Bay Qld	0	0	0	0	0
RL51	Wide Bay Qld	9	0	0	0	0
RL54	Wide Bay Qld	4	0	0	107	0
RL60	Wide Bay Qld	0	0	0	0	0
GS01	Wide Bay Qld	52	0	56	131	0
GS02	Wide Bay Qld	0	0	0	0	0
GS03	Wide Bay Qld	0	0	0	0	0
GS04A	Wide Bay Qld	204	0	0	393	0
GS04B	Wide Bay Qld	7044	0	0	0	0
GS04C	Wide Bay Qld	0	0	0	0	0
GS05	Wide Bay Qld	148	0	219	0	0
GS06	Wide Bay Qld	19	0	0	0	0
GS07	Wide Bay Qld	0	0	0	0	0
GS08	Wide Bay Qld	257	0	88	0	0
GS09	Wide Bay Qld	76	0	0	0	0
GS10	Wide Bay Qld	0	0	0	0	0
RL18	northern NSW	319	0	25	828	0
RL19	northern NSW	250	3	173	0	0
RL20	northern NSW	4	4	0	0	0
RL21	northern NSW	5	0	0	0	0
RL22	northern NSW	9	2	0	0	0
RL23	northern NSW	0	0	0	0	0

RL24	northern NSW	4	0	0	0	0
RL25	northern NSW	10	0	0	0	0
RL45	northern NSW	66	2	0	110	0
RL46	northern NSW	1	0	0	0	0
RL47	northern NSW	118	0	162	0	0
RL53	northern NSW	11	0	0	0	0
EC02	northern NSW		0	0	0	0
EC04	northern NSW	314	0	0	548	0
EC05	northern NSW	4	2	0	0	0
EC06	northern NSW	24	2	0	68	0
RL11	Central Qld	0	0	0	0	0
RL12	Central Qld	12	0	0	0	0
RL13	Central Qld	568	0	732	0	0
RL14	Central Qld	6	0	0	0	0
RL15	Central Qld	8	0	0	0	0
RL16	Central Qld	10	0	0	0	0
RL17	Central Qld	131	0	188	0	0
RL55	Central Qld	0	0	0	0	0
RL56	Central Qld	0	0	0	0	0
RL57	Central Qld	12	0	0	0	0
RL58	Central Qld	0	0	0	0	0
RL59	Central Qld	0	0	0	0	0
RL42	southern Qld	0	0	0	0	0
RL43	southern Qld	0	0	50	0	0
RL44	southern Qld	8	0	0	0	0
EC07	southern Qld	0	0	0	0	0
EC08	southern Qld	17	0	0	0	0
EC09	southern Qld	19	4	0	0	0
MH01	North Qld	0	0	0	0	0
MH003	North Qld	0	0	0	0	0
MH004	North Qld	512	0	697	0	0
MH005	North Qld	442	0	0	939	0

Table 3 Results of the SARDI molecular identification of *Meloidogyne* spp. (root-knot nematode) summarised into sweetpotato growing regions from soils surveyed during initial surveys conducted

<i>Meloidogyne</i> spp. ID	Sweetpotato growing regions				
	Wide Bay (n=43)	northern NSW (n=16)	Central QLD (n=12)	southern Qld (n=6)	North Qld (n=4)
DNA positive sites	28	14	7	4	2
<i>M. javanica</i>	13 (46%)	1 (7%)	2 (29%)	1 (25%)	1 (50%)
<i>M. incognita</i>	3 (11%)	1 (7%)	N/A	N/A	1 (50%)
<i>M. arenaria</i>	N/A	N/A	N/A	N/A	N/A
<i>M. hapla</i>	N/A	3 (22%)	N/A	1 (25%)	N/A
<i>M. javanica, M. incognita</i>	2 (7%)	1 (7%)	N/A	N/A	N/A
<i>M. javanica, M. arenaria</i>	1 (4%)	N/A	N/A	N/A	N/A
<i>M. javanica, M. hapla</i>	N/A	1 (7%)	N/A	N/A	N/A
<i>M. incognita, M. hapla</i>	N/A	2 (14%)	N/A	N/A	N/A
No identification	9 (32%)	5 (36%)	5 (71%)	2 (50%)	0 (0%)

Conference presentation

Cobon, J.A., O'Neill, W.T., Shuey, T., Langenbaker, R., Dennien, S., 2022. Plant-parasitic nematodes in sweetpotato production areas in Australia. Oral presentation at the 11th Australasian Soilborne Disease Symposium, Cairns, August 2022.



Image 1 Some blocks were sampled at the end of cover crop rotations.



Image 2 Some blocks were sampled post-harvest.



Image 3 Block sampled some weeks after-harvesting showing potential volunteer roots.



Image 4 Some blocks were sampled prior to or just after planting.



Image 5 This block was sampled prior to bed forming.



Image 6 Project team member Mike Hughes and grower soil sampling in a block.