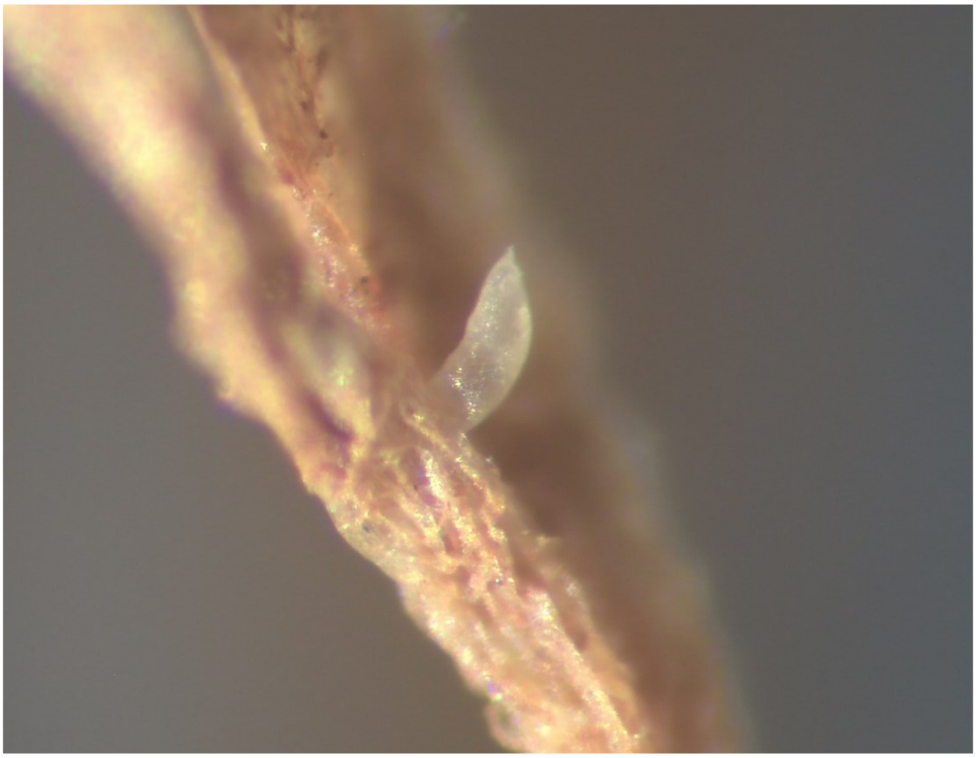
The effects of *Rotylenchulus reniformis* on two sweetpotato cultivars



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PW17001 Final report Appendix 19 Integrated pest management of nematodes in sweetpotato

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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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Introduction

In 1960 *R. reniformis* was found pathogenic to sweetpotato (Martin 1960) and has since become an important pest in the United States (US) sweetpotato production (Smith et al 2017). The literature describing the damage on sweetpotato from *R. reniformis* is limited. Robinson (2002), Abel et al. (2007) and Smith et al. (2017) reported *R. reniformis* causes yield decreases, with minimal visual symptoms. On the other hand, Thomas (1982), Walters and Barker (1993) and Dutta et al. (2018) found *R. reniformis* reduced yields with visual symptoms of root cracking, root distortion, root necrosis and foliage stunting and yellowing. Stirling (2022) adds that problems caused by *R. reniformis* are difficult to diagnose as distinctive symptoms on roots are not produced.

R. reniformis can survive in air-dried soil stored at 20-25°C for seven months (Reddy 2021). Under drought conditions they can enter an anhydrobiotic state which can keep the nematode alive for up to two years outside of a host plant (Robinson et al 1997, Wang 2001). A trait which enhances survival and makes the pest more difficult to control.

In the state of Louisiana and Georgia in the US, the *R. reniformis* has proven to be a problematic nematode affecting sweetpotato production. Previously *M. incognita* was considered the most important parasite of sweetpotato, but the increase and spread of *R. reniformis* populations has seen the rise of prominence of this nematode (Smith et al 2017).

R. reniformis is present in Australia. It has long been established on horticultural crops in tropical parts of the country and was detected in soils of cotton farming systems of Emerald in 2003 (Roughly & Smith 2015). The nematode has also been found in Queensland's sweetpotato production areas (Stirling 2022, Dennien et al 2022a). An integrated pest management project aimed at nematodes found that *R. reniformis* populations may increase as root-knot nematode populations decrease (Dennien et al 2022b). Thomas, alone (1982), then later with Clark (1983), observed a competitive and inhibitive dynamic between the two nematode species that would often see one species dominate the other.

The aim of this pot trial was to assess the effects of *R. reniformis* on two popular Australian sweetpotato cultivars, Beauregard and Bellevue, to determine the damage the nematode causes to storage roots. The cultivars were chosen due to their nematode resistance. Bellevue, developed by Louisiana Agricultural Experiment Station, is considered highly resistant to southern root-knot nematode, *Meloidogyne incognita* (La Bonte et al 2015). Beauregard is a susceptible variety with Walters and Barker (1993) describing the cultivar as an excellent host for nematodes.

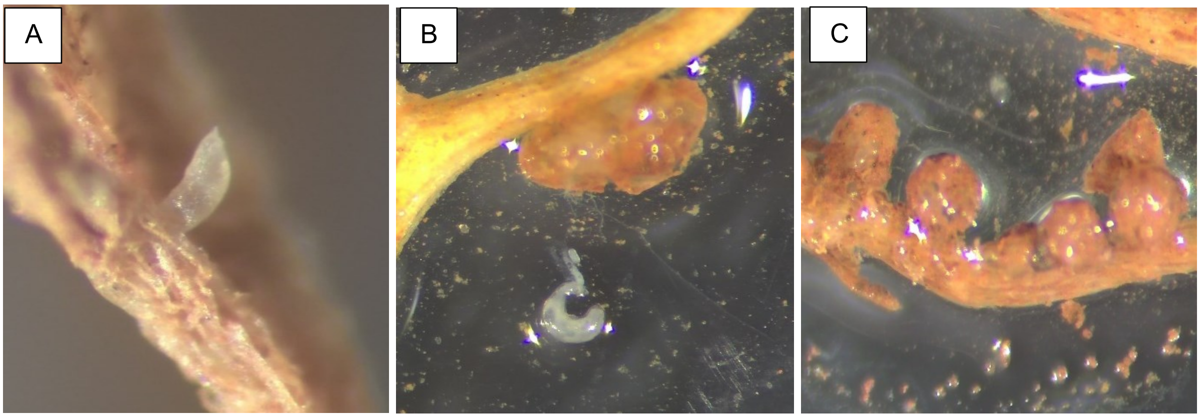

Image 1. a) *R reniformis* attached to a fibrous root; b) detached *R. reniformis* alongside an egg mass attached to the fibrous root; c) *R. reniformis* egg masses on fibrous roots.

Methodology

Experimental Set Up

This experiment was a randomised pot trial grown in pasteurised soil in an insect proof plant house. The trial consisted of two sweetpotato cultivars (Bellevue and Beauregard) each with two treatments, a nematode treatment (pots inoculated with a known number of juvenile *R. reniformis* nematodes) and a control treatment, (no inoculation) and six replicates of each cultivar/nematode treatment (Table 1). Twelve cuttings of each cultivar were grown in individual pots giving a total of 24 pots. This pot trial was grown according to best sweetpotato practice for 132 days, approximately the duration of a commercial crop.

Table 1. Pot trial design

Cultivar	**Number of plants**	**Treatment**	
Beauregard	12	6 x inoculated with *R. reniformis*	6 x control
Bellevue	12	6 x inoculated with *R. reniformis*	6 x control

The vines were planted on the 27th of September 2022 (Image 2a & 2b). The inoculation of *R. reniformis* occurred 16 days later, once the vines had established a thriving root system, ensuring an effective delivery of the nematodes onto plants.

Image 2. a) Beauregard (back) and Bellevue (front) vine; b) vine is laid in the furrow with all 4 nodes buried; c) inoculum mix evenly distributed into the furrows.

Inoculation was delivered by applying a bag of sand and root mixture infested with *R. reniformis* derived from a pure population into furrows dug 5cm deep either side of the vine (Image 2c). Each bag consisted of 100g of infested roots mixed with 200ml of nematology sand mix. The approximate reniform egg count being delivered to each pot was 156,800 eggs/pot (5807 eggs per litre of soil).

The trial was harvested on the 6 February 2023. The above ground biomass was removed, and roots obtained from each pot were washed free of soil. A representative soil sample was collected from each pot and sent to DAF nematology experts to determine the nematode populations per pot.

Assessment and Measurements

Roots harvested from each pot were individually inspected for damage according to sweetpotato nematode assessment protocols. Individual root weight, length, and diameter were recorded as was an overall weight of fibrous roots. While weights were taken, grading was done by damage level using industry standards to determine first grade, second grade or non-marketable sweetpotatoes.

Data was collected;

- Quantitative measurements using balance and calipers.
- Qualitative measurements;

Damage was rated using the proportion of skin surface area affected:

- Low: 0 – 33% of the sweetpotato surface area
- Medium: 34 – 66% of the sweetpotato surface area
- High: 67 – 100% of the sweetpotato surface area

Presence / Absence of listed defects were also recorded.

Data analysis

The total root weight, mean root weight, mean root length, mean root diameter, and fibrous root weight were analysed using analysis of variance (ANOVA). The proportion of roots with the different types of damage were analysed using a generalised linear model (GLM). The number of roots in each pot were analysed using a Poisson GLM with a log link function. Analysis results were deemed significant at the 0.05 level. Where a significant effect was found, the 95% least significant difference (lsd) was used to make pairwise comparisons.

Results

Nematode Counts

All inoculated pots had high numbers of R. reniformis in the soil samples, indicating that the pest had established and reproduced. Counts ranged from 3455 to 22467 per 200g of soil (dry weight). The variety Beauregard had a mean count of 13 432/200g soil whereas Bellevue had a mean of 7021. This indicates that Bellevue's resistance to root-knot nematode may also confer some partial resistance to R. reniformis. Further data analysis is required to determine statistical significance of this finding.

Root Count

There was a noticeable difference of treatment effect on root count. Both Beauregard and Bellevue produced less roots in the nematode inoculated pots. However, the analysis showed this was not significant ($p = 0.260$). When comparing the treatment effect without cultivar influence, a marginally significant effect of treatment is found suggesting the nil treatments produced more roots per pot than the nematode treatment (Table 2).

Table 1 Root count by treatment

Root Count by Treatment		
Treatment	**Pred Mean**	**se**
Nematode	7.6	0.79
Nil	10.0	0.91

Root weight and size

Although there were no significant differences in total root weight by treatment ($p > 0.05$), the mean individual root weight was significantly higher in the nematode treated pots than compared with the nil treatments (Table 3). While not significant ($p > 0.05$), the roots from the nematode treatments had a higher mean root length and mean root diameter (Table 4).

Table 2 Mean individual root weight by treatment.

Treatment	Pred Mean
Nematode	116.2 a
Nil	91.8 b
p-value	0.031
$F_{(1,15)}$	5.66
se	7.26
95% lsd	21.87

Table 3 Mean root length and mean root diameter per cultivar / treatment.

Cultivar	Treatment	Mean Root Length	Mean Root Diameter
Beauregard	Nematode	134.8	38.8
	Nil	128.9	37.7
Bellevue	Nematode	149.4	36.5
	Nil	141.0	34.0

Darkened Lateral Root Scar

Darkened lateral feeder root scars (DLRS) were found on both cultivars. For both cultivars, the nematode treated pots had significantly higher mean proportion of DLRS than the nil treatments (Figure 1). The analysis suggests the incidence of darkened lateral feeder root scars is driven by treatment.

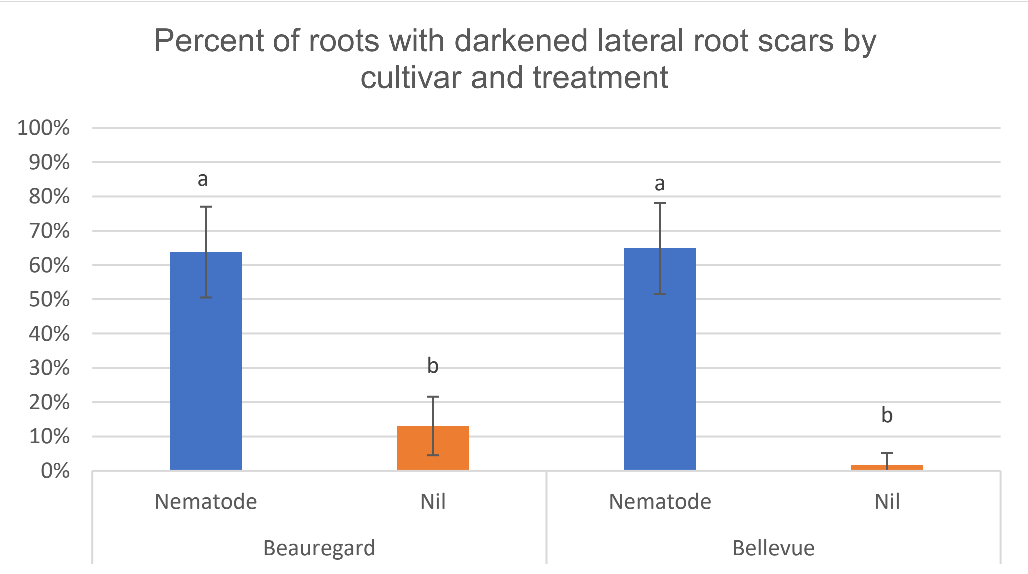

Figure 1 Percent of DLRS by cultivar and treatment

Black Pimple

Black pimples were another visual defect that was detected on both cultivars. Analysis on the occurrence of black pimples was marginally significant when comparing inoculated pots with nil treatment (Table 5).

Table 5. Mean incidences of black pimple by treatment

Treatment	Pred Mean	se

Nematode	0.2218	0.08028
Nil	0.0414	0.03345

Discussion

Nematode treated pots produced a lower quantity of roots. However, the roots produced had a higher mean root weight, length, and diameters (i.e. were larger roots). This trend was evident in both cultivars. *R. reniformis* may reduce the number of developing storage roots. Thomas (1982) observed a significant root growth stimulation in *R. reniformis* infested plants. Reducing the number of developing roots could direct more nutrients and energy to fewer roots, leading to an increase in size but a reduced yield overall. The nematode free pots have more roots competing for space and nutrients. Overly large sweetpotatoes in a commercial crop are not desirable and are downgraded as "Jumbos". This experiment shows that the presence of *R. reniformis* lead to fewer, larger storage roots and so could cause economic losses that are not obvious to a grower.

Two visual defects that affect marketability were found to be related to the presence of *R.reniformis* in this experiment. DLRS occur when the lateral roots are damaged, and a wound response is initiated. The result is an indent on the root surface filled with a darkened scab-like layer on the periderm. Nematode treated pots had a significantly higher level of DLRS. As DLRS were still found on the nil treatments, this may a naturally occurring event that nematodes exacerbate. *R. reniformis* were not observed with microscopic examination of the DLRS.

While only marginally significant, higher levels of black pimples were found on nematode treated roots. Finding black pimples on nil treated roots also suggests this is a natural defect. Higher proportions on nematode treated roots may indicate that *R. reniformis* intensifies the occurrence.

There were no cracks or rots recorded in this trial. This does not rule out the possibility that *R. reniformis* may cause these defects in the field. Barnacle defects (extensive areas of raised lesions) were found exclusively on Beauregard roots and were not significant, suggesting this may not be caused by *R. reniformis* but instead may be a cultivar issue. Raised pimples, elongated lenticles and sunken lenticles while found on both cultivars and treatments showed no significant relationships.

Conclusion

While it is difficult to definitively distinguish the damage caused by *R. reniformis,* this experiment indicates that the nematode has an impact on the quantity and quality of sweetpotato crops. *R. reniformis* will reduce the number of roots a plant can produce though the remaining roots may be larger due to less roots competing for resources. The reduction in root numbers and the inclination for *R. reniformis* to feed on fibrous roots, suggest that the most damage comes while the roots are still forming and therefore prevent development. This observation supports the findings of Clark and Wright (1983) who suggested that R. reniformis won't develop on storage roots once they enlarge past approximately 5 – 10mm in diameter.

DLRS and black pimple will be found in higher proportions than is naturally occurring when *R. reniformis* is present, reducing the quality of sweetpotato. The presence of the nematode will reduce the quantity and quality of sweetpotato harvests. It is recommended that the current best practice for nematode management be followed to ensure the harvest of quality sweetpotatoes.

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