Sustainable farming systems trials - Nematode population monitoring

PW17001 Final report Appendix 15 Integrated pest management of nematodes in sweetpotato

Wayne O'Neill August 2023

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Summary

Two long-term field trials were conducted at Bundaberg Research Facility to test the feasibility of using integrated management tactics to minimise losses caused by root-knot nematode. An intensive trial (with 4 crops over 5 years) was managed close to grower best practice, and also included some treatments with high rates of organic amendments. The extensive trial was more experimental and included pre-formed beds to minimize tillage prior to planting, as well as a wider range of rotation crops. There were 3 crops over 5 years in this trial.

Statistically significant suppression of root-knot nematode was achieved for 3 consecutive harvests by the organic matter (chicken manure + sawdust) amendment treatment in the intensive trial. The compost amendment treatment also had significantly less root-knot than the nil and nematicide treatments at the 3rd and 4th harvests.

There were no significant differences in root-knot nematode counts between treatments at any harvest in the extensive trial, although there was a trend for lower root-knot numbers in treatments with organic amendments. The pre-formed bed system meant that most of the organic amendments had to be applied a long time prior to the sweetpotato crop, with an apparent drop in efficacy of those treatments. This was reflected by reductions in free-living nematode numbers and less organic carbon accumulation during the course of the crop, which contrasted with stable/increased free-living nematodes numbers and greater organic carbon gains in comparable treatments in the intensive trial.

The early bed formation system in the extensive trial was not successful at enhancing root-knot nematode suppression, and the v-furrow of amendment treatment (tested in both trials) also did not provide any significant benefits in terms of nematode suppression or crop yield/quality. Resistant rotation crops, including forage sorghum, sunn hemp, white French millet and pasture grasses were all successful in dramatically reducing root-knot nematode populations between sweetpotato crops. No treatment in either trial was successful in suppressing reniform nematode, another sweetpotato pest which became widespread in both trials during the 5 years of the field trials.

At most harvests, organic amendment treatments had the highest marketable yields or there was no difference compared with other treatments. Organic amendment treatments often had fewer nematode defects but sometimes had higher incidence of other defects.

Organic amendments have the potential for effective root-knot control as well as improved yield and long-term soil health benefits for growers willing to make them part of their system. The cost is comparable to nematicide treatment. On-farm evaluation of locally available materials would be recommended to determine if the practice will achieve consistency in results in a farming system.

Outputs

Farmer field days and grower updates on field trials

Project Reference Group Updates on field trials

ASPG annual general meeting updates on field trials

Conferences

O'Neill, W.T., Cobon, J.A., Shuey, T., Langenbaker, R., Dennien, S.E., 2022. Integrated management of Root-Knot nematode in sweetpotato. Oral presentation at the 11th Australasian Soilborne Disease Symposium, Cairns, August 2022.

Shuey, TA., O'Neill, W.O., Cobon, J.A., Langenbaker, R., B Day2, Bobby, J., Firrell, M., Hughes, M., Corner, R.D., Pattison, A.B., and Dennien, S.E. 2023. Suppression of Root-knot Nematode in Modified Commercial Sweetpotato Production Systems. Poster Presentation at the 24th Australasian Plant Pathology Society Conference, Adelaide, November 2023

Outcomes/ Take home message/key findings.

High rates of certain organic amendments have the potential for effective root-knot control as well as improved yield and long-term soil health benefits. Statistically significant root-knot nematode control was achieved by treatments comprising high rates of banded organic amendments (applied just prior to planting at bed formation), combined with a resistant rotation crop.

Ideally, these practices should be combined with other components - such as nematode monitoring, volunteer control and use of resistant sweetpotato cultivars where required - into an integrated nematode management program to deliver consistent crop yield and quality. Vigilance in on-farm biosecurity is critical to avoid introduction of new nematode pests which may be more difficult to manage.

Introduction

Long-term field trials were conducted over the course of the project to test the feasibility of using integrated management tactics to minimise losses caused by root-knot nematode (and potentially other plant-parasitic nematodes), while improving (rather than depleting) soil biological health. Longer term trials were required for these investigations as improvements in soil biological health and suppression of plant-parasitic nematodes may not be apparent in one crop cycle. Management practices included in the trials included:

- Inputs of organic matter from cover crops
- Use of diverse (largely root-knot resistant) rotation crops including legumes
- Minimum tillage, controlled traffic
- Organic amendments

Intensive Trial

Introduction

The intensive trial was designed to be similar to conventional best practice currently used by many sweetpotato growers. The aim was to assess the nematode control and soil health benefits provided by relatively high rates of organic amendments applied at bed formation, just prior to planting. A forage sorghum rotation was utilised in all plots between sweetpotato crops.

Methodology

The intensive sweetpotato trial had five replicates of 5 treatments laid out as a randomised complete block. Four harvests were completed in the 5 years of the trial. The five treatments were:

Treatment A	Organic Matter	band of sawdust + chicken manure
Treatment B	Compost	band of compost
Treatment C	V-furrow	Compost applied in a v-shaped furrow
Treatment D	Nil	no treatment control
Treatment E	Nematicide	Nimitz (fluensulfone)

Image 1. Double discs used to create v-furrow (right) and compost amendment in v-furrow (right)

Soil samples were collected by taking approximately 10 sub-sample along the length of each plot (5 – 15cm depth), which were well mixed by hand. To extract nematodes, two Whitehead trays per sample (approximately 200mL each) were set up for four days. The solutions from each tray were sieved twice through a 38µm sieve and then examined under a compound microscope for the presence of nematodes which were identified morphologically and quantified. Soil weights were recorded, and a subsample dried to calculate moisture content. Nematode counts were standardized and reported as the number per 200g dry weight equivalent soil.

Results and Discussion

In this trial, root-knot pressure was high in the first 3 crops but low for the 4th. There were no significant differences in root-knot numbers between the treatments at the first harvest (June 2020). However, at harvest 2 (June 2021), the organic matter amendment treatment (band of sawdust and chicken manure) had significantly less root-knot compared with all other treatments (a 109% reduction compared with the nil control treatment). At the third harvest (June 2022), both the organic matter treatment and the compost treatment had significantly less root-knot than the other three treatments (155% and 128% reduction respectively cf. nil). At the final harvest (May 2023), root-knot numbers were low across the trial, but the organic matter treatment still had significantly lower root-knot than all other treatments (166% reduction cf. nil). Figure 1 shows mean root-knot nematode counts for each treatment at the 4 harvests.

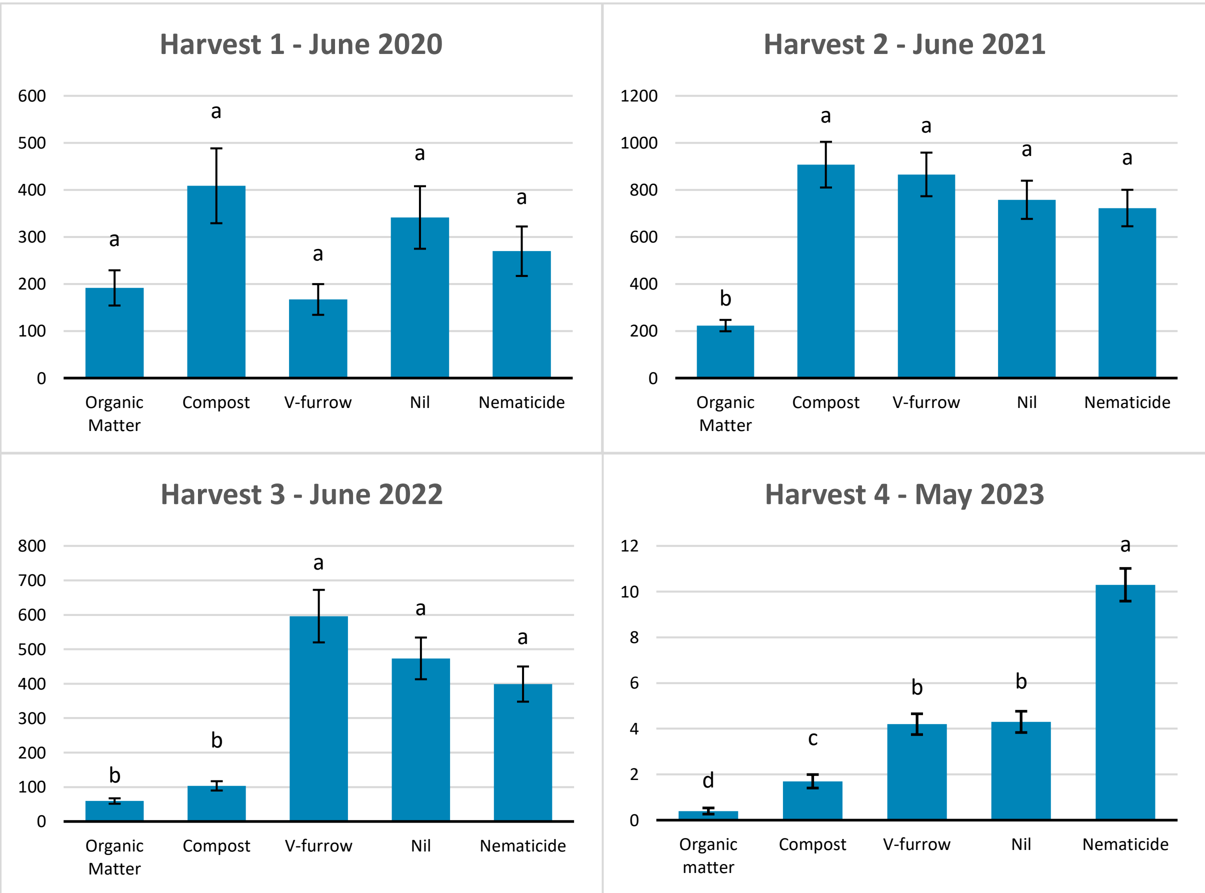

Figure 1. Mean root-knot nematode counts (per 200g dry soil) in the intensive field trial, at each of the four harvests.

This root-knot suppression in the organic matter treatment, which commenced at harvest 2, coincided with a boost in total free-living (TFL) nematode numbers which was first detected just prior to planting the second crop. At the January 2021, June 2021, January 2022 and June 2022 samplings, the organic matter treatment had a significantly higher mean count of TFL than all other treatments. Figure 2 illustrates the inverse relationship between root-knot nematode counts and TFL counts, in this case at harvest 2.

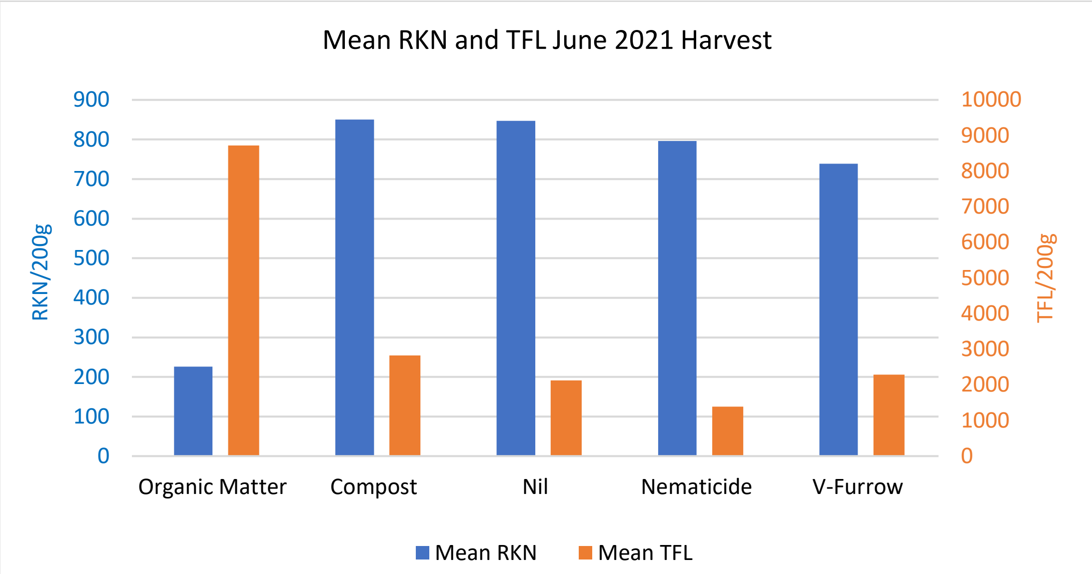

Figure 2.Mean root-knot and total free-living nematode counts (per 200g dry soil) at the second intensive trial harvest, June 2021. The organic matter treatment has significantly less root-knot and significantly more free-living nematodes than all other treatments

The banded compost treatment was variable in its performance; it was equivalent to the organic matter treatment at the June 2022 harvest and had significantly less root-knot than all other treatments (except organic matter) at the May 2023 harvest, but in the first two crops it was not as efficacious. Composts can be highly variable products and in some studies their application has increased numbers of plant-parasitic nematodes (see Thoden et al., 2011).

There was no significant difference for root-knot and TFL means between the nil and v-furrow treatments at any assessment (except for the final TFL count in May 2023), indicating that the v-furrow amendment application provided no appreciable advantage. The organic amendment used in the v-furrow treatment in this trial was compost.

Reniform nematode (Rotylenchulus reniformis) was detected in few plots at low numbers at the start of the field trial but was present in moderate to high numbers in all plots by the final sampling. There was no significant treatment effect at any assessment for reniform nematode in the intensive trial.

Extensive Trial

Introduction

The extensive trial was more experimental than the intensive trial in its design. The aim was to assess the nematode control and soil health benefits provided by farming systems that incorporate minimum tillage (pre-formed beds) as well as crop rotation and organic amendments.

Methodology

The trial had 4 replicates of 10 treatments made up as a factorial of 2 factors. The trial was laid out as a randomised complete block. Three harvests were completed in the 5 years of the trial. The 10 treatments were:

	Method		Rotation Crops
Treatment 1	Nematicide	Vydate (oxamyl)	Grass/Brassica
Treatment 2	Nil	no treatment control	Grass/Brassica
Treatment 3	V-furrow	sawdust + chicken manure in v-furrow	Grass/Brassica
Treatment 4	Incorporated	band of sugarcane mulch + chicken manure/compost	Grass/Brassica
Treatment 5	Double	incorporated + v-furrow treatments combined	Grass/Brassica
Treatment 6	Nematicide	Vydate (oxamyl)	Grass/Legume
Treatment 7	Nil	no treatment control	Grass/Legume
Treatment 8	V-furrow	sawdust + chicken manure in v-furrow	Grass/Legume
Treatment 9	Incorporated	band of sugarcane mulch + chicken manure/compost	Grass/Legume
Treatment 10	Double	incorporated + v-furrow treatments combined	Grass/Legume

Image 2. Banded amendments (sugarcane mulch + chicken manure) in the extensive trial at bed forming.

Results and Discussion

In this trial there were no significant differences in root-knot numbers between treatments at any of the three harvests, considering factors or individual treatments. However, there were significant differences in root-knot counts at some mid-rotation or pre-plant samplings, where the banded organic amendment treatments (“incorporated” or “double”) had lower numbers. Total free-living nematode counts tended to be significantly higher for banded amendment treatments at mid-rotation or pre-plant samplings, but this effect didn’t always persist through to harvest. For example, at the November 2022 pre-plant sampling, the incorporated and double amendment treatments had significantly higher TFL counts than all other treatments, but there was no difference between treatments at harvest.

No significant difference was found between the mean root-knot counts for the nematicide, nil and V-furrow treatments at any assessment, indicating that there was no advantage in implementing the V-furrow practice in this trial. The organic amendment used in the v-furrow treatment in this trial was sawdust + chicken manure.

Reniform nematode (*Rotylenchulus reniformis*) was initially absent or in low numbers throughout most of the extensive trial, although there were four plots at the northern end with moderate numbers. Like the intensive trial, reniform nematode became dominant over time and was present in moderate to high numbers in all plots by the final sampling. There was no significant treatment effect (considering factorial structure or just individual treatments) at any sampling date for this nematode.

A variety of rotation crops were tested in the extensive trial, with half of the treatments being a brassica/grass sequence and the other half legume/grass, in between sweetpotato crops. All rotations were selected on the basis of having moderate or better resistance to root-knot, so performed as expected in reducing populations between crops. The only significant difference between the brassica and legume crops detected during the course of the field trial was significantly less root-knot after the legume sunn hemp compared with the brassica blend Nemsol (178% reduction in comparison).

Discussion (both trials)

The intensive trial has demonstrated successful control of root-knot nematode through a farming system of a resistant rotation crop (in this case forage sorghum) plus the application of suitable organic amendments. Statistically significant control was achieved with the organic amendment treatment (a band of sawdust and chicken manure) by the second harvest and was maintained for all subsequent harvests. In contrast, the nematicide treatment (Nimitz) was no better than the nil control treatment at any of the four harvests, although nematicide trials have demonstrated that plant parasite numbers can rebound to high levels after initial chemical suppression in sweetpotato crops.

Suppression of root-knot nematode by the organic amendment treatment corresponded to a significantly higher mean count of free-living nematodes at almost all sampling dates from January 2021 onwards. Chicken litter (manure plus bedding material) is widely used as an amendment in agricultural soils (horticulture, pastures/turf, broadacre cropping) because it provides essential nutrients and benefits soil health due to its high organic carbon content (Wiedemann, 2015). Chicken litter or a chicken manure plus sawdust blend has successfully been trialled in the past for root-knot nematode control (e.g. Stirling 1989).

Sweetpotato yield and quality were enhanced by the organic amendment treatment at some harvests, but this effect was not consistent. For example, this treatment had a significantly higher total and marketable yield at harvest 1 and higher marketable yield at harvest 3. However, at harvest 2, total yield was not significantly different from other treatments and marketable yield was less than for the nematicide treatment. Full details of yield and quality results are given in appendix 18.

The root-knot control demonstrated in this trial was achieved with the susceptible variety Beauregard, which was used in both trials to better show differences between treatments. In a true integrated nematode management system, it would be preferable to utilise a more resistant variety, which would result in better control of root-knot numbers through the crop cycle, when combined with the other elements of the regime.

The banded amendment treatments were applied at a rate of 40 tonnes per hectare, calculated for the width of the band on top of the bed. This was approximately half of the row spacing, so the true per hectare rate for costing would be around 20t/ha. Sufficient hardwood sawdust and chicken manure for 20 tonnes of a 60/40 blend - the most successful treatment in the intensive trial - would cost approximately $1700 delivered, if the products are available in the local district (based on quotes from bulk suppliers: 12 tonnes hardwood sawdust @ $35/m3, 2.85 m3 per tonne; 8 tonnes chicken litter @ $30/m3, 2.2 m3 per tonne). This is comparable to the cost of some nematicides (others are less expensive) but the cost of organic amendments is also offset by additional benefits. So high rates of organic amendments can potentially offer superior root-knot nematode control, be cost competitive with nematicides, and have other advantages in terms of nutrient input and improvements to soil physical, chemical and biological soil health. However, organic amendments are variable in their availability, and transport may add considerably to cost if suitable products are not available in the local area. Some food safety schemes have withholding periods for untreated manures for some crops, so growers would need to check that the use of chicken manure is compliant with the relevant scheme. Composted/processed alternatives are available, although they may be more expensive than raw products.

The extensive trial was not as successful in suppressing root-knot nematode, despite the use of similar organic amendments. The sweetpotato system poses many challenges for implementing soil health practices, related to the marketable product being underground (e.g., major disturbance required for harvest, storage roots subject to direct effects of amendments/pests). The early bed formation utilised in the extensive trial was an attempt to introduce a minimize tillage practice into the system, by completing bed formation straight after the previous crop so no major soil disruption occurred just prior to planting. Unfortunately, this also meant that the banded organic amendments also had to be incorporated at bed formation, a long time prior to sweetpotato planting. The root-knot and TFL data indicate that the beneficial effects of the banded organic amendments were diminished by the time the sweetpotato crop was planted. The average time between amendment incorporation and planting in the intensive trial was only 12 days, whereas it was 317 days for the extensive trial. This delay inevitably meant that there was some depletion of carbon and other beneficial inputs from the amendments prior to planting and development of the sweetpotato crop. As a comparison, the incorporated treatment in this trial had a mean total organic carbon increase of 17% over the nil control at harvest, whereas the comparable organic matter treatment in the Intensive trial had a 30% mean increase at harvest.

The v-furrow treatment did not provide nematode control or yield/quality benefits in either trial. Other studies have demonstrated root-knot nematode control and yield benefits from similar practices in field and pot trials (Stirling 2020, Stirling et al 2020), with various amendments including sawdust, compost and sawdust/chicken manure blends. The objective is to provide a zone of high biological activity/suppression of plant-parasitic nematodes for developing roots. In our trials, compost was the amendment used in the intensive trial and sawdust/chicken manure in the extensive trial, but results were not encouraging. Stirling (2020) does caution that chicken manure can be detrimental to early root development and recommends delaying planting if high rates are used.

All rotation crops utilised in both trials were successful in reducing root-knot populations between sweetpotato crops. The legume sunn hemp performed especially well, reducing root-knot to extremely low numbers during its three-month rotation. Sunn hemp appears to be an excellent rotation option, quickly producing a large amount of biomass, fixing nitrogen, shading weeds and is a very poor host for root-knot.

Image 3 Dense stands of the rotation crops sunn hemp (left) and forage sorghum (right) in the extensive and intensive trials, respectively.

In both trials, there was no significant treatment effect at any assessment for reniform nematode. This nematode came to be the dominant plant parasite in both trials, even in treatments where root-knot nematode was suppressed. Initially, it was mainly confined to a few plots at the northern end of the extensive trial, with low numbers in a few other scattered plots. Despite machinery movements being along rows, across the short axis of the trials, reniform nematode became established throughout the full length of both trials and was present in high numbers in almost all plots by early to mid-2022 (see Figures 3 and 4, Heat Maps of Reniform Nematode). This demonstrates that management strategies that may work for one nematode pest won't necessarily control another. Compared with root-knot, reniform nematode is more difficult to control as it becomes metabolically inactive in dry conditions (enabling it to survive in soil for long periods of time) and it also can move very deep in the soil profile, avoiding the effects of nematicides and biological suppression near the soil surface. It can then reinvade a susceptible crop from the deeper soil layers. Competitive interactions between reniform and root-knot nematode has been reported in the literature, and one pest may dominate the other, depending on field conditions which interact with aspects of their differing life cycles. Thomas and Clark (1983) state that where root-knot is survival is reduced by fallowing (or resistant rotations in our field trials) between susceptible crops, the greater survival capacity of reniform will favour its predominance in a field.

In summary, the long-term field trials have successfully demonstrated most of the major components of an integrated nematode management program detailed below. Statistically significant root-knot nematode control was achieved by treatments comprising high rates of banded organic amendments (applied just prior to planting at bed formation) combined with a resistant rotation crop. In situations with high root-knot nematode pressure growers should also utilise a more resistant sweetpotato cultivar than was employed in these demonstration trials, as this would further suppress the pest and help to deliver consistent crop yield and quality. The rapid domination of reniform nematode in these trials, and the recent detection of guava root-knot nematode in Queensland, reinforce the importance of on-farm biosecurity for excluding pests not yet present on a farm.

Elements of an integrated nematode management program:

1. Monitoring – knowledge of plant parasite species present (therefore suitable resistant rotations), pre-plant levels, targeted use of "soft" nematicides
2. Organic amendments
3. Resistant cultivars
4. Minimising crop stress (irrigation, other pests, etc)
5. Crop rotation, volunteer & weed control
6. On-farm biosecurity (keep out what you don't have yet).

References

Stirling GR, 1989. Organic amendments for control of root-knot nematode (Meloidogyne incognita) on ginger. Australasian Plant Pathology 18:39–44

Stirling GR 2020. Surrounding the swollen roots of sweetpotato with a decomposing band of an organic amendment enhances nematode-suppressive services and reduces damage caused by root-knot nematode. Australasian Plant Pathology (2021) 50:151–168

Stirling GR, Stirling AM, Prichard M 2020. Sustainable sweetpotato farming systems to improve soil health and reduce losses caused by root-knot nematodes. Australasian Plant Pathology 49:591–604

Thoden TC, Korthals GW, Termorshuizen AJ, 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? Nematology, Vol. 13(2), 133-153

Thomas RJ and Clark CA, 1983. Population dynamics of Meloidogyne incognita and Rotylenchulus reniformis alone and in combination and their effects on sweet potato. Journal of Nematology, Vol. 15(2), 204-211

Weidemann SG 2015. Land application of chicken litter: A guide for users. RIRDC Publication No. 14/094

Heat Maps of Reniform Nematode

Heat maps illustrate the increasing dominance of *Rotylenchulus reniformis* in both field trials over time. From initial patchy distribution at low levels the nematode becomes widespread at high levels throughout both trials.

Extensive Trial

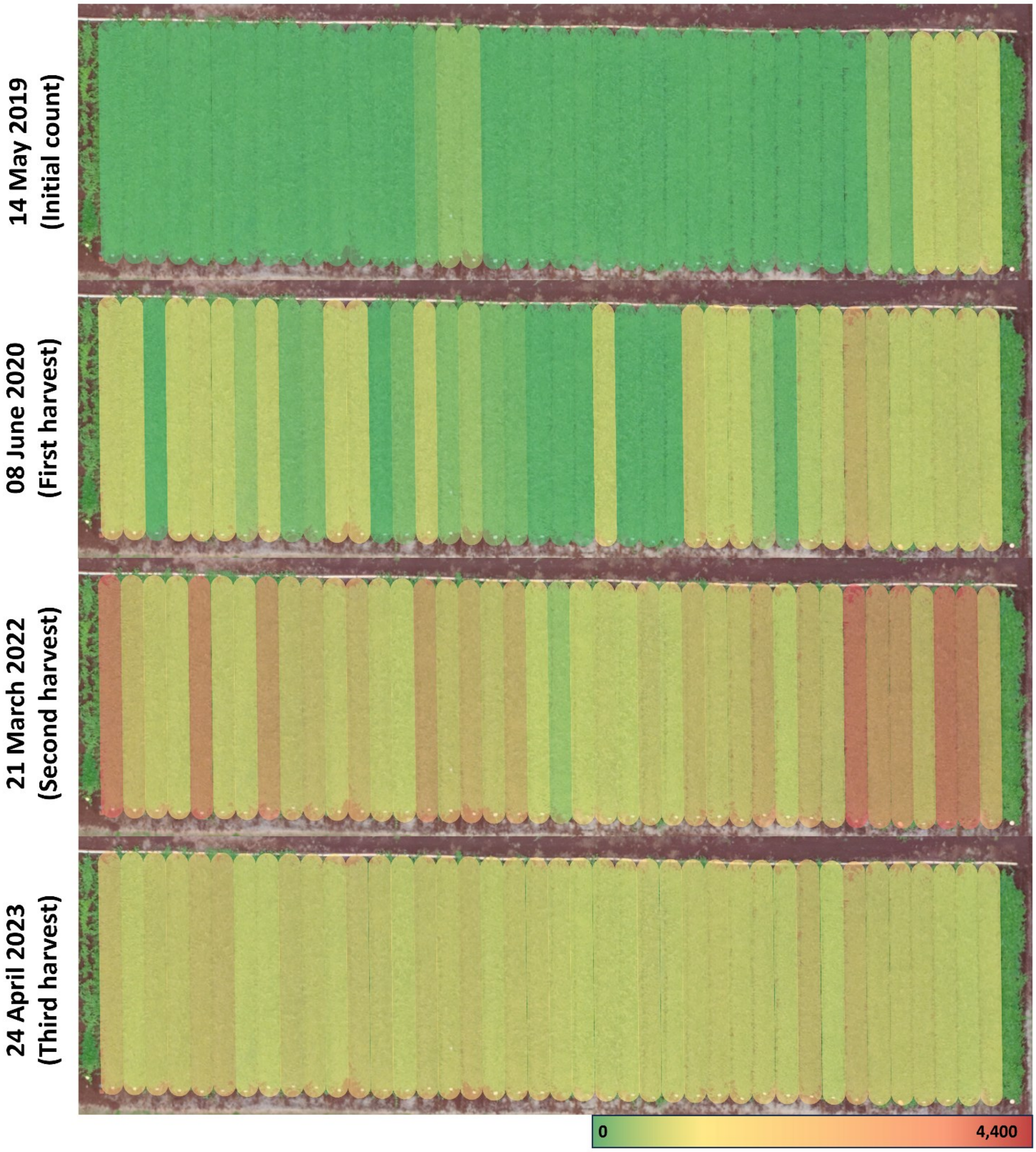

Figure 3. Mean reniform counts (per 200g dry soil) over the life of the Extensive trial.

Intensive Trial

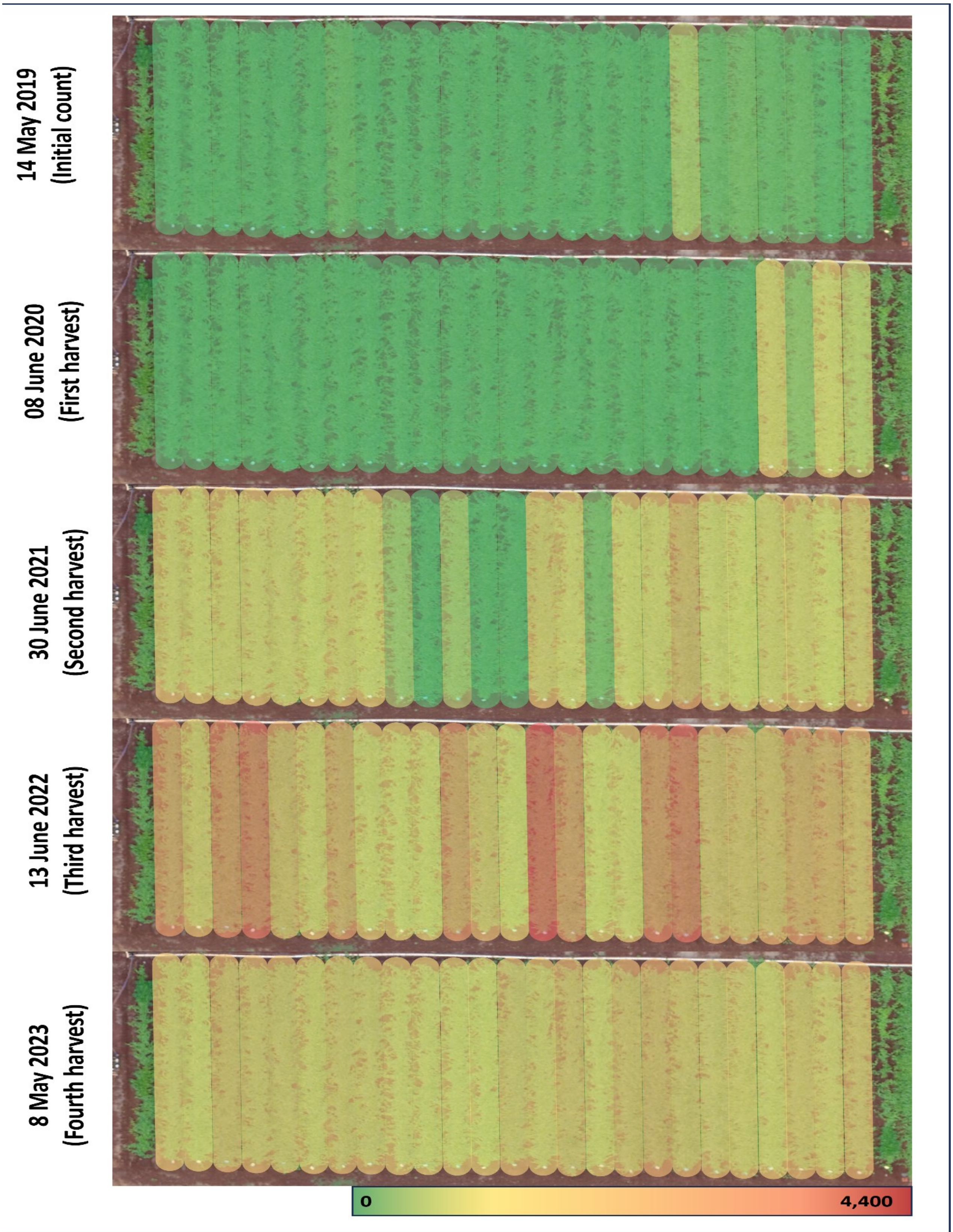

Figure 4. Mean reniform counts (per 200g dry soil) over the life of the Intensive trial.

Assays to Investigate Biological Suppression of Plant-parasitic Nematodes in Intensive Long-term Field Trial.

Introduction

General (non-specific) biological suppression of soilborne diseases (including plant-parasitic nematodes) is most commonly seen in soils with high levels of organic matter. This type of suppression results from many antagonistic organisms acting as predators, parasites, and competitors of the pest and disease organisms. Soils can also have specific suppression to certain nematode pests, resulting from a small number of antagonists (Stirling et al., 2016).

Following the addition of high rates of organic matter, some treatments in the intensive sweetpotato field trial demonstrated apparent suppression of root-knot nematode. Suppression assays were conducted to investigate the nature of this suppression.

Methodology

Soil samples were collected from the intensive trial at BRF on 17/04/23, just prior to harvest. Samples were collected in two ways for two different suppression assays:

- Intact cores: PVC tubes (internal diameter 100 mm X 50 mm = 196 mL volume) were pushed into the soil until level with the surface in the centre of each plot. One soil core was collected per plot, and an extra core was collected from 5 plots (one for each treatment). The tubes were carefully withdrawn to retain all of the soil and were then bagged tightly to hold the soil core in place.
- Composite samples for bioassays: 10 subsamples were collected and mixed to form one bulked sample per plot. Extra soil was collected from 5 plots (one for each treatment).

Intact Core Assays

The weight of each core was recorded. The 5 extra cores were heated at 80°C for one hour. After the heated cores had cooled to room temperature, all cores were inoculated with 1500 Pratylenchus zeae (mixture of adults, juveniles and eggs) in one mL of water. P. zeae was chosen as the test organism as it is rarely encountered in samples from the field site, so there is no background population to conflate results. The cores were then incubated at room temperature for 12 days. Soil was then removed from each core and set up in a Whitehead tray for extraction of nematodes over 3 days. Surviving P. zeae in each sample were identified and quantified using a compound microscope.

Image 4 Inoculated core assays incubating in the laboratory.

Pot Bioassays

The 5 extra soil samples were heated at 80°C for 2 hours. Pots (100 mm) were filled with the soil samples from each plot plus 5 extra pots of the heat-treated soils. Four days later a tomato seedling (cv. Tiny Tim) was planted into each pot. Eight days later each pot was inoculated with 6000 *Meloidogyne javanica* (root-knot nematode) eggs and the tomato plants were then grown for 7 weeks in the glasshouse. At the conclusion of the experiment, root systems were washed free of soil, galling was rated for each plant on a 1–5 scale, and root-knot nematode eggs were stripped from the roots by soaking in 0.1% NaOCl for 5 minutes and passing the solution through a 38 µm sieve. Egg numbers were then quantified by counting under a compound microscope.

Results and discussion

Intact Core Assays

Analysis of the P. zeae counts showed no significant difference between the trial treatments, but all treatments had significantly less surviving P. zeae than the heat-treated soils (Figure 1). This indicates that biological factors are suppressing nematodes, as the heat treatment regime is sufficient to kill most organisms in the soil. However, the suppression of P. zeae was either equal in all of the trial treatments, or this assay wasn't sensitive enough to show a difference between treatments. The organic matter treatment (which demonstrated the best root-knot nematode control) had the lowest mean P. zeae count (Table 1), but as previously mentioned, differences between trial treatments were not significant in this assay. Of the 2 predominant plant-parasitic species encountered in the field trial (root-knot and reniform nematodes), only root-knot nematode was being controlled by the trial treatments, so it could be that the biological suppression is of a more specific nature (controlling root-knot nematode) and not general to all plant parasite species.

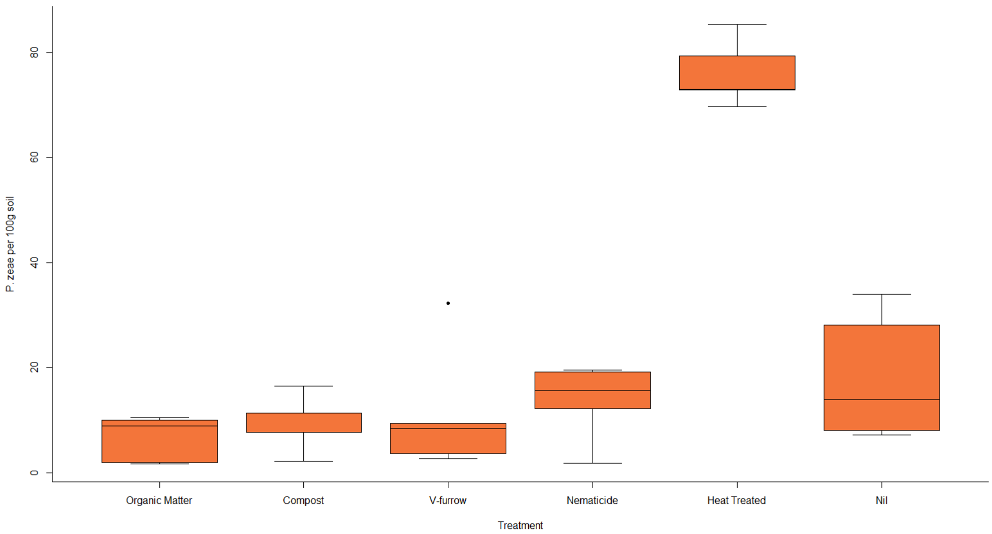

Figure 5. Box and whisker plot illustrating increased survival of P. zeae following heat treatment of soil.

Table 1. Mean numbers of *P. zeae* per 100g soil recovered from the cores after 12 days.

Treatment	**Mean *P. zeae* per 100g soil**
Organic Matter	6.6 b
Compost	9.1 b
V-furrow	11.3 b
Nil	18.2 b
Nematicide	14.4 b
Heat Treated	76.1 a

Means followed by the same letter are not significantly different ($P \leq 0.05$)

Pot Bioassays

Analysis of the root-knot nematode egg numbers recovered from the root systems of the bioassay plants showed no significant difference between the heat-treated soils and the non-heated soils, nor between any of the field trial treatments. However, there was significantly greater root galling in the plants grown in heat-treated soil (Table 2), which supports a biological mechanism of root-knot nematode suppression at the trial site. It was hoped that the pot bioassay might show enhanced levels of suppression of root-knot nematode by some of the trial treatments, but like the core assays, the bioassay wasn't able to demonstrate any difference in the level of suppression between treatments. Root-knot nematode numbers were very low across the trial in this final crop compared with the previous three (numbers at the time of sampling were quantified to check for the likelihood of confounding effects in the bioassay). In specific suppression, populations of parasites that are adapted to using a specific pest as a food source fluctuate rapidly in response to pest numbers (Stirling et al., 2016). It may be that soil samples for this bioassay were collected at a time which coincided with low levels of specific suppression organisms in response to the low root-knot nematode population.

Table 2. Mean gall rating of bioassay plants (1–5 scale), 7 weeks after inoculation with Meloidogyne javanica

Treatment	Mean Gall Rating
Organic Matter	1.8 b
Compost	1.6 b
V-furrow	1.75 b
Nil	1.7 b
Nematicide	1.5 b
Heat Treated	3.2 a

Means followed by the same letter are not significantly different ($P \leq 0.05$)

Reference

Stirling G, Hayden H, Pattison T & Stirling M. 2016. Soil Health, Soil Biology, Soilborne Diseases and Sustainable Agriculture: A Guide. CSIRO Publishing.

Summary Statistical Analysis of Nematode Counts from Long-Term Field Trials

Carole Wright

Intensive Trial

Counts of root-knot nematode (RKN), reniform nematode (Rr) and total free-living nematodes (TFL) were recorded at 6-month intervals from January 2020. The counts have been analysed using a generalised linear model (GLM). The replicate block is fitted as the first term in the model, followed by the treatment term. Initial analyses using a generalised linear mixed model (GLMM) often resulted in the random model being bound, therefore a GLM was fitted. The presence of over-dispersion was detected when a Poisson distribution was assumed (except May 2023) and therefore a Negative Binomial distribution is applied. Over-dispersion is not uncommon and occurs when there is more variation present than expected by the Poisson distribution. The dispersion parameter is estimated in all models. For RKN in May 2023, under-dispersion was detected. This suggests there is less variation than expected by the Poisson distribution. To account for this the dispersion parameter is fixed at 1. All significance testing is performed at the 0.05 level. Where a significant treatment effect is detected, the 95% least significant difference (lsd) is used to make pairwise comparisons.

Root-Knot Nematode (RKN)

Significant differences between the treatments were found for June 2021, January 2022, and June 2022.

- In June 2021, organic matter had a significantly lower mean RKN count than all other treatments.
- In January 2022, V-furrow had a significantly higher mean RKN count than compost and nil treatments. Nil had a significantly lower mean RKN count than nematicide and V-furrow.
- In June 2022, compost and organic matter had a significantly lower mean RKN count than the other three treatments.
- Significant differences between the treatments were found for May 2023 but not in December 2022. In May 2023 nematicide had a significantly higher mean count compared to all other treatments and treatment organic matter had a significantly lower mean count.

Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

January 2020	pred	s.e.	
Treatment			
Compost	12.3	3.94	
Nematicide	11.0	3.58	
Nil	7.5	2.59	
Organic Matter	11.3	3.66	
V-furrow	22.0	6.63	$F_{(4,16)}$ = 1.46; p = 0.261; av 95% lsd = 12.50

June 2020	pred	s.e.	
Treatment			
Compost	408.8	159.16	
Nematicide	269.7	105.19	
Nil	341.4	133.03	
Organic Matter	191.7	74.90	
V-furrow	167.1	65.36	$F_{(4,16)}$ = 0.87; p = 0.505; av 95% lsd = 329.45

January 2021	pred	s.e.
Treatment		
Compost	39.2	12.43

Nematicide	34.9		11.12	
Nil	28.6		9.21	
Organic Matter	36.5		11.61	
V-furrow	34.6		11.02	$F_{(4,16)}$ = 0.13; p = 0.970; av 95% lsd = 33.11

June 2021	pred		s.e.	
Treatment				
Compost	907.4	a	194.33	
Nematicide	723.0	a	154.97	
Nil	758.0	a	162.46	
Organic Matter	223.2	b	48.36	
V-furrow	865.9	a	185.47	$F_{(4,16)}$ = 5.68; p = **0.005;** av 95% lsd = 467.01

January 2022	pred		se	
Treatment				
Compost	6.7	bc	2.29	
Nematicide	19.7	ab	5.80	
Nil	6.5	c	2.23	
Organic Matter	10.6	abc	3.35	
V-furrow	21.3	a	6.22	$F_{(4,16)}$ = 3.18; p = **0.042;** av 95% lsd = 12.54

June 2022	pred		se	
Treatment				
Compost	103.4	b	27.01	
Nematicide	398.9	a	102.20	
Nil	473.5	a	121.17	
Organic Matter	59.4	b	15.81	
V-furrow	596.2	a	152.40	$F_{(4,16)}$ = 12.48; p**<0.001;** av 95% lsd = 279.32

December 2022	pred		se	
Treatment				
Compost	1.9		1.17	
Nematicide	3.6		1.90	
Nil	3.5		1.83	
Organic Matter	1.8		1.13	
V-furrow	5.2		2.57	$F_{(4,16)}$ = 0.62; p = 0.658**;** av 95% lsd = 5.27

May 2023	pred		se	
Treatment				
Compost	1.7	c	0.59	
Nematicide	10.3	a	1.43	
Nil	4.3	b	0.93	
Organic Matter	0.4	d	0.27	
V-furrow	4.2	b	0.91	$F_{(4,16)}$ = 17.45; p < 0.001**;** av 95% lsd = 2.65

Reniform Nematode (Rr)

Only 4 plots recorded Rr in June 2020 and therefore this data has not been analysed. The plots with Rr in June 2020 all occurred in replicate 5 and included treatments Compost, V-Furrow, Nil and Nematicide. No organic matter plots recorded Rr in June 2020. Over-dispersion was present in the counts for both December 2022 and May 2023. Therefore, a Negative Binomial distribution is assumed.

Results suggest there is no significant treatment effect at any assessment (p > 0.05). Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

January 2020	pred	se	
Treatment			
Compost	1.35	0.713	
Nematicide	1.22	0.675	
Nil	0.74	0.515	
Organic Matter	1.79	0.833	
V-furrow	3.03	1.125	$F_{(4,16)}$ = 1.13; p = 0.375; av 95% lsd = 2.361

January 2021	pred	se	
Treatment			
Compost	20.8	16.80	
Nematicide	30.5	24.43	
Nil	35.1	28.07	
Organic Matter	11.1	9.10	
V-furrow	9.6	7.94	$F_{(4,16)}$ = 0.40; p = 0.803; av 95% lsd = 53.20

June 2021	pred	se	
Treatment			
Compost	304.8	260.27	
Nematicide	513.5	438.17	
Nil	435.3	371.51	
Organic Matter	101.2	86.60	
V-furrow	125.4	107.26	$F_{(4,16)}$ = 0.55; p = 0.702; av 95% lsd = 810.06

January 2022	pred	se	
Treatment			
Compost	68.8	43.25	
Nematicide	53.4	33.65	
Nil	96.7	60.63	
Organic Matter	39.0	24.70	
V-furrow	30.9	19.65	$F_{(4,16)}$ = 0.46; p = 0.765; av 95% lsd = 112.09

June 2022	pred	se	
Treatment			
Compost	1645.0	875.15	
Nematicide	1200.8	638.96	
Nil	3020.2	1606.45	
Organic Matter	712.2	379.13	
V-furrow	917.5	488.30	$F_{(4,16)}$ = 1.13; p = 0.378; av 95% lsd = 2533.98

December 2022	pred	se	
Treatment			
Compost	81.5	39.00	
Nematicide	39.8	19.27	
Nil	142.1	67.63	
Organic Matter	36.6	17.77	
V-furrow	102.2	48.74	$F_{(4,16)}$ = 1.45; p = 0.265; av 95% lsd = 121.38

May 2023	pred	se	
Treatment			
Compost	479.1	161.59	
Nematicide	534.6	180.24	
Nil	958.7	322.80	
Organic Matter	764.4	257.49	
V-furrow	463.5	156.33	$F_{(4,16)}$ = 0.85; p = 0.516; av 95% lsd = 657.04

Total Free-Living Nematodes (TFL)

Significant differences between the treatments were found for all assessments since January 2021 inclusive ($P < 0.05$), except December 2022. Over-dispersion was present in the counts for both December 2022 and May 2023. Therefore, a Negative Binomial distribution is assumed.

- In January 2021, June 2021, January 2022 and June 2022, organic matter had a significantly higher mean count of TFL than all other treatments.
- In June 2021, nematicide had a significantly lower mean count of TFL than all other treatments.
- In May 2023, treatment organic matter had a significantly higher mean count than all other treatments and nil and nematicide had significantly lower mean counts.
- There was no significant difference between the nil and V-furrow treatments at any assessment, except May 2023.

Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

January 2020	pred		se	
Treatment				
Compost	3017		579.1	
Nematicide	2621		503.2	
Nil	2226		427.4	
Organic Matter	2982		572.4	
V-furrow	3309		634.9	$F_{(4,16)} = 0.62$; p = 0.656; av 95% lsd = 1632.1

June 2020	pred		se	
Treatment				
Compost	2338		377.9	
Nematicide	1779		287.8	
Nil	1572		254.4	
Organic Matter	1930		312.2	
V-furrow	1461		236.5	$F_{(4,16)} = 1.32$; p = 0.304; av 95% lsd = 881.4

January 2021	pred		se	
Treatment				
Compost	3812	b	355.2	
Nematicide	3049	b	284.4	
Nil	3404	b	317.3	
Organic Matter	5325	a	495.5	
V-furrow	3210	b	299.4	$F_{(4,16)} = 6.08$; p = **0.004**; av 95% lsd = 1060.6

June 2021	pred		se	
Treatment				
Compost	2826	b	259.0	
Nematicide	1376	d	126.9	
Nil	2131	c	195.7	
Organic Matter	8835	a	805.9	
V-furrow	2271	bc	208.4	$F_{(4,16)} = 66.38$; p**<0.001**; av 95% lsd = 1066.5

January 2022	pred		se	
Treatment				
Compost	1697	b	195.6	
Nematicide	1729	b	199.3	
Nil	1821	b	209.9	
Organic Matter	2925	a	336.2	
V-furrow	1744	b	201.0	$F_{(4,16)} = 4.42$; p = **0.013**; av 95% lsd = 693.8

June 2022	pred		se	
Treatment				
Compost	2032	b	346.6	
Nematicide	618	c	106.0	
Nil	910	c	155.7	
Organic Matter	5305	a	903.3	
V-furrow	1030	c	176.2	$F_{(4,16)}$ = 26.37; p**<0.001**; av 95% lsd = 1159.5

December 2022	pred	se	
Treatment			
Compost	1951.8	397.70	
Nematicide	947.9	193.55	
Nil	1848.1	376.62	
Organic Matter	1542.6	314.50	
V-furrow	1375.1	280.44	$F_{(4,16)}$ = 1.79; p = 0.180; av 95% lsd = 949.61

May 2023	pred		se	
Treatment				
Compost	776.4	b	165.21	
Nematicide	333.9	c	71.56	
Nil	326.7	c	70.04	
Organic Matter	1951.6	a	413.91	
V-furrow	714.5	b	152.11	$F_{(4,16)}$ = 12.28; **p < 0.001**; av 95% lsd = 573.26

Extensive Trial

Counts of root-knot nematode (RKN), and reniform nematode (Rr) were recorded at 9 occasions from May 2019 through to April 2023. Total free-living nematodes (TFL) were recorded on 8 occasions from August 2019 through to April 2023. For consistency with the analyses of the intensive trial, the counts have been analysed using a generalised linear model (GLM). The replicate block is fitted as the first term in the model, followed by the treatment terms. The treatment term has been fitted acknowledging the factorial treatment structure and also ignoring the factorial treatment structure. In the first analysis, the main effect of Method and Crop are fitted first, followed by the interaction of Method and Crop. If the interaction of Method and Crop is not significant, the term is dropped from the model and only the main effects are fitted. In the second analysis, a single treatment term with 10 levels is fitted ignoring the factorial structure. The presence of over-dispersion was detected when a Poisson distribution was assumed and therefore a Negative Binomial distribution is applied. Over-dispersion is not uncommon and occurs when there is more variation present than expected by the Poisson distribution. The dispersion parameter is estimated in all Negative Binomial models. There was no evidence of over-dispersion for RKN counts in September 2021 and therefore a Poisson distribution was assumed. All significance testing is performed at the 0.05 level. Where a significant treatment effect is detected, the 95% least significant difference (lsd) is used to make pairwise comparisons.

Root-Knot Nematode (RKN)

The counts observed in September 2021 were substantially lower than all other assessments. There was no evidence of over-dispersion and therefore a Poisson distribution was assumed for these counts. In fact, under-dispersion was observed and therefore the dispersion parameter was fixed at one.

A significant main effect of crop was detected in February 2021 and September 2021. A significant main effect of method was found in August 2019 and September 2021. The only significant interaction of method and crop was observed in January 2020.

- No significant difference was found between the mean RKN counts for the nematicide, nil and V-furrow treatments at any assessment. In August 2019, the mean RKN count for these three treatments was significantly higher than the double and incorporated treatments.
- In September 2021, the nematicide and nil treatments had significantly higher mean RKN counts than the double and incorporated treatments.
- In February 2021 and September 2021, the overall grass/brassica treatment mean RKN count was significantly higher than the mean for the grass/legume treatments.
- In January 2020, a significant interaction was found, but no significant difference was observed between the crops for each treatment. The only significant difference within the grass/brassica crop was incorporated had a significantly lower mean RKN count than the nil treatment. Within the grass/legume crop, the double and incorporated treatments had significantly lower mean RKN counts than the other treatments.

Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

May 2019	pred	se	
Method			
Double	912.8	125.49	
Incorporated	989.9	136.04	
Nematicide	827.8	113.86	
Nil	730.3	100.51	
V-Furrow	819.8	112.76	$F_{(4,31)} = 0.71$; p = 0.591; av 95% lsd = 340.03

May 2019	pred	se	
Crop			
Grass/Brassica	858.6	75.10	
Grass/Legume	853.6	74.66	$F_{(1,31)} = 0.00$; p = 0.961; av 95% lsd = 214.36

August 2019	pred	se

Method				
Double	8.6	b	1.65	
Incorporated	3.9	c	0.99	
Nematicide	111.8	a	14.53	
Nil	110.9	a	14.42	
V-Furrow	89.0	a	11.71	$F_{(4,31)}$ = 76.31; p **< 0.001**; av 95% lsd = 28.81

August 2019	pred	se	
Crop			
Grass/Brassica	70.9	7.01	
Grass/Legume	58.8	5.88	$F_{(1,31)}$ = 1.90; p = 0.178; av 95% lsd = 17.92

January 2020	pred			
Crop	Grass/Brassica		Grass/Legume	
Method				
Double	37.7	cde	14.7	e
Incorporated	17.9	e	25.3	de
Nematicide	52.1	cde	108.5	abc
Nil	68.5	abcd	190.5	a
V-Furrow	53.6	bcde	186.0	ab

January 2020	se	
Crop	Grass/Brassica	Grass/Legume
Method		
Double	13.10	5.48
Incorporated	6.54	8.98
Nematicide	17.90	36.56
Nil	23.33	63.75
V-Furrow	18.39	62.24

$F_{(4,31)}$ = 2.79; p = **0.046**; av 95% lsd = 83.62

June 2020	pred	se	
Method			
Double	269.6	70.97	
Incorporated	431.6	113.42	
Nematicide	564.4	148.21	
Nil	600.5	157.65	
V-Furrow	470.0	123.46	$F_{(4,31)}$ = 1.36; p = 0.271; av 95% lsd = 359.13

June 2020	pred	se	
Crop			
Grass/Brassica	506.5	86.07	
Grass/Legume	427.9	72.75	$F_{(1,31)}$ = 0.49; p = 0.490; av 95% lsd = 223.38

February 2021	pred	se	
Method			
Double	9.4	6.62	
Incorporated	21.0	14.33	
Nematicide	80.1	53.53	
Nil	42.1	28.30	
V-Furrow	30.1	20.35	$F_{(4,31)}$ = 1.54; p = 0.215; av 95% lsd = 74.31

February 2021	pred		se	
Crop				
Grass/Brassica	69.0	a	30.82	
Grass/Legume	4.1	b	1.89	$F_{(1,31)}$ = 18.92; p **< 0.001**; av 95% lsd = 62.11

September 2021	pred		se
Method			
Double	0.16	b	0.139

Incorporated	0.08	b	0.098	
Nematicide	1.15	a	0.380	
Nil	0.92	a	0.339	
V-Furrow	0.39	ab	0.220	$F_{(4,31)}$ = 3.59; p = **0.006**; av 95% lsd = 0.722

September 2021	pred		se	
Crop				
Grass/Brassica	0.95	a	0.214	
Grass/Legume	0.12	b	0.078	$F_{(1,31)}$ = 14.54; p **< 0.001**; av 95% lsd = 0.473

March 2022	pred	se	
Method			
Double	325.3	69.74	
Incorporated	382.0	81.83	
Nematicide	473.2	101.24	
Nil	518.1	110.80	
V-Furrow	522.3	111.69	$F_{(4,31)}$ = 0.92; p = 0.465; av 95% lsd = 272.64

March 2022	pred	se	
Crop			
Grass/Brassica	433.0	60.41	
Grass/Legume	455.3	63.51	$F_{(1,31)}$ = 0.06; p = 0.802; av 95% lsd = 170.74

November 2022	pred	se	
Method			
Double	2.0	1.25	
Incorporated	2.8	1.68	
Nematicide	7.0	3.88	
Nil	16.1	8.60	
V-Furrow	8.1	4.48	$F_{(4,31)}$ = 2.32; p = 0.078; av 95% lsd = 11.99

November 2022	pred	se	
Crop			
Grass/Brassica	4.4	1.86	
Grass/Legume	10.0	4.16	$F_{(1,31)}$ = 2.70; p = 0.110; 95% lsd = 7.89

April 2023	pred	se	
Method			
Double	93.4	47.59	
Incorporated	97.5	49.65	
Nematicide	132.1	67.19	
Nil	99.9	50.86	
V-Furrow	72.5	36.99	$F_{(4,31)}$ = 0.17; p = 0.953; av 95% lsd = 145.47

April 2023	pred	se	
Crop			
Grass/Brassica	79.9	26.19	
Grass/Legume	118.3	38.74	$F_{(1,31)}$ = 0.64; p = 0.432; 95% lsd = 92.84

When the factorial treatment structure is ignored, a significant treatment effect is found for August 2019, January 2020, February 2021 and September 2021. Significant effects were also found in the analysis above for these months.

- In August 2019, treatments 4, 5, 9 and 10 have significantly lower mean RKN counts than the other treatments. These are the incorporated and double treatments.
- Although a significant F-test was obtained in February 2021, no significant differences were detected using the 95% lsd. This is a reasonably rare event, although has previously occurred with nematode data. The overall F-test and the pairwise comparisons are independent tests and addressing slightly different questions. It is recommended to state that the overall F-test was significant, but no pairwise contrasts were found to be significant.

- In September 2021, treatments 1 and 2 had significantly higher mean RKN counts than treatments 4 to 10.

Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

May 2019	pred		se	
Treatment				
1	726.8		139.02	
2	599.0		114.75	
3	943.8		180.21	
4	1009.0		192.60	
5	1045.9		199.61	
6	929.6		177.53	
7	863.1		164.90	
8	694.9		132.96	
9	972.6		185.70	
10	782.4		149.58	$F_{(9,27)}$ = 0.89; p = 0.547; av 95% lsd = 478.15

August 2019	pred		se	
Treatment				
1	125.0	a	22.15	
2	118.3	ab	21.02	
3	112.2	ab	20.00	
4	2.9	d	1.20	
5	8.3	c	2.32	
6	98.8	ab	17.75	
7	102.7	ab	18.41	
8	68.1	b	12.58	
9	5.0	cd	1.66	
10	8.8	c	2.41	$F_{(9,27)}$ = 35.29; p **<0.001**; av 95% lsd = 39.47

January 2020	pred		se	
Treatment				
1	52.1	cde	17.90	
2	68.5	abcd	23.33	
3	53.6	bcde	18.39	
4	17.9	e	6.54	
5	37.7	cde	13.10	
6	108.5	abc	36.56	
7	190.5	a	63.75	
8	186.0	ab	62.24	
9	25.3	de	8.98	
10	14.7	e	5.48	$F_{(9,27)}$ = 6.46; p **<0.001**; av 95% lsd = 83.62

June 2020	pred		se	
Treatment				
1	522.3		195.24	
2	871.6		325.43	
3	607.2		226.88	
4	411.0		153.75	
5	230.1		86.33	
6	607.7		227.07	
7	368.8		138.00	
8	352.0		131.75	
9	449.0		167.92	
10	304.0		113.86	$F_{(9,27)}$ = 1.03; p = 0.444; av 95% lsd = 582.17

February 2021	pred		se	
Treatment				
1	176.3	a	157.19	
2	94.5	a	84.44	
3	48.3	a	43.29	
4	22.7	a	20.53	
5	21.5	a	19.17	
6	5.2	a	4.98	
7	4.0	a	3.90	
8	3.8	a	3.67	
9	3.4	a	3.33	
10	0.7	a	0.85	$F_{(9,27)}$ = 2.57; p = **0.028**; av 95% lsd = 123.83

November 2022	pred	se	
Treatment			
1	4.8	3.70	
2	8.5	6.22	
3	2.3	1.98	
4	2.5	2.14	
5	1.2	1.17	
6	7.9	5.84	
7	21.6	14.94	
8	14.7	10.39	
9	1.8	1.61	
10	2.6	2.21	$F_{(9,27)}$ = 1.46; p = 0.211; av 95% lsd = 16.11

April 2023	pred	se	
Treatment			
1	118.8	87.00	
2	34.9	25.83	
3	56.0	41.22	
4	103.7	75.97	
5	93.5	68.53	
6	153.9	112.61	
7	199.1	145.64	
8	108.6	79.55	
9	76.7	56.29	
10	72.1	52.91	$F_{(9,27)}$ = 0.39; p = 0.931; av 95% lsd = 225.72

September 2021	pred		se	
Treatment				
1	2.00	a	0.707	
2	1.84	a	0.679	
3	0.62	ab	0.394	
4	0.00	b	0.002	
5	0.31	b	0.279	
6	0.31	b	0.277	
7	0.00	b	0.002	
8	0.15	b	0.196	
9	0.16	b	0.199	
10	0.00	b	0.002	$F_{(9,27)}$ = 3.77; p **<0.001**; av 95% lsd = 0.935

March 2022	pred	se
Treatment		
1	459.1	140.18
2	569.0	173.57
3	543.4	165.78
4	253.4	77.71

5	341.1	104.38	
6	484.1	147.78	
7	453.5	138.50	
8	481.0	146.83	
9	510.7	155.85	
10	301.1	92.21	$F_{(9,27)} = 0.73$; $p = 0.682$; av 95% lsd = 392.79

Reniform Nematode (Rr)

Insufficient non-zero data was obtained in May 2019 for counts of Rr and this data has not been analysed. Non-zero data was found in 3 plots in replicate 2 (treatments 1, 2, 10) and 6 plots in replicate 4 (treatments 1, 2, 3, 4, 8, 9). The raw means for each treatment are shown below.

Treatment	Mean
1	3.683
2	13.505
3	57.704
4	2.455
5	0.000
6	0.000
7	0.000
8	9.822
9	7.366
10	1.228

The models for June 2020 would not converge. It is unclear why this has occurred but may be due to the large variability within each treatment. The following table shows the minimum and maximum Rr counts within each treatment. Six of the 10 treatments recorded at least one plot with no Rr recorded. No results are presented for this assessment.

Treatment	Minimum	Maximum
1	0.000	301.9
2	8.221	186.8
3	0.000	339.3
4	4.583	782.4
5	0.000	212.5
6	0.000	1528.3
7	17.169	235.7
8	0.000	607.3
9	0.000	168.9
10	3.371	143.9

The interaction of crop and method was not significant for any assessment and therefore this term has been dropped from all models. Across all assessments, the only significant effect of method was in August 2019. Unfortunately, the pairwise comparisons of the method means did not detect any significant differences. Again, it is recommended to state that the overall F-test was significant, but no pairwise contrasts were found to be significant. Arithmetically the nil treatment has a higher mean Rr count than the other treatments in August 2019. Over-dispersion was present for counts recorded in both November 2022 and April 2023. The GLM fitted therefore assumed a Negative Binomial distribution. Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

August 2019	pred		se
Method			
Double	5.3	a	2.97

Incorporated	0.7	a	0.55	
Nematicide	7.1	a	3.92	
Nil	24.6	a	12.85	
V-furrow	6.9	a	3.81	$F_{(4,31)}$ = 4.12; p = **0.009**; av 95% lsd = 15.35

August 2019	pred	se	
Crop			
Grass/Brassica	7.7	3.39	
Grass/Legume	10.1	4.44	$F_{(1,31)}$ = 0.32; p = 0.575; av 95% lsd = 8.67

January 2020	pred	se	
Method			
Double	13.7	5.92	
Incorporated	9.1	4.01	
Nematicide	16.3	7.02	
Nil	21.0	8.98	
V-furrow	27.8	11.77	$F_{(4,31)}$ = 0.84; p = 0.509; av 95% lsd = 22.11

January 2020	pred	se	
Crop			
Grass/Brassica	13.0	3.73	
Grass/Legume	22.1	6.26	$F_{(1,31)}$ = 1.56; p = 0.221; av 95% lsd = 14.04

February 2021	pred	se	
Method			
Double	10.0	4.24	
Incorporated	9.6	4.09	
Nematicide	24.3	9.97	
Nil	31.7	12.91	
V-furrow	10.5	4.46	$F_{(4,31)}$ = 1.94; p = 0.129; av 95% lsd = 21.55

February 2021	pred	se	
Crop			
Grass/Brassica	14.8	4.26	
Grass/Legume	19.7	5.63	$F_{(1,31)}$ = 0.45; p = 0.508; av 95% lsd = 13.00

September 2021	pred	se	
Method			
Double	14.8	7.99	
Incorporated	8.8	4.84	
Nematicide	44.3	23.31	
Nil	42.8	22.51	
V-furrow	35.1	18.53	$F_{(4,31)}$ = 1.55; p = 0.211; av 95% lsd = 46.83

September 2021	pred	se	
Crop			
Grass/Brassica	30.57	11.31	
Grass/Legume	27.74	10.27	$F_{(1,31)}$ = 0.05; p = 0.833; av 95% lsd = 27.52

March 2022	pred	se	
Method			
Double	896.1	359.75	
Incorporated	1352.6	542.85	
Nematicide	1818.1	729.61	
Nil	1463.5	587.34	
V-furrow	1076.1	431.94	
			$F_{(4,31)}$ = 0.43; p = 0.784; av 95% lsd = 1523.75

March 2022	pred	se
Crop		

Grass/Brassica	1262.4	335.18	
Grass/Legume	1380.2	366.45	$F_{(1,31)} = 0.06$; $p = 0.811$; av 95% lsd = 946.69

November 2022	pred	se	
Method			
Double	81.9	26.83	
Incorporated	132.0	42.99	
Nematicide	221.8	71.98	
Nil	249.2	80.81	
V-furrow	178.8	58.08	$F_{(4,31)} = 1.86$; $p = 0.143$; av 95% lsd = 163.02

November 2022	pred	se	
Crop			
Grass/Brassica	176.2	39.05	
Grass/Legume	169.3	37.52	$F_{(1,31)} = 0.02$; $p = 0.886$; 95% lsd = 98.91

April 2023	pred	se	
Method			
Double	453.8	115.88	
Incorporated	487.2	124.38	
Nematicide	679.2	173.26	
Nil	617.8	157.62	
V-furrow	376.1	96.10	$F_{(4,31)} = 0.81$; $p = 0.527$; av 95% lsd = 387.31

April 2023	pred	se	
Crop			
Grass/Brassica	490.1	80.80	
Grass/Legume	555.5	91.57	$F_{(1,31)} = 0.27$; $p = 0.607$; 95% lsd = 242.14

When the factorial treatment structure is ignored, no significant differences between the treatments is detected ($p > 0.05$). Below are the predicted means (pred), standard errors (se), F-test, p-value and average 95% lsd.

August 2019	pred	se	
Treatment			
1	9.4	6.99	
2	35.1	24.26	
3	6.1	4.76	
4	0.3	0.38	
5	2.2	1.99	
6	5.8	4.54	
7	20.3	14.37	
8	8.3	6.25	
9	1.2	1.23	
10	11.5	8.40	$F_{(1,31)} = 2.15$; $p = 0.060$; av 95% lsd = 23.81

January 2020	pred	se	
Treatment			
1	13.8	8.08	
2	25.6	14.68	
3	17.6	10.22	
4	4.0	2.64	
5	6.9	4.24	
6	17.5	10.16	
7	9.1	5.48	
8	40.9	23.16	
9	16.1	9.39	
10	21.4	12.36	$F_{(1,31)} = 1.14$; $p = 0.373$; av 95% lsd = 31.01

February 2021	pred	se	
Treatment			
1	22.0	13.21	
2	23.7	14.16	
3	6.6	4.24	
4	11.8	7.27	
5	8.7	5.46	
6	25.7	15.35	
7	42.3	24.94	
8	15.7	9.57	
9	7.6	4.83	
10	11.6	7.18	$F_{(1,31)}$ = 0.96; p = 0.491; av 95% lsd = 32.69

September 2021	pred	se	
Treatment			
1	51.6	39.94	
2	57.5	44.51	
3	37.9	29.48	
4	7.9	6.54	
5	11.9	9.62	
6	38.8	30.18	
7	33.2	25.92	
8	32.4	25.27	
9	9.6	7.83	
10	18.2	14.40	$F_{(1,31)}$ = 0.67; p = 0.729; av 95% lsd = 72.06

March 2022	pred	se	
Treatment			
1	1835.6	1057.01	
2	1331.9	767.06	
3	675.4	389.23	
4	2106.9	1213.16	
5	830.0	478.19	
6	1762.5	1014.91	
7	1777.1	1023.32	
8	1597.5	919.91	
9	749.1	431.61	
10	1122.7	646.64	$F_{(1,31)}$ = 0.48; p = 0.875; av 95% lsd = 2353.60

November 2022	pred	se	
Treatment			
1	241.9	105.43	
2	291.1	126.72	
3	153.8	67.31	
4	237.4	103.50	
5	50.7	22.68	
6	223.2	97.33	
7	254.2	110.74	
8	225.0	98.14	
9	60.5	26.94	
10	117.9	51.80	$F_{(9,27)}$ = 1.458 p = 0.172; av 95% lsd = 243.87

April 2023	pred	se
Treatment		
1	713.3	258.59
2	483.2	175.37

3	296.3	107.76	
4	621.6	225.43	
5	366.4	133.13	
6	638.6	231.58	
7	771.7	279.73	
8	466.2	169.20	
9	334.2	121.45	
10	549.8	199.43	$F_{(9,27)} = 0.77$; $p = 0.647$; av 95% lsd = 562.84

Total Free-Living Nematode (TFL)

A significant effect of method was detected in August 2019, June 2020, February 2021, September 2021, and March 2022. There was no significant effect of crop at any assessment. A significant interaction was identified for the assessment in January 2020.

- In January 2020, there was a significant effect of crop on the nil treatment. The grass/brassica treatment had a significant higher mean count of TFL than the nil treatment with grass/legume. The double and incorporated treatments had the highest mean counts for both crops. For grass/brassica the nematicide treatment had a significantly lower mean count than double, incorporated, and nil treatments. For grass/legume the nil and V-furrow treatments had a significantly lower mean count than the double and incorporated treatments.
- In August 2019, double and incorporated treatments had significantly higher mean TFL counts than all other treatments.
- Double, incorporated and V-Furrow treatments consistently had the highest mean TFL for all assessments from June 2020 inclusive.
- In June 2020 and February 2021, the nil and nematicide treatments had significantly lower mean TFL counts than the other treatments.
- In September 2021 and March 2022, the nil treatment had a significantly lower mean TFL count, although it was not significantly lower than nematicide in March 2022.
- The main effect of method was significant for November 2022, but no significant main effects were detected in April 2023. In November 2022 the double and incorporated treatments had significantly higher mean counts of TFL compared to the other three treatments.

Below are the predicted means (pred), standard errors (s.e.), F-test, p-value and average 95% lsd.

August 2019 Method	pred		se	
Double	11364.3	a	833.81	
Incorporated	9713.2	a	712.83	
Nematicide	2487.0	b	183.34	
Nil	2215.0	b	163.41	
V-furrow	2040.9	b	150.65	$F_{(4,31)}$ = 134.54; p **< 0.001**; av 95% lsd = 1335.11

August 2019 Crop	pred	se	
Grass/Brassica	5640.2	295.73	
Grass/Legume	5488.0	287.76	$F_{(1,31)}$ = 0.17; p = 0.681; av 95% lsd = 746.76

January 2020 pred — Crop / Method	Grass/Brassica		Grass/Legume	
Double	6988.7	a	5546.7	ab
Incorporated	7394.6	a	4975.6	abc
Nematicide	1453.6	de	2399.6	cde
Nil	4175.7	abc	1321.3	e
V-furrow	3014.9	bcd	1803.0	de

	se	
Crop	Grass/Brassica	Grass/Legume
Method		
Double	1619.57	1285.58
Incorporated	1713.59	1153.28
Nematicide	337.50	556.64
Nil	968.01	306.86
V-furrow	699.16	418.44

$F_{(4,27)}$ = 1.46; p = **0.030**; av 95% lsd = 2824.76

June 2020	pred		se	
Method				
Double	5253.2	a	637.39	
Incorporated	2432.5	c	295.52	
Nematicide	893.8	d	109.01	
Nil	1183.5	d	144.13	
V-furrow	3572.1	b	433.64	$F_{(4,31)}$ = 35.31; p **< 0.001**; av 95% lsd = 1022.95

June 2020	pred	se	
Crop			
Grass/Brassica	2510.2	210.29	
Grass/Legume	2823.8	236.51	$F_{(1,31)}$ = 1.17; p = 0.288; av 95% lsd = 590.99

February 2021	pred		se	
Method				
Double	2516.4	a	311.41	
Incorporated	2646.1	a	327.42	
Nematicide	812.0	c	100.94	
Nil	786.2	c	97.75	
V-furrow	1648.2	b	204.20	$F_{(4,31)}$ = 20.98; p **< 0.001**; av 95% lsd = 640.78

February 2021	pred	se	
Crop			
Grass/Brassica	1722.6	142.43	
Grass/Legume	1641.0	135.71	$F_{(1,31)}$ = 0.19; p = 0.668; av 95% lsd = 380.39

September 2021	pred		se	
Method				
Double	2436.8	b	309.60	
Incorporated	3970.0	a	503.97	
Nematicide	1685.8	b	214.39	
Nil	1073.3	c	136.74	
V-furrow	1811.0	b	230.26	$F_{(4,31)}$ = 14.00; p **< 0.001**; av 95% lsd = 842.31

September 2021	pred	se	
Crop			
Grass/Brassica	2006.8	170.48	
Grass/Legume	2383.9	202.44	$F_{(1,31)}$ = 2.28; p = 0.142; av 95% lsd = 508.02

March 2022	pred		se	
Method				
Double	1394.8	ab	208.24	
Incorporated	1753.5	a	261.64	
Nematicide	1104.1	bc	164.94	
Nil	886.2	c	132.51	
V-furrow	1493.3	ab	222.90	$F_{(4,31)} = 3.11$; p = **0.029**; av 95% lsd = 579.86

March 2022	pred	se	
Crop			
Grass/Brassica	1183.3	113.10	
Grass/Legume	1469.5	140.37	$F_{(1,31)} = 2.58$; p = 0.118; av 95% lsd = 363.07

November 2022	pred		se	
Method				
Double	4064.7	a	433.17	
Incorporated	4174.1	a	444.81	
Nematicide	1724.6	b	184.23	
Nil	2165.4	b	231.12	
V-furrow	1762.0	b	188.20	$F_{(4,31)} = 17.24$; **$p < 0.001$**; av 95% lsd = 891.76

November 2022	pred	se	
Crop			
Grass/Brassica	2787.7	195.72	
Grass/Legume	2768.6	194.38	$F_{(1,31)} = 0.01$; p = 0.942; 95% lsd = 540.00

April 2023	pred	se	
Method			
Double	1567.6	234.62	
Incorporated	2150.5	321.65	
Nematicide	1438.5	215.34	
Nil	1575.4	235.78	
V-furrow	1442.4	215.92	$F_{(4,31)} = 1.16$; p = 0.348; av 95% lsd = 707.75

April 2023	pred	se	
Crop			
Grass/Brassica	1757.2	168.24	
Grass/Legume	1512.6	144.87	$F_{(1,31)} = 1.20$; p = 0.283; 95% lsd = 445.65

When the factorial treatment structure is ignored, significant differences between treatments were detected at all assessments except March 2022.

- In August 2019, treatments 4, 5, 9 and 10 had significantly higher mean TFL counts than all other treatments. These are the incorporated and double treatments.
- In January 2020, treatments 4, 5 had significantly higher mean TFL counts than all treatments except 9 and 10. These are the incorporated and double treatments.
- In June 2020 and February 2021, treatments 1, 2, 6 and 7 had significantly lower means than all other treatments. These are the nematicide and nil treatments.
- In November 2022, treatments 4, 5 and 9 had significantly higher mean TFL counts than all other treatments except treatment 10. These are the incorporated and double amendment treatments for the two crops. In November 2022, treatments 1, 2, 3, 6, and 8 had significantly lower mean TFL counts than all other treatments except treatment 7. These are the nematicide, nil and V-furrow treatments for the two crops.
-

August 2019	pred		se
Treatment			
1	2561.2	b	265.27
2	2337.7	bc	242.28
3	2270.3	bc	235.35
4	9291.8	a	957.63

5	10422.1	a	1073.89	
6	2413.0	bc	250.03	
7	2095.2	bc	217.33	
8	1819.1	c	188.93	
9	10123.3	a	1043.15	
10	12282.1	a	1265.23	$F_{(9,27)}$ = 61.08; p **<0.001**; av 95% lsd = 1862.12

January 2020	pred		se	
Treatment				
1	1453.6	de	337.50	
2	4175.7	abc	968.01	
3	3014.9	bcd	699.16	
4	7394.6	a	1713.59	
5	6988.7	a	1619.57	
6	2399.6	cde	556.64	
7	1321.3	e	306.86	
8	1803.0	de	418.44	
9	4975.6	abc	1153.28	
10	5546.7	ab	1285.58	$F_{(9,27)}$ = 7.06; p **<0.001**; av 95% lsd = 2823.76

June 2020	pred		se	
Treatment				
1	876.0	e	155.16	
2	1252.0	e	221.29	
3	2905.5	bcd	512.11	
4	2285.3	d	403.03	
5	4830.2	ab	850.61	
6	908.3	e	160.84	
7	1101.7	e	194.85	
8	4305.9	abc	758.41	
9	2579.8	cd	454.81	
10	5694.0	a	1002.54	$F_{(9,27)}$ = 15.23; p **<0.001**; av 95% lsd = 1488.46

February 2021	pred		se	
Treatment				
1	919.3	c	167.53	
2	742.6	c	135.53	
3	1630.6	b	296.34	
4	2918.3	a	529.55	
5	2384.6	ab	432.89	
6	708.0	c	129.25	
7	824.2	c	150.30	
8	1662.0	b	302.02	
9	2385.0	ab	432.96	
10	2641.1	ab	479.34	$F_{(9,27)}$ = 8.88; p **<0.001**; av 95% lsd = 940.23

September 2021	pred		se	
Treatment				
1	1620	cde	281.3	
2	898	f	156.5	
3	1356	def	235.7	
4	4410	a	764.0	
5	2215	bcd	384.3	
6	1730	cde	300.4	
7	1258	ef	218.7	
8	2317	bc	401.9	
9	3385	ab	586.7	
10	2657	abc	460.8	$F_{(9,27)}$ = 7.55; p **<0.001**; av 95% lsd = 1152.24

March 2022	pred		se

Treatment				
1	1057		229.3	
2	719		156.3	
3	1186		257.1	
4	1500		324.9	
5	1454		315.0	
6	1137		246.6	
7	1069		231.7	
8	1833		396.9	
9	2022		437.8	
10	1285		278.4	$F_{(9,27)} = 1.84$; $p = 0.106$; av 95% lsd = 850.73

November 2022	pred		se	
Treatment				
1	1752.4	c	265.08	
2	1871.9	c	283.07	
3	1667.0	c	252.23	
4	4339.1	a	654.52	
5	4657.3	a	702.42	
6	1694.3	c	256.34	
7	2454.9	bc	370.84	
8	1858.0	c	280.97	
9	4001.4	a	603.67	
10	3478.8	ab	524.99	$F_{(9,27)} = 8.07$; $\mathbf{p < 0.001}$; av 95% lsd = 1267.17

April 2023	pred		se	
Treatment				
1	2032.1		400.57	
2	1545.1		304.81	
3	1464.8		289.02	
4	1808.5		356.61	
5	1766.9		348.41	
6	916.5		181.21	
7	1585.0		312.65	
8	1409.3		278.10	
9	2427.6		478.34	
10	1378.8		272.10	$F_{(9,27)} = 1.69$; $p = 0.142$; av 95% lsd = 947.29