NeoBiota 36: 1–16 (2017)
doi: 10.3897/neobiota.36.11418
http://neobiota.pensoft.net

DATA PAPER

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Plant parasitic nematode survival and detection to inform biosecurity risk assessment

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Academic editor: *R. Shaw* | Received 7 December 2016 | Accepted 23 May 2017 | Published 26 June 2017

Citation: Aalders LT, McNeill MR, Bell NL, Cameron C (2017) Plant parasitic nematode survival and detection to inform biosecurity risk assessment. NeoBiota 36: 1–16. https://doi.org/10.3897/neobiota.36.11418

Abstract

Plant parasitic nematodes (PPN) are known to survive periods of desiccation, an ability that increases the risk of them surviving unintentional transport between countries. To investigate nematode survival in soil subject to prolonged storage, soil collected from a native forest and an organic orchard was stored separately in cupboards at ambient temperature for 36 months. Subsamples were taken at 0, 3, 6, 12, 13, 24 and 36 months to determine the presence of plant parasitic and total nematodes using a standard misting technique. *Pratylenchus* was used as a model to determine if PPNs that had been under prolonged storage were able to infect plant hosts at 13, 24 and 36 months.

Overall, the total number of nematodes recovered from stored soil declined over time, with differences in species diversity determined by molecular methods, related to soil origin. No PPN were recovered in soil stored beyond 13 months using the three-day misting technique. By comparison, *Pratylenchus* nematodes, using a baiting method, were found to successfully invade host plant roots (ryegrass and white clover) even after 36 months storage and were observed to produce offspring at 13 months. Baiting was not effective for *Pratylenchus* found in soil originally collected from the forest but was for orchard soil, a result attributed to the lack of suitable host plants for the *Pratylenchus* species found in forest soil.

This study demonstrated, that in protected environments, nematodes could survive for at least 36 months and were observed to produce offspring at 13 months. Baiting with a host plant was more sensitive in detecting nematodes than using the misting extraction technique, although this approach only works where the host plant is known. Without *a priori* knowledge of the nematode-plant host association, plant baiting may also produce false negatives. In the context of plant biosecurity and providing an accurate risk assessment in soil contaminants, the development of a generic test for PPN that induces nematodes in a resting stage to emerge and respond to a cue would enhance the probability of detection. However, as assessments at the border are often time limited, a molecular based bioassay that can be used to indicate the presence of multiple species of live PPN species may be a more feasible option for risk assessments.

Keywords
invasion pathways, soil risk, plant biosecurity, screening tool, international trade, molecular diagnostics, invasive pests

Introduction

Trade and tourism are important to the economic wellbeing of the world's global economy, but carry with it the real risk of introducing unwanted organisms that threaten the productive sectors of individual countries or regions because of production losses due to direct yield reduction or cost for pest control (Mack et al. 2000, Pimentel et al. 2000, Work et al. 2005, Hulme 2014). For natural habitats and native biota, invasive species can have both direct and indirect impacts through modification, displacement or eradication costs along with a general loss of biodiversity (Lee and Lee 2015). Impacts can therefore be economic, ecological and social with the impacts and costs prolonged for intractable invasive species. Plant parasitic nematodes (PPN) are estimated to cause billions of dollars (USD) of crop damage worldwide each year, many of which have known or potential phytosanitary importance (Singh et al. 2013).

Amongst PPNs there are three main types of parasitism, ectoparasitic, endoparasitic and semi-endoparasitic (Decraemer and Hunt 2006). For ectoparasites (e.g. *Paratylenchus*, Criconematidae), the nematodes remain within the soil feeding externally on plant roots. Two types of endoparasites feed within the roots; there are migratory ones such as *Pratylenchus* which do not form a permanent feeding site and can move in and out of the plant; and sedentary ones such as *Globodera* and *Heterodera* which form a permanent feeding site except for the infective second stage juveniles which are mobile within the soil. For semiendoparasites, such as *Helicotylenchus*, only the anterior portion of the nematode penetrates the root with the posterior portion remaining in the soil (Decraemer and Hunt 2006).

While their minute size and cryptic nature in plants and soil makes discovery more difficult when transported, the ability of many PPN species to survive periods of desiccation (Norton 1978), makes these nematodes a biosecurity issue as it increases the probability of establishment when inadvertently transported from one country to another via trade and tourism routes. PPNs have been detected in soil associated with shipments of imported seeds (Lal and Lal 2006), plant material (Tenente et al. 1996), contaminated footwear (McNeill et al. 2011), used machinery (Hughes et al. 2011, Aalders et al. 2012) and sea containers (Gadgil et al. 2000, McNeill et al. unpublished data).

In an effort to improve predictions on which PPN species will become invasive in a country or region before they arrive, a Pest Screening and Targeting (PeST) framework has been developed to provide a more structured and systematic approach for screening large numbers of species and identifying species likely to become invasive (Singh et al. 2015). PeST integrates heterogeneous information and data on species biogeography, biotic and abiotic factors to first determine a preliminary risk index. While species with better survival adaptations pose greater risks than those without the capability, a

paucity of information on survival in transit represents an important knowledge gap when developing a pest risk profile for PPNs (Singh et al. 2015).

In this current study, soil was collected and stored in cupboards to mimic soil contaminants that may be stored in a protected environment (e.g. contaminated footwear, used equipment or camping gear) for a period of time before reuse. The study assessed not only nematode survival but the viability of nematodes recovered from soil that had been stored in a cupboard over a 36 month period. While the research commenced prior to the development of the PeST framework proposed by Singh et al (2015), this work provides a valuable contribution to our understanding of nematode survival over time. The hypothesis tested was that nematodes surviving long term desiccation would be able to subsequently invade plant roots ('baiting'), thus presenting a heightened biosecurity risk.

Methods

Collection and processing of soil

Soil was collected in late winter (23 August 2011) from two sites in the Canterbury region of New Zealand; a native forest reserve in Prices Valley, Banks Peninsula (S43.7669, E172.7140) and an organic orchard at Lincoln (S43.6508; E172.4559). At each site, a spade square soil sample (140 mm × 140 mm) was taken to a depth of 5 cm at three randomly chosen points within a 3 m radius of an arbitrarily designated central point. The soil sampled from these three locations at each site were treated separately throughout the experiment. Any vegetation was cut to ground level with scissors and loose litter was removed from the sample point prior to collection. The individual soil samples were mixed separately in a stainless steel tray and transferred to a plastic bag. The spade was cleaned with 70% ethanol between each site and location. Disposable laboratory gloves were worn at all times, and changed between sites. The work presented in this paper is part of a project published in McNeill et al. (2017), methods for collecting and processing the soil are the same as described in that paper.

In the laboratory, the soil was sieved (10 mm sieve) and a subsample taken for nematode counts and identification. The remaining soil from each site and sampling location (six individual soil sources) was divided amongst stainless steel steam trays (dimensions c. 400 × 200 × 50 mm (300 mm × 240 mm internal dimensions)), in which twenty × 4 mm drainage holes had been drilled into the base, then allocated to treatments (c. 700 g of soil per tray). The soil was spread roughly evenly onto the tray surface and gently pressed with a stainless steel pan to lightly compact the soil, resulting in a soil depth of approximately 40 mm (McNeill et al. 2017). Soil from all sites contained plant root material.

The uncovered tray was then placed within a cupboard situated indoors at ambient temperature at Lincoln (S43.6279, E172.4704). The soil in the trays was subsampled at 3, 6, 12, 13, 24 and 36 months. Approximately 75 g soil was collected from each tray using a stainless steel spoon and placed in a 100 ml plastic screw cap container. The spoon was cleaned with 70% ethanol between sampling each tray.

In addition to the above, the two original locations were resampled at 3, 6, 12, 13, 24 and 36 months. This was to monitor the natural population in relation to counts taken from the stored soil to ensure that any decrease in population was due to storage. As per the original sampling strategy, at the three locations within each of the two original sites, soil was collected using 20 × 25 mm diameter × 100 mm deep cores, hand crumbled and mixed.

Extraction methodology

There are a range of accepted nematode extraction techniques (Hooper 1986, Hooper and Evans 1993, Hunt and De Ley 1996). Techniques are classified as either active methods such as the Whitehead and Hemming tray and misting, or passive such as centrifugation and flotation - sieving (McSorley and Walter 1991, Hooper and Evans 1993, Hunt and De Ley 1996). The misting method was chosen because it provided the capability for high throughput of the large number of samples. The limitation was that the method would not have extracted nematode cysts, but was the most efficient and cost effective method available.

Nematode survival and viability

To determine nematode survival over the duration of the study, at each storage time, a 25 g soil subsample was placed in a mistifier funnel for extraction and misted for 30 sec every 5 min over 72 hours at a water temperature of 20 °C. The water from the mister flushes the nematodes through the soil and into a test tube where they are collected. For the original day zero samples 100 g of fresh soil was placed on to extraction trays (Bell and Watson 2001) and extracted over a 72 hr period. At the three month sampling time the two methods of extraction were compared and no significant difference found (data unpublished). For the 36 month bioassay, samples were extracted for an additional 48 hours, in the expectation that prolonged storage could result in poor physiological condition so that more time may be required to extract surviving nematodes. Counts were taken for all nematodes (bacterivores, fungivores, omnivores, predators and plant parasites); PPNs were identified to genera where possible.

The endoparasitic nematode *Pratylenchus* was the only nematode extracted from soil after 12 months, so at 13, 24 and 36 months, the ability of *Pratylenchus* to invade plant roots was tested using both white clover (*Trifolium repens* L.) and ryegrass (*Lolium* spp.) as bait plants. To determine viability, plastic pots (50 mm × 50 mm × 120 mm), were part filled with 140 g of oven dried sand and topped with 46 g of soil from each ca. 75 g sample. The six original sites were also sampled to check plant host suitability of the sown seed for the nematode species present. This resulted in 24 pots of cupboard soil and 12 pots of fresh soil collected from the original six sites. Each pot was sown with three nil-endophyte ryegrass *Lolium multiflorum* (cv. Moata for

2012) and *L. perenne* (cv. Samson for 2013 and 2014) and 6–8 white clover *Trifolium repens* (cv. Sustain) seeds. The pots were randomised, placed into two forestry crates, maintained in a 20°C controlled environment room with a light: dark photoperiod of 16: 8 hours and watered as required. The forestry crates enabled the pots to be held separately from each other and above the bench to avoid cross contamination.

Twenty four days post-sowing, the ryegrass and white clover seedlings were removed from each pot, gently washed to remove adhering soil and counted before the shoots and roots were separated and weighed. For each pot, ryegrass and white clover roots were stained using aniline blue (Rohan et al. 2006), to determine if *Pratylenchus* nematodes had infected the seedling roots.

Molecular identification of PPNs

DNA was extracted from single nematode specimens using the prepGEM™ tissue kit (ZyGEM Corporation Ltd, New Zealand) according to manufacturer's instructions.

DNA was amplified in 25 µl reactions using 1x buffer (Thermo Scientific Finnzymes), 0.2mM dNTPs, 0.3 µM of each primer, 0.2 mg /ml BSA and 0.5 units of Phusion Hot Start II Hi-Fi DNA polymerase (Thermo Scientific Finnzymes). Thermo cycling included an initial denaturing at 98 °C for 2 min, then 40 cycles of 98 °C for 10 sec, 57 °C for 30 seconds, and 72 °C for 60 °C, with a final extension step of 72 °C for 5 minutes. The product was purified using the GeneJET PCR Purification Kit (Thermo Scientific™). The fragments were sequenced by Massey Genome Service (Massey University, New Zealand) and cleaned using the computer programme Geneious™ 8.1.5 (Kearse et al. 2012). The sequences were compared to nematode sequences in the BLAST (Basic Local Alignment Search Tool) database (http://www.ncbi.nlm.nih.gov/blast.cgi).

Restriction fragment length polymorphism analysis (RFLPs) of the internal transcribed spacer (ITS) regions of ribosomal DNA was used to try and distinguish between the closely related *Heterodera* species to identify the *Heterodera* specimen isolated from the orchard soil in this study. Three reference sequences for each of *H. trifolii*, *H. schachtii* and *H. betae* were imported into Geneious to compare. *In silico*, the restriction enzyme *Msp*I generated a RFLP profile that showed the

Table 1. The primers used for sequencing of the plant parasitic nematodes.

Nematode taxa	Primer code	Amplified region of the rDNA gene	Reference
Criconematidae	SSU_F_07 / 18P	18S	(Blaxter et al. 1998)
Globodera / *Heterodera*	TW81 /AB28	ITS1 – 5.8S – ITS2	(Joyce et al. 1994)
Paratylenchus / *Pratylenchus* / *Helicotylenchus*/ *Rotylenchus*	D2A / D3B	D2 – D3 segment of the 28S	(De Ley et al. 1999)

sequence of this *Heterodera* nematode was not *H. schachtii*, but did not distinguish *H. trifolii* from *H. betae*. *H. trifolii* is widespread throughout New Zealand while *H. betae* has not been described from New Zealand.

Soil Moisture

To determine soil moisture at the 6 and 12 month bioassay, a separate 20 g sub-sample of soil was taken from each sample and oven dried at 80 °C for 48 hours. The availability of the remaining soil was limited at 13, 24 and 36 months, so soil moisture was determined using the 25 g of soil following processing in the mistifier funnel. As with the earlier samples, the soil was oven dried at 80 °C for 48 hours.

Temperature and humidity in the cupboards was measured using a Tinytag Ultra Temperature/Humidity logger (Gemini Data Loggers (UK) Ltd.).

Analysis

Data was analysed by split plot analysis of variance using GenStat (16th edition). Soil samples were the main plots and replicate trays the sub plots. Nematode data were log transformed to equalize the variance to better meet the normality assumptions of the analysis.

Results

Temperature and humidity in the cupboards averaged 12.5 °C (range 0.8–25.9 °C) and 76.9 % (38.4–100 %), respectively, over the course of the 36 month experiment. Soil moisture at the beginning of the experiment (day zero) was 34–38 % and 30–32 % for the forest and orchard soils respectively. At 13 months, the forest soil moisture ranged from 4.2–4.6 % compared to 3.3–3.5 % for the orchard soil ($P < 0.001$), with no significant change in moisture content from 13 to 36 months.

Total nematode numbers

The total number of nematodes extracted from the freshly collected forest and orchard soils was variable within each site (mean of 37.9 and 43.4/ g dry soil for forest and orchard, respectively), but with no significant difference between the two sites or the six different sampling times (Figure 1).

By comparison, for the stored samples, there was a difference between soil origin with the forest soil having significantly less nematodes than the orchard soil at 6, 12, 13 and 24 months ($P < 0.001$) storage. After 36 months of storage, nematodes were only extracted from one sample and that was from orchard soil (1 of 12 trays) (Figure 1).

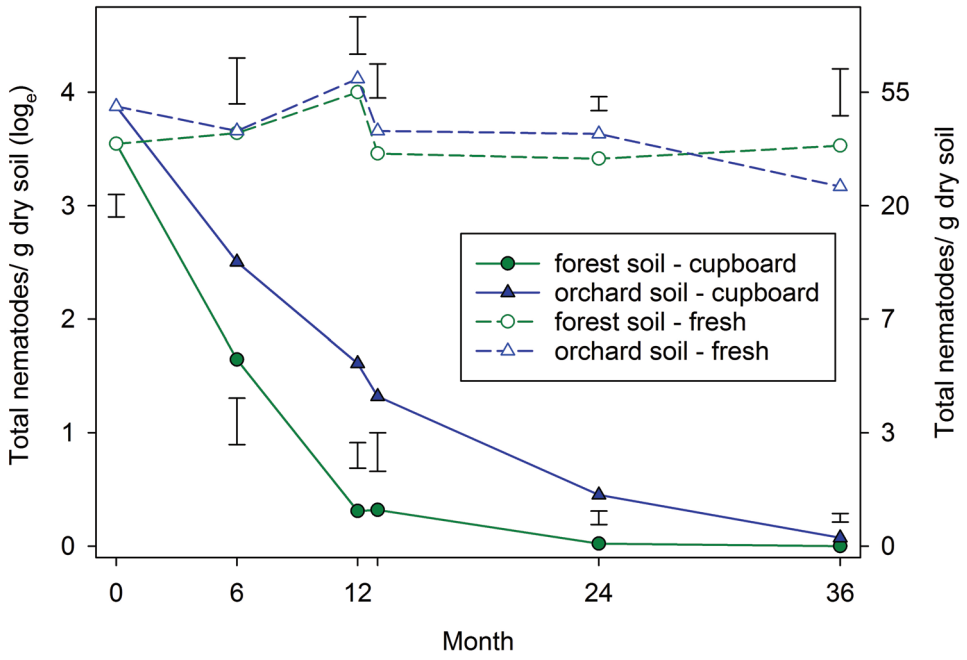

Figure 1. Mean total number of nematodes per gram of dry soil (log$_e$ transformed) collected from either forest or orchard and stored in cupboards for up to 36 months or freshly collected from the original sites. Error bars are SEDs. Note: transformed data presented with back-transformed scale on right hand side for ease of conversion to actual numbers /g.

Plant parasitic nematodes

Fresh soil collected from the forest site contained the highest diversity of plant parasitic genera with a mean/g of dry soil of 1.9 *Pratylenchus*, 2.3 *Paratylenchus*, 0.3 *Globodera*, 0.3 *Helicotylenchus* / *Rotylenchus* and 0.1 for Criconematidae. By comparison, in the orchard soils, the plant parasitic genera consisted of 1.5 *Pratylenchus*, 1.8 *Paratylenchus* and 0.1 *Heterodera* spp. / g dry soil from the orchard site.

Over all sample times, *Pratylenchus* comprised 4.4% and 10.4% of the total nematode fauna in the forest and orchard soils, respectively. PPN populations were substantially larger in the fresh soil than were observed in stored soil (results not shown), especially so for the orchard samples.

Stored samples

Of the PPN taxa recovered at three months, *Pratylenchus* was the most common, found in seven of the twelve forest soil samples (58%) and in all of the orchard samples (12/12) (Table 2). Small numbers of *Paratylenchus*, *Globodera*, *Helicotylenchus* /*Rotylenchus* and Criconematidae were also recovered from the forest soil at three months.

Table 2. Age of soil from which plant parasitic nematode taxa were extracted using the three day misting technique, from 25 g of soil collected from either the forest or orchard location and stored in cupboards for 36 months.

	Months			
	3	**6**	**12 /13**	**24–36**
Forest				
Pratylenchus	Present	Present		
Paratylenchus	Present			
Globodera	Present			
Helicotylenchus /*Rotylenchus*	Present			
Criconematidae	Present	Present		
Orchard				
Heterodera	Present			
Pratylenchus	Present	Present	Present	
Paratylenchus		Present		

Heterodera were present in very low numbers from the orchard site, with 1–10 nematodes extracted at the 3 month sampling (3/12 samples) despite none being extracted from the original sample (day zero). At six months, with the exception of a single Criconematidae from the forest soil and two *Paratylenchus* nematodes from the orchard soil, *Pratylenchus* was the only plant parasitic nematode extracted (Table 2). At both the 12 and 13 month sampling, the only PPN recovered were *Pratylenchus* spp.

Thirteen months after soil had been placed into cupboards, *Pratylenchus* were the only PPN recovered using the misting technique, and then only from the orchard soil. Of those recovered, both female and juvenile stages were present.

The number of *Pratylenchus* recovered over time decreased substantially using a three day misting interval for extraction, with no specimens detected from soil stored for 24 and 36 months (Figure 2). An extra two days extraction time at 36 months did result in three *Pratylenchus* nematodes from one sample, all three of which were females.

Plant baiting

Sowing white clover and ryegrass seed resulted in *Pratylenchus* being recovered from more samples than with mistifier extraction at the 13, 24 and 36 month sampling intervals. At 13 months, *Pratylenchus* were found in four root samples (4 of 12 samples, 33%), but not in their respective misting samples. At 24 months, *Pratylenchus* were detected in five root samples (42%) and at 36 months in three samples (25%).

Reproductively mature *Pratylenchus* were evident in soil that had been stored for 13 months with eggs observed in white clover plant roots from two (c. 17%) of the stored orchard soil samples, demonstrating that not only could these nematodes survive in stored soil without a host plant but could also subsequently infect and reproduce in plant roots. No other PPN genera were detected using the plant baiting method.

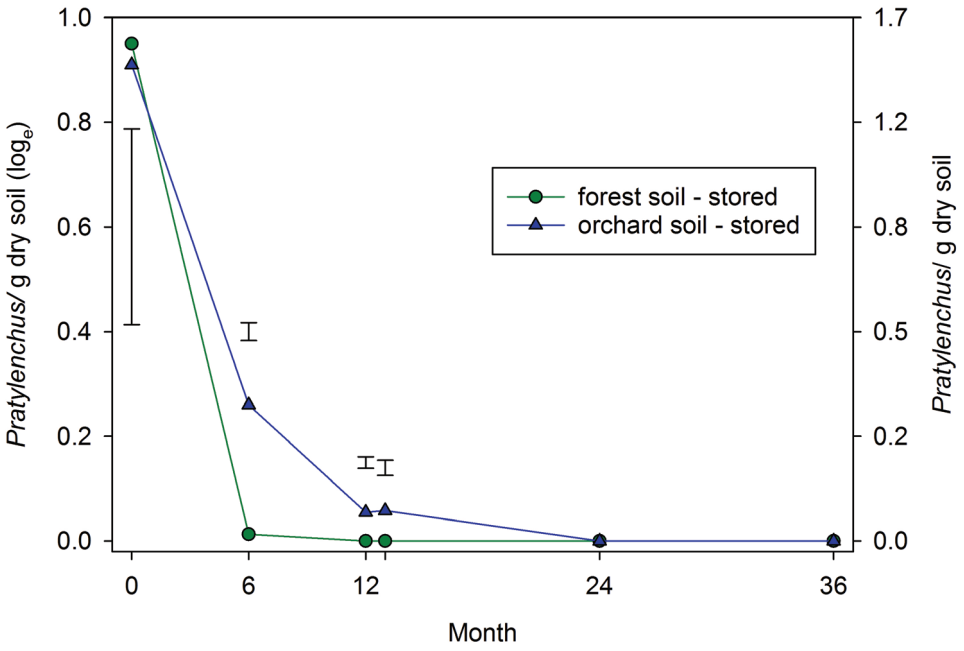

Figure 2. *Pratylenchus* per gram of dry soil (Log$_e$ transformed) collected from either forest or orchard and stored in cupboards for 36 months. Error bars are SEDs. Note: transformed data presented with back-transformed scale on right hand side for ease of conversion to actual numbers /g.

Molecular identification of PPNs

The *Pratylenchus* DNA sequences from the forest soil matched *P. bolivianus* from the NCBI database and specimens were preserved to be confirmed morphologically. *Pratylenchus* sequences from the orchard soil indicated the presence of at least four species: *P. crenatus*, *P. thornei*, *P. penetrans*, and an unidentified *Pratylenchus* that had a poor match to *Pratylenchus* currently in the database (Table 3).

Specimens found in the orchard soil at the start of the experiment were most commonly *P. penetrans* with the unknown *Pratylenchus* sp. also being isolated, while *P. crenatus* and *P. thornei* were only isolated once the soil had begun to desiccate (Table 4). Specimens *of P. crenatus* were isolated at six and 13 months after storage but not from the 36 month samples. *P. thornei*, along with the unidentified *Pratylenchus* sp., were isolated at 12 and 36 months post storage.

When comparing the number of *Pratylenchus* present in the roots of white clover grown in fresh soil collected from the two original sampling sites, there was a significant difference between the two locations at 13 months ($P = 0.004$), 24 and 36 months (both $P<0.001$). For ryegrass, the number of *Pratylenchus* present in the roots grown in fresh soil was significantly different ($P<0.001$), at all three sample times.

Fewer *Pratylenchus* were found in the white clover grown in the fresh forest soil samples with a mean, median and range of 5.2, 4 and 1–17, compared to the fresh orchard soil (130.7, 144 and 39–224, respectively).

Table 3. *Pratylenchus* specimens isolated and identified from orchard and forest soil using the closest matching BLAST reference (accessed August 2016).

***Pratylenchus* species**	**Soil source**	**BLAST reference**	**Match**
bolivianus[a]	forest	KP780256	99.9%
crenatus	orchard	KM580535	99.5%
penetrans	orchard	JX046990	99.9%
thornei	orchard	JX261954	99.9%
unidentified sp.	orchard	JX046999	92.0%

[a] to be confirmed morphologically

Table 4. *Pratylenchus* species extracted from orchard soil stored in cupboards and identified using D2/D3 primers for the 28S gene of rDNA.

Species	**Months**				
	3	**6**	**12 and 13**	**24**	**36**
P. penetrans	Present	Present			
P. crenatus		Present	Present		
P. thornei		Present	Present		Present[a]
Pratylenchus sp.		Present	Present		Present[a]

[a] five day extraction process

Table 5. Plant parasitic nematodes (excluding *Pratylenchus* spp.) isolated and identified from orchard and forest soil using the closest matching BLAST reference (accessed Aug 2016).

Plant parasitic nematode	**Soil origin**	**BLAST reference**	**Match**
Mesocriconema xenoplax	forest	KJ934180	96.3%
Rotylenchus conicaudatus	forest	HQ700698	93.8%
Globodera zelandica	forest	HQ260411	99.5%
Paratylenchus leptos	forest	KR270602	87%
Paratylenchus nanus	orchard	KF242196	100%
Heterodera trifolii[a]	orchard	LC030417	99.2%

[a]DNA sequencing did not give a distinction between *H. trifolii*, *H. schachtii* and *H. betae*.

Similar results were obtained for ryegrass growing in forest soil with a mean, median and range of 0.7, 0.5 and 0–3, respectively. This compared to a mean, median and range of 135.4, 115.5 and 24–291, respectively for orchard soils. For freshly collected forest soil, more *Pratylenchus* were recovered using the misting method than the baiting method.

For the forest soil, with the exception of *Globodera zelandica*, the PPNs were a poor match to the sequences found in the NCBI website (Table 5). Morphological and molecular identification found that the spiral nematodes observed in the samples were a mixed population consisting of both *Helicotylenchus* and *Rotylenchus* species. No *Helicotylenchus* specimens were sequenced.

For the *Heterodera* nematode extracted from the orchard soil, the DNA sequence did not give a clear distinction between *H. trifolii*, *H. schachtii* and *H. betae*. The sequence was compared to three reference sequences from NCBI of each species analysed in Geneious™ using the restriction site *Msp*I. It produced a similar profile to *H. trifolii* and *H. betae* but not *H. schachtii*.

Discussion

This study has confirmed the hypothesis that not only are *Pratylenchus* species able to survive soil desiccation, but after prolonged storage are able to successfully reproduce on host plants.

The ability of nematodes to survive desiccation has been known for some time (Norton 1978). Nematodes that can achieve anhydrobiosis have been divided into two groups, slow-dehydration and fast-dehydration strategists (Womersley 1987). The majority of nematodes require a slow, controlled rate of water loss to achieve anhydrobiosis (Womersley et al. 1998). The soil in this study was stored in cupboards reducing air flow over the samples slowing the rate of desiccation, allowing any nematodes present that had the ability to survive water deficits to achieve anhydrobiosis. The survival of PPNs was greater in soil stored in cupboards than soil stored in sea containers (McNeill et al. 2017).

Nematodes have developed a number of means by which they can survive desiccation, which include survival stages such as eggs, cysts, and dauer larvae (Womersley et al. 1998; Wharton 2002). Nematodes from *Globodera* and *Heterodera* genera form cysts which can allow them to survive in the soil for many years, and some cyst species will not hatch unless stimulated by host root diffusates (Turner and Rowe 2006). This could potentially be the case for the *G. zelandica* and *Heterodera* juveniles that were extracted from the forest and orchard soil respectively that had been stored for three months. The other PPNs observed in this study, *Paratylenchus*, *Helicotylenchus*, *Rotylenchus* and Criconematidae also showed an ability to survive in stored soil albeit for a shorter period of time than *Pratylenchus*. Species from each of these genera have been found in previous studies to survive desiccation (Norton 1978). Other methods employed to slow the rate of water loss may include remaining in senescing plant tissue, swarming or forming aggregates and coiling (Womersley et al. 1998). Coiling has been observed in *P. penetrans* (Townshend 1984) and *P. thornei* (Glazer and Orion 1983). The soil in this current study included root fragments so it is possible they were a source of *Pratylenchus* nematodes able to withstand desiccation.

Pratylenchus species including *P. penetrans* and *P. thornei* have been recorded exhibiting anhydrobiosis (Glazer and Orion 1981; Townshend 1984; Townshend 1987; Anon 1997; Talavera et al. 1998; Ghaderi and Bideh 2011), but there is less information on the ability of *P. crenatus* to survive desiccation. Interestingly, *Pratylenchus crenatus* has been isolated from small quantities of soil associated with imported seed (Lal and Lal 2006). Survival of *P. penetrans* and *P. thornei* in air dried soil for up to 11 months has been pre-

viously recorded and listed in a review by Norton (1978). Talavera et al. (1998) found *P. thornei* was able to penetrate roots after 75 days of desiccation. The current study isolated *P. thornei* from soil stored in a cupboard for 36 months (1097 days), but as an unidentified *Pratylenchus* species was also found after 36 months of storage, it is unclear which of the two species infected perennial ryegrass roots in the "baiting" experiment. Townshend (1984) found *P. penetrans* in slowly dried soil could survive up to 770 days (25.3 months) and that their infectivity and reproduction at 207 days was not affected by anhydrobiosis. The current study did not however find *P. penetrans* in the soil beyond six months (180 days). Conversely, *P. crenatus* was detected in soil that had been stored for 13 months but not at 24 or 36 months. Of the known *Pratylenchus* species identified from this study all three have a broad host range, particularly *P. penetrans* (Castillo and Vovlas 2007; Singh et al. 2013). However, *Lolium perenne* and *L. multiflorum* are considered less favourable hosts (Kimpinski et al. 1984; Townshend et al. 1984) than legumes such as red (*Trifolium pratense* L.) (Willis et al. 1982; Kimpinski et al. 1984) or white clover (*T. repens* L.) (L. Aalders, unpublished data). The plant host preference of the unidentified *Pratylenchus* species isolated from the orchard soil has yet to be determined.

The *Pratylenchus* isolated from the forest soils and tentatively identified as *P. bolivianus,* was only detected up to six months. Both white clover and ryegrass proved to be unfavourable hosts for this *Pratylenchus* sp. with root infection rates considerably lower than numbers extracted from soil using the misting technique.

Pratylenchus crenatus, *P. penetrans* and *P. thornei* are each regulated pests for at least one country globally (Singh et al. 2013), and this study has shown that *P. crenatus* and an unidentified species of *Pratylenchus*, along with *P. penetrans* and *P. thornei*, can also survive prolonged periods of desiccation. According to a review by Jones et al. (2013), *Pratylenchus* are ranked third only to *Meloidogyne* and Heteroderidae (includes *Globodera* and *Heterodera*) nematodes as having the greatest impact on crops worldwide, and coupled with their ability to survive desiccation their status as a biosecurity risk is increased with more than 80 *Pratylenchus* species described (Siddiqi 2000).

The study showed that for disturbed soil stored in protected environments *Pratylenchus* nematode populations can survive prolonged storage for up to 36 months (1095 days) and that in the presence of a suitable host plant, 'baiting' was a more sensitive technique in detecting *Pratylenchus* spp. than the misting extraction technique. However, this study demonstrated that the approach only works if a suitable host plant is available. Without *a priori* knowledge of the PPN-plant host association, plant baiting may also produce false negatives. For other PPN, the lack of a suitable host plant meant that the mistifier extraction method was more accurate. Where the host plant was not known, this provided the best option to assess presence /absence, although this method may not extract cyst nematodes. Extraction using flotation /sugar centrifugation would have extracted cysts as well as vermiform stages but the technique was not feasible with the high numbers of soil samples. Furthermore, examining only the roots of bait plants for parasitic nematodes will only show those endo-parasitic species present, it cannot be used to assess external root feeding species, which would require that the soil surrounding bait plants is also checked.

In the context of plant biosecurity and providing an accurate risk assessment for soil contaminants, the development of a generic test for PPN that induces nematodes in a resting stage to emerge and respond to a cue would enhance the probability of detection. Having a better understanding of PPN survival in soil inadvertently transported with commodities, freight, used machinery or humans (e.g. footwear) is important in the development of both scientifically valid pest risk analysis as well as cost-effective management strategies (Colunga-Garcia et al. 2013, Singh et al. 2015, McNeill et al. 2017).

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors contribution

LA: Developed the research concept, led and carried out the extraction and identification of nematodes, contributed to manuscript writing. MM: Developed the research concept, carried out the soil sampling and contributed to manuscript writing, NB: Contributed to development of baiting technique, identification of nematodes and writing of the manuscript. CC: Analyzed data.

Acknowledgements

The research was funded by AgResearch via the Better Border Biosecurity research collaboration (www.b3nz.org). The authors thank Dr Barbara Barratt, Dr Scott Hardwick, Dr Alison Popay and Dr Michael Wilson (AgResearch) for reviewing the draft document.

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