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Identification and reproduction of dagger nematode, *Xiphinema americanum*, in potato

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Summary – Plant-parasitic nematodes limit potato production by feeding on roots, reducing tuber yield and quality, and resulting in poor growth. Dagger nematodes (*Xiphinema* spp.) can pose a significant threat to crop production even in low numbers. Dagger nematodes have been reported in North Dakota, USA, potato fields. In this study, a dagger nematode species was identified and its reproduction potential in 21 potato cultivars was evaluated. The dagger nematode was identified as *X. americanum via* morphometric measurements and DNA sequence analysis. At the molecular level, D2-D3 of 28S ribosomal RNA (rRNA) and internal transcribed spacer (ITS)-regions of rDNA were targeted. Sequencing data of D2-D3 (749 bp) showed 98.18% similarity with three isolates of *X. americanum*. ITS sequence (860 bp) analysis showed 97.68% similarity with an isolate of *X. americanum* but lower similarity with other species of *Xiphinema*. The phylogenetic analysis of ITS region further confirmed the species of dagger nematode as *X. americanum*. Using naturally infested soil, two glasshouse experiments were conducted to assess the reproduction rate of *X. americanum* on 21 potato cultivars belonging to four distinct market potato classes (yellow, red, white and russet). The reproduction rates were found to vary among the potato classes and cultivars. Numerically, *X. americanum* population density was higher in 'Soraya', whereas 'Ranger Russet', 'Manistee', 'Kennebec', 'Russet Norkotah 278', 'Modoc', 'Pomerelle Russet' and 'Dakota Rose' reduced nematode reproduction in both trials. This is the first study demonstrating the reproduction of *X. americanum* in potato cultivars and could be helpful to manage dagger nematodes.

Keywords – molecular, morphology, morphometrics, potato market classes, reproductive factor, species identification.

Plant-parasitic nematodes are microscopic, thread-like organisms that belong to the phylum Nematoda. From feeding on root tissues and transmitting viruses, plant-parasitic nematodes cause disease that results in approximately 10% yield reduction of the world's crops (Eriksson, 1997). Beside direct feeding, some nematodes in the families Trichodoridae and Longidoridae are able to transmit viruses to various crops. Plant-parasitic nematodes can cause significant damage to the crops in tropical, subtropical and temperate conditions (Bahadur *et al.*, 2021; Pulavarty *et al.*, 2021). Varying environmental conditions of these geographic regions could favour specific nematodes in specific fields (Lima *et al.*, 2018), resulting in different potential to damage crops. Identifying and measuring plant-parasitic nematode population densities in crop fields is important for guiding farmers and understanding the effects of management practices on the nematodes (Berry *et al.*, 2008).

Dagger nematodes, *Xiphinema* spp., belonging to the family Longidoridae, are ranked eighth among plant-parasitic nematodes in terms of causing significant damage to agricultural crops grown all over the world (Jones *et al.*, 2013). *Xiphinema* spp. have the potential to cause great yield loss even when present in relatively lower numbers in field soils. Dagger nematodes are ectoparasitic, which means their body remains in the soil as their odontostyle penetrates the root cortex to feed. As a result, up to 65% of root cells can collapse, leading to plant death (Heve *et al.*, 2015). *Xiphinema* spp. have a wide host range, affecting a large number of crops such as

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apple, apricot, grapevine, litchi, peach, pear, plum, sugarcane, citrus, strawberry, tomato, tobacco, maize, soybean and other woody, as well as herbaceous, plants (Heve *et al.*, 2015) with varying capacity to reproduce and cause damage. According to research by Brodie *et al.* (1970), marigold was found to increase *X. americanum* reproduction 75 times, and cotton, indigo and millet were also able to increase *X. americanum* population by ten times. On the other hand, sudan grass, crotalaria and beggar weed did not support the reproduction of *X. americanum*.

In addition to direct feeding, dagger nematodes have also been reported to transmit viral diseases such as Grapevine fanleaf virus reducing grapevine yields substantially by up to 80% (Andret-Link *et al.*, 2004; Van Zyl *et al.*, 2012; Jones *et al.*, 2013), Tomato ringspot virus reducing tomato yields by 50%, and Cherry rasp leaf virus, Peach rosette mosaic virus and Tobacco ringspot virus that cause damage to many vegetable and fruit trees (Taylor & Brown, 1997). There is no cultivar reported with resistance to dagger nematodes and it is difficult to manage the damage caused by *Xiphinema* spp. without using resistant cultivars (Heve *et al.*, 2015).

Distinct species of dagger nematode can differentially cause losses in crop production. Thus, for efficient management, it is important to know the specific identity of the nematodes. There are numerous species of dagger nematodes, such as *X. index*, *X. americanum*, *X. diversicaudatum*, *X. vuittenezi*, *X. italiae* and many more (Coomans *et al.*, 2001). Until now, *X. americanum* is the most prevalent and harmful nematode species of dagger nematode (Taylor & Brown, 1997; Gozel *et al.*, 2006). *Xiphinema americanum* feeds on the epidermal cells of plant roots, resulting in shortened, swollen roots with dark lesions (Di Sanzo & Rohde, 1969) and has been identified in fields of tomato, grape, oak, sea grapes, pines, hackberry, Brazilian pepper and citrus (Gozel *et al.*, 2006; Mokrini *et al.*, 2014). *Xiphinema americanum* has also been reported to transmit nepovirus in many crops, leading to further reduction in crop productivity (Brown *et al.*, 1993).

According to our knowledge, there have been no reports where *X. americanum* has been found to cause direct damage to potato cultivars. Potato is an economically significant crop, grown worldwide (Wasilewska-Nascimento *et al.*, 2020). The potato output of the USA is fifth in the world, producing 23 million metric tons of potatoes annually (FAO, 2020). North Dakota ranks fifth in potato production in the USA, and, together with Minnesota, produced 2.1 million metric tons in 2019 (USDA-NASS, 2020). Donald & Hosford (1980) reported that *X. americanum* was found in sugar beet fields of North Dakota. In the 40 years since its initial identification, no report has been found of this species of dagger nematode in North Dakota. In some field trials conducted in North Dakota, dagger nematode was detected in soil samples of potato fields in Sargent County. Although dagger nematode has been found in potato fields of North Dakota, there has been no specific study of the reproductive ability of dagger nematode in potato cultivars.

The presence of dagger nematode in North Dakota necessitated species identification and the assessment of its reproduction ability on potato. The main objectives of this research paper were: *i*) to identify the species of dagger nematode from potato field trials in North Dakota; and *ii*) to quantify the reproductive factor of dagger nematode in 21 potato cultivars. To do so, we identified the dagger nematode morphometrically by taking measurements, and molecularly by sequencing two genomic regions (D2-D3 of 28S rRNA and ITS of rDNA).. Additionally, using the naturally infested soil from North Dakota potato fields, we screened potato cultivars in glasshouse trials. This is the first research to evaluate the reproduction ability of dagger nematode in potato cultivars.

Materials and methods

Soil sample collection and nematode extraction from the soil

Soil samples were collected from potato fields of Sargent County, ND, USA, where dagger nematodes were found previously. Soil samples were mixed thoroughly to ensure uniform distribution of nematodes. The nematodes were then extracted from the soil samples by modified sugar centrifugal flotation method (Jenkins, 1964; Plaisance & Yan, 2015). The extracted nematodes were collected in 50 ml vials in the form of nematode suspension.

Morphological identification of dagger nematode

From the nematode suspension, dagger nematodes were picked and identified based on morphological features using an inverted transmitted light microscope (Mai *et al.*, 1996). Morphological characteristics observed for identifying *Xiphinema* spp. at genus level were long body length (1.4-1.7 mm), smooth and flat to rounded lip area,

Table 1. The primers of different regions are used for amplifying the DNA of dagger nematodes.

Primer	Direction	Sequence ($5' \rightarrow 3'$)	Target region	Source
18S	Forward	TTGATTACGTCCCTGCCCTTT	ITS1-5.8S-ITS2 rDNA	Vrain *et al.* (1992)
26S	Reverse	TTTCACTCG CCGTTACTAAGG		Yan & Smiley (2010)
D2A	Forward	ACAAGTACCGTGAGGGAAAGTTG	D2-D3 of 28S rRNA	Nunn (1992)
D3B	Reverse	TCGGAAGGAACCAGCTACTA		De Ley *et al.* (1999)

slightly offset head, long needle-like stylet (odontostyle and odontophore), flanges, and guiding ring (Goodey *et al.*, 1960; Brown & Topham, 1984, 1985; Siddiqi & Lenne, 1990; Decraemer & Gerart, 2006). Further morphometric measurements were also carried out to identify the species of dagger nematode as shown in the section of results.

Molecular identification of dagger nematode at species level

DNA extraction

Once the identity of dagger nematode was confirmed morphologically at the genus level, the specimens were taken from the collected nematode suspensions for species identification. DNA was extracted from adult female nematodes using the proteinase K method (Kumari & Subbotin, 2012; Huang & Yan, 2017). In this method, dagger nematodes were picked under a dissecting microscope (Zeiss Stemi 305 compact stereo microscope) using a dental pick, and placed in a concave slide (Chalex), containing 10 μl distilled water. The nematode was cut into two pieces using the same dental pick under a dissecting microscope in the concave slide. The cut pieces of nematode along with water were added into 0.5 ml sterile Eppendorf tube. In the same tube, 2 μl proteinase K (600 μg ml^{-1}), 2 μl extraction buffer (10×PCR buffer) and 6 μl of double distilled water (ddH_2O) was added. The tube was then placed into refrigerator at -20°C for 30 min, followed by incubation at 65°C for 1 h in the thermal cycler, where the master mix needs to be vortexed after every 30 min, followed by 95°C for 10 min. The DNA was then stored at -20°C for future use.

PCR amplification

The extracted DNA of dagger nematode was amplified using universal primers in polymerase chain reaction (PCR), targeting two genomic regions, D2-D3 of 28S rRNA and ITS of rDNA (Table 1). In this study, 18S and 26S were used as primers for amplifying ITS1, 5.8S and ITS2 of rDNA. A reaction of 20 μl volume was prepared in a 0.5 ml microcentrifuge tube, comprising 3 μl of DNA extracts, 0.5 μM each of forward and reverse primer, 1 × Colorless GoTaq Flexi Buffer with 1.5 mM $MgCl_2$, 0.5 mM $MgCl_2$, 0.32 μl of 0.2 mM dNTP, 1.25 U of GoTaq Flexi DNA Polymerase (Promega), and nuclease free water. The amplification was carried out in a Bio-Rad T100 Thermal Cycler (Bio-Rad), with denaturation at 94°C for 2 min, followed by 94°C for 1 min, 60°C for 50 s and 72°C for 1 min (40 cycles) and extension for 7 min at 72°C (Vrain *et al.*, 1992; Yan & Smiley, 2010).

The 28S rRNA region was amplified with the primers D2A and D3B (Table 1). The master mix of 25 μl volume was prepared in a microcentrifuge tube of 0.5 ml, comprising 2 μl of DNA extracts, 0.5 μM each of forward and reverse primer, 1 × Colorless GoTaq Flexi Buffer with 1.5 mM $MgCl_2$, 0.5 mM $MgCl_2$, 0.32 μl of 0.2 mM dNTP, 0.75 U of GoTaq Flexi DNA Polymerase (Promega), and nuclease free water. The amplification was carried out in a Bio-Rad T100 Thermal Cycler (Bio-Rad), with denaturation at 94°C for 3 min, followed by 94°C for 30 s, 55°C for 1 min and 72°C for 2 min (40 cycles) and extension for 10 min at 7°C (Nunn, 1992; De Ley *et al.*, 1999).

The PCR products were run on agarose gel electrophoresis to confirm the amplification of DNA (Huang *et al.*, 2018). Agarose gel electrophoresis was carried out by loading PCR products in solidified agarose gel (2%) in TAE buffer and 2 μl ethidium bromide placed in a buffer tank (Owl EasyCast Wide-Format Horizontal Electrophoresis Systems, Model D13-14 System; Thermofisher Scientific). The PCR samples were run on gel for 35 min at 80 V. The bands on gel were observed using AlphaImager Gel Documentation System (Protein Simple). PCR products having dagger nematode DNA showed amplicon bands under ultraviolet light whereas no bands were observed in non-template control. PCR products showing bands confirm the amplification of DNA and

were further purified using the E.Z.N.A.® Cycle Pure kit (Omega).

Sanger sequencing and analysis

The purified samples were sent for Sanger sequencing to the Molecular Cloning Laboratories (MCLAB). Sequencing was done in both directions for all the purified PCR products. The sequencing data obtained from MCLAB were analysed using the BioEdit sequence alignment editor (Hall, 2011). The sequencing data were in the form of chromatographs. The ambiguous peaks in starting and end of each chromatograph were removed to obtain high-quality sequences. These cleaned sequences were aligned using Clustal/Multi Alignment tool in BioEdit (Hall, 2011) and the consensus sequence for each amplified gene was created, and deposited into GenBank to obtain the accession numbers (OQ974799 and OQ969990). All consensus sequences were further compared with other sequences in GenBank at NCBI using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis

The phylogenetic relationships among the dagger nematodes used in this study were explored by comparing with the reference sequences of dagger nematode deposited in the NCBI GenBank. Using molecular evolutionary genetics analysis (MEGA 11) software, a maximum-likelihood (ML) tree was constructed for the ITS region (Kumar *et al.*, 2016). Sequences obtained in this study and retrieved from GenBank were aligned by MUSCLE (v3.8.31) tool available in MEGA 7 software with default settings. The best suited model to construct the ML tree for aligned sequences was determined by the MODELS tool of the MEGA 11 software. Accordingly, Kimura-2 parameter model using gamma-distributed invariant sites was used as suggested by MODELS tool and ML bootstrapping tree was generated with 1000 replicate numbers (Kimura,1980).

Reproduction of dagger nematode using infested field soil

Initial nematode density determination in soil

Soil samples that were positively identified to contain dagger nematodes by sugar centrifugal flotation and manual counting were combined and then mixed to achieve a uniform distribution. The uniformly mixed soil was used for growing potato tubers in the glasshouse. Before planting, five sub-samples were collected randomly from this mixed soil to determine the density of nematodes in soil. The tray method (Whitehead & Hemming, 1965) was used for extracting live nematodes from soil. Nematodes were counted by placing 1 ml suspension in Peters 1 ml gridded slide (Chalex) and observing the nematodes in the slide under an inverted transmitted light microscope.

Table 2. List of all the potato cultivars used for evaluating the reproduction ability of dagger nematode, *Xiphinema americanum*, under controlled glasshouse conditions.

Potato cultivar	Market class	Utilisation
'Dakota Ruby'	Red	Fresh
'Sangre'	Red	Baking and boiling
'Modoc'	Red	Fresh market
'Colorado Rose'	Red	Table stock
'Dakota Rose'	Red	Baking, boiling, fresh
'Dark Red Chieftain'	Red	Table stock
'Silverton Russet'	Russet	Fries or fresh
'Clearwater'	Russet	Processing or fresh
'Ranger Russet'	Russet	Fries or fresh
'Russet Burbank'	Russet	Baking, mashing, fries
'Russet Norkotah 278'	Russet	Baking, fries, chips
'Russet Norkotah'	Russet	Baking, boiling, fresh
'Pomerelle Russet'	Russet	Baking, boiling
'Manistee'	White	Chipper
'Cascade'	White	Processing or fresh
'Kennebec'	White	Baking, boiling, fresh
'Soraya'	Yellow	Salads and soups
'Yukon Gold'	Yellow	Boiling, fresh, baking
'Satina'	Yellow	Boiling, roasting
'Milva'	Yellow	Fresh, cooking
'Columba'	Yellow	Fresh or fries

Potato cultivars

A total of 21 potato cultivars were used for the evaluation of reproduction potential of dagger nematode (Table 2). Potato cultivars belonging to four market classes: yellow, white, russet, and red were selected. Potato tubers were pre-sprouted before planting in the glasshouse by placing them on a moist paper towel in plastic trays for a few days at room temperature (*ca* 22°C). The sprouted potato tubers were sliced into two or three sections, with at least three or more sprouted bud eyes in each cut tuber, to ensure optimal growth in glasshouse conditions. Before planting, the cut tubers with sprouts were kept at room temperature for 2 or 3 days to suberise.

Glasshouse trials

Sprouted potato tubers were grown in plastic pots (15 cm top diam. × 15 cm height; Dillen Products)

using uniformly mixed soil containing dagger nematodes. Planting was done in a completely randomised design in the glasshouse (North Dakota State University Agricultural Experiment Station- Jack Dalrymple Agricultural Research Complex, Fargo, ND, USA) at *ca* 22°C and 16 h daylight with five replications of each cultivar and non-planted control. The potato plants were watered once a day. Thus, a total of 110 pots were used with 1 kg soil in each pot. Potato plants were harvested after about 12 weeks. The soil samples were processed using sugar centrifugal and flotation method. The dagger nematodes were quantified to obtain the final population density by manual counting with an inverted transmitted light microscope (Zeiss Axiovert 25; Carl Zeiss). Reproductive factor (RF) was calculated by dividing the final nematode population density by the initial population density (Oostenbrink, 1966). Two glasshouse trials were conducted. Planting for the first trial was done on June 17, 2021, using soil with initial dagger nematode population density of 700 nematodes (kg soil)$^{-1}$, and the potato plants were harvested on September 9, 2021. For the second trial, initial nematode population density was 350 nemtodes (kg soil)$^{-1}$, and planting was done using the same potato cultivars on November 5, 2021, and potato plants were harvested on January 29, 2022.

Data analysis

The final population densities from all the cultivars and RF values of dagger nematode for the potato cultivars and market classes were analysed using SAS 9.4 (SAS Institute). Analysis of variance (ANOVA) was conducted followed by *post-hoc* Tukey's test to discern the significant differences among the potato cultivars and market classes for final population densities and RF values of dagger nematode (Neupane & Yan, 2023). The analysis of two trials for final population density and RF values was done separately because the data were not homogenous for both the trials.

Results

Species identification

Morphometric measurements

Based on the morphological features of the nematodes that were isolated from field samples, the presence of dagger nematodes was found in the potato fields of North Dakota. The morphometric measurements of females (n = 10) including body length, odontostyle, odontophore, total stylet, guiding ring from oral aperture, tail, body diam. at anus, J, body diam. at beginning of J, body diam. at 5 μm from tail terminus, and V% are presented in Table 3. The morphological characteristics and morphometric measurements of adult females isolated from soil samples indicated the identity of the dagger nematode species as *Xiphinema americanum* Cobb, 1913.

Table 3. Morphometric measurements of dagger nematode for identification of the dagger nematode species at morphological level. All measurements are in μm and in the form: mean ± s.d. (range) except V%.

Character	
Body length	1648.0 ± 77.1 (1448-1707)
Odontostyle length	72.7 ± 3.4 (65-75)
Odontophore length	49.3 ± 1.6 (45-50)
Total stylet	121.9 ± 4.8 (110-125)
Guiding ring from oral aperture	62.1 ± 2.1 (62-65)
Tail length	35.0 ± 2.3 (30-40)
Body diam. at anus	21.2 ± 0.9 (20-22)
J	7.9 ± 0.7 (7-9)
Body diam. at beginning of J	8.5 ± 1.0 (7-11)
Body diam. at 5 μm from tail terminus	7.2 ± 0.6 (7-8)
V%	50.4 ± 0.7 (50-52)

Molecular diagnosis

The molecular diagnosis to identify and confirm the dagger nematode species was based on the analysis of sequencing data of amplified dagger nematode DNA from two genomic regions (D2-D3 of 28S rRNA, and ITS of rDNA). The sequence obtained (749 bp; GenBank accession no. OQ974799) from primer pair D2A/D3B (D2-D3 of 28S rRNA) had 98.18% similarity with three *X. americanum* isolates: CD100 (KX263175.1) and XA32 (KX263174.1) from Texas, and CD1078 (KX263173.1) from California. Other than *X. americanum*, the highest similarity of D2-D3 region of 28S rRNA was 97.59% with *X. californicum*. Additionally, the consensus sequence of ITS region obtained (860 bp; OQ969990) using 18S/26S primer pair was 97.68% identical to a population of *X. americanum* from the UK (AY430189.1). The highest similarity of the ITS region-based consensus sequence with other dagger nematode species was 95.15% (*e.g.*, L2 isolate of *X. laevistriatum* (DQ299529.1)) or below 95.15%. Thus, the sequence analysis of PCR products confirms the species identity of dagger nematode as *X. americanum*.

Phylogenetic relationship

The phylogenetic relationship was established using sequences of the ITS region. Evolutionary history was inferred by using the maximum likelihood method and Kimura 2-parameter model (Kimura, 1980). The phylogenetic tree of ITS rDNA displayed two well-supported clades: (clade I) ten sequences, including isolates of *X. americanum*, *X. citricolum*, *X. georgianum*, *X. peruvianum*, *X. oxycaudatum*, *X. thornei*, *X. californicum* and *X. diffusum*; and (clade II) six sequences including isolates of *X. index*, *X. bernardi*, *X. coxi europaeum*, *X. belmontense*, *X. baetica* and *X. globosum* (Fig. 3). The *Xiphinema americanum* (OQ969990) sequence obtained in this study displayed a close relationship with isolates of *X. americanum*, AY430189 from the UK, and AY430188 from Belgium, as compared to the other species of the *X. americanum* group species within the clade.

GLASSHOUSE TRIALS

In Trial 1, variations in the final population density of dagger nematodes in 21 potato cultivars were observed. The initial population density (P_i) of dagger nematode was 700 nematodes (kg soil)$^{-1}$. After a 12-week period, the mean final population (P_f) of *X. americanum* (kg soil)$^{-1}$ was higher than the initial inoculum in two cultivars, 'Dakota Ruby' ($P_f = 870$) and 'Soraya' ($P_f = 780$), but all other cultivars had P_f lower than the P_i. The lowest P_f was observed in 'Columba' and 'Kennebec' with both showing 60 nematodes (kg soil)$^{-1}$. Both 'Columba' and 'Kennebec' had significantly lower ($P < 0.05$) P_f than 'Soraya' and 'Dakota Ruby'. All the other cultivars had P_f in the range of 120 to 420 nematodes (kg soil)$^{-1}$.

Similarly to Trial 1, there were variations in final population densities of dagger nematode in Trial 2. Among the 21 cultivars used with the P_i of 350 nematodes (kg soil)$^{-1}$, the highest P_f was observed in 'Soraya' with almost double the P_i (612 nematodes (kg soil)$^{-1}$), followed by 'Dark Red Chieftain' (610 nematodes (kg soil)$^{-1}$), 'Columba' (555 nematodes (kg soil)$^{-1}$) and 'Satina' (482 nematodes (kg soil)$^{-1}$). 'Milva', 'Silverton Russet' and 'Cascade' showed slightly higher numbers of dagger nematodes (388, 376 and 355 nematodes (kg soil)$^{-1}$, respectively) than the P_i. In 'Dakota Rose' and 'Pomerelle Russet', the nematode population was reduced to 40 and 45 nematodes (kg soil)$^{-1}$, respectively. Statistically significant differences were not observed ($P \geqslant 0.05$) for Trial 2.

Based on the RF values, it was observed that the overall reproduction rate was greater in the second trial (Fig. 1, bottom panel) than the first trial (Fig. 1, top panel). 'Soraya' had high average RF values in both the trials (RF = 1.1 in the first trial and RF = 1.7 in the second trial). 'Yukon Gold' showed consistent RF results in both the trials (RF = 0.6). Similarly, 'Ranger Russet', 'Manistee', 'Kennebec', 'Russet Norkotah 278', 'Modoc', 'Pomerelle Russet' and 'Dakota Rose' showed RF values less than 0.5 in both the trials. All the above cultivars that had RF values lower than 1, indicating that they are poor hosts for *X. americanum*. By contrast, 'Soraya' had RF values greater than 1 and can be considered a relatively susceptible cultivar.

COMPARISON OF MARKET CLASSES OF POTATO CULTIVARS BASED ON THE RF VALUES OF DAGGER NEMATODES

Dagger nematode reproduction ability was compared on four market classes (Table 2) of potatoes by measuring the RF value (Fig. 2). For both the trials, numerically the mean RF values for market classes were in order of yellow $\geqslant$ red $>$ russet $>$ white. From the statistical analysis no significant differences were found among the four different market classes for the first trial ($P \geqslant 0.05$), but for the second trial, the yellow type of potato cultivars displayed significantly higher reproductive rate ($P < 0.05$) than the russet and white skinned cultivars. Red skinned cultivars showed no statistical differences with yellow, russet and white type of potatoes.

Discussion

This study identified the dagger nematode species, *Xiphenema americanum*, in potato fields of North Dakota, USA. Also, the research described the reproduction potential of *X. americanum* in 21 potato cultivars. This is the first study of testing the reproduction of *X. americanum* on potato cultivars in controlled glasshouse conditions.

The economic loss in the crop varies according to the species, crop, location and soil type (Ravichandra, 2014), and the population density of that species (Koenning *et al.*, 1999). According to a report by Michigan State University in 1993, the threshold damage of dagger nematode in apple, cherry and peach was as low as 10 dagger nematodes (kg soil)$^{-1}$ (Bird & Melakeberhan *et al.*, 1993), whereas according to the Nematode Guidelines for South

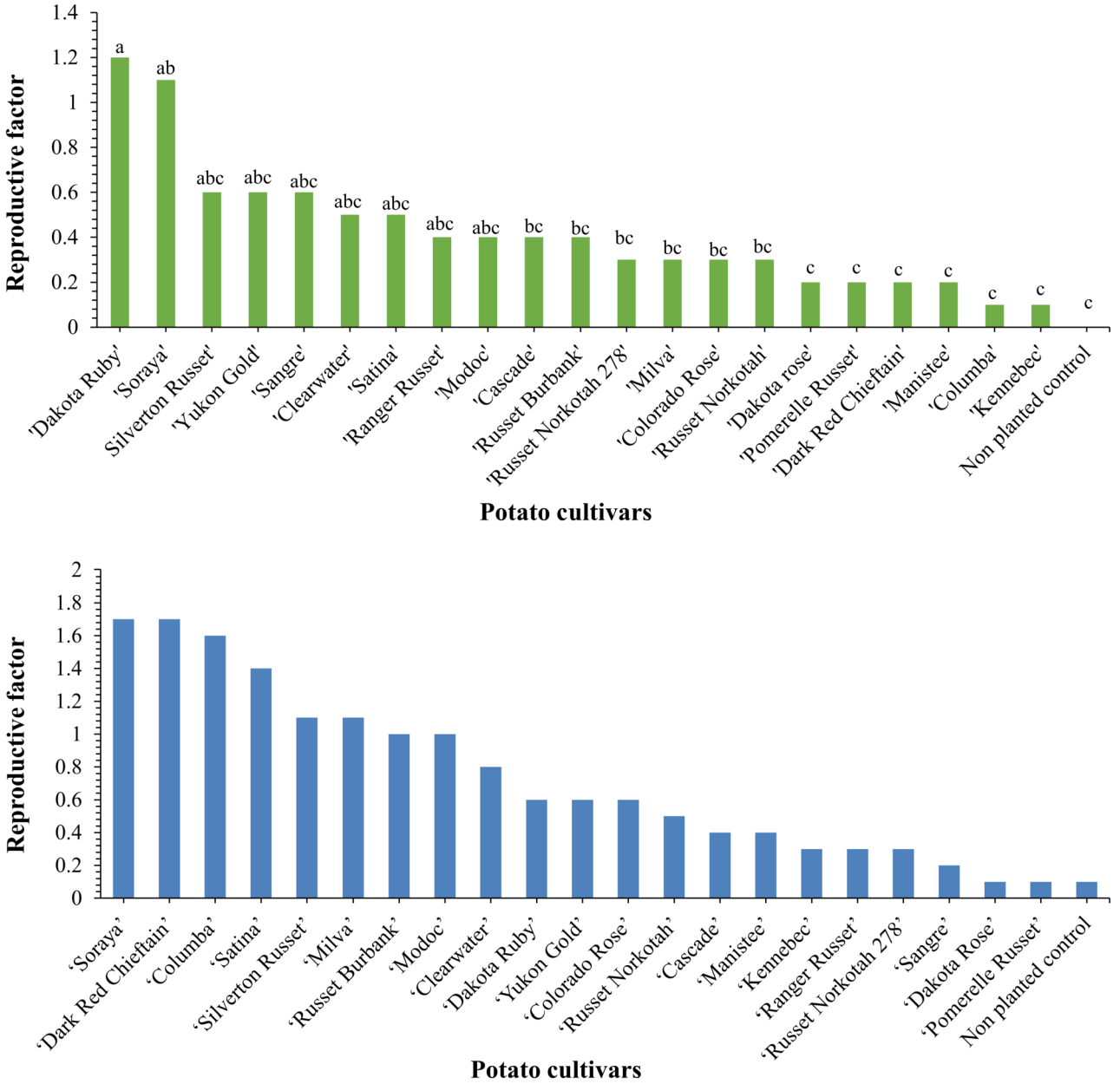

Fig. 1. Reproductive factor (RF) values of dagger nematode, *Xiphinema americanum*, on potato cultivars grown in infested soil under the glasshouse conditions. RF values are the mean of five replications. Final nematode density in each pot with single plant was determined after harvesting the trials 12 weeks after planting. Top panel: RF values from Trial 1. Initial dagger nematode density for *X. americanum* was 700 kg^{-1} soil at the time of planting in the glasshouse. Bars with the same letter are not significantly different according to the Tukey's HSD test ($P \geqslant 0.05$); Bottom panel: RF values from Trial 2. Initial dagger nematode density for *X. americanum* was 350 kg^{-1} soil at the time of planting in the glasshouse. There were no significant differences according to the Tukey's HSD test ($P \geqslant 0.05$).

Carolina by the Clemson University Cooperative Extension Service (Dickerson *et al.*, 2000), the threshold values of dagger nematodes for most of the crops varied from 500 to 2000 nematodes (kg soil)$^{-1}$. The economic threshold of dagger nematode for potato has not been established in previous literature. Previously, *X. americanum* was found

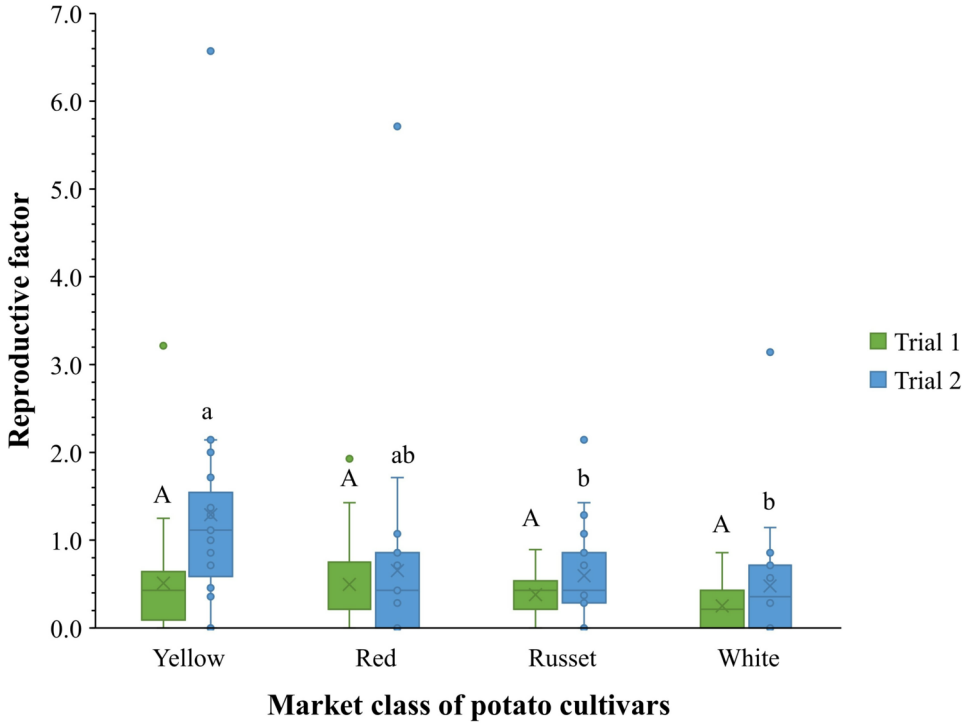

Fig. 2. Reproductive factor (RF) of potato cultivars belonging to four different market classes: yellow, red, russet and white type for Trial 1 and Trial 2. Mean RF of the potato cultivars in every class has been represented by 'x'. For Trial 1 (green boxes), the means of RF of yellow and red type potato cultivars were followed by russet type and white type. The market classes for Trial 1 with same capital letters were not significantly different ($P \geqslant 0.05$). For Trial 2 (blue boxes), mean RF of yellow type was the highest, followed by red, russet and white type. The RF values of market classes for Trial 2 with same letters were not significantly different from each other ($P \geqslant 0.05$).

in sugar beet fields of North Dakota (Donald & Hosford, 1980). Robbins (1993) reported that *X. thornei* was found in North Dakota, but the report did not mention the crop or the location of dagger nematode.

It is challenging to identify the species within *Xiphinema* genus (Orlando *et al.*, 2016; Daramola *et al.*, 2019) because of similar overlapping morphological features. A combined morphological and molecular analysis with phylogenetic inference was used to identify the nematode species (Daramola *et al.*, 2019). In this study, naturally infested field soils were collected from potato field plots in North Dakota. Using morphological visualisations under the microscope as described by Lamberti & Bleve-Zacheo (1979) and Lamberti & Carone (1991), dagger nematode presence (at genus level) in potato fields was confirmed. The morphometric measurements indicated the species as *X. americanum*. Further molecular analysis identified the species of dagger nematode based on the sequence analysis. Sequences of the ITS region showed 97.68% identity match with *X. americanum*, and D2-D3 of 28S region showed 98.18% match with *X. americanum* sequences in GenBank. Sequences are available for D2-D3 expansion segments of numerous Tylenchid (Subbotin *et al.*, 2006) and Longidorid (Floyd *et al.*, 2002; He *et al.*, 2005; Rubtsova *et al.*, 2005) nematodes. To distinguish dagger nematodes at species level, ITS and D2-D3 genomic regions were used (He *et al.*, 2005; Orlando *et al.*, 2016), allowing us to identify the DNA sequences belonging to *X. americanum*. The sequences obtained in this study for species delimitation indicated that the dagger nematode belongs to the *X. americanum* group species. The monophylectic relationship was observed among the *X. americanum* complex species and *X. non-americanum* complex species. The evolutionary relations among the ITS sequences of the dagger nematode obtained in this study clearly distinguished it from the species of *X. non-americanum* group and outgroups and showed a close relationship with the isolates of *X. americanum* species.

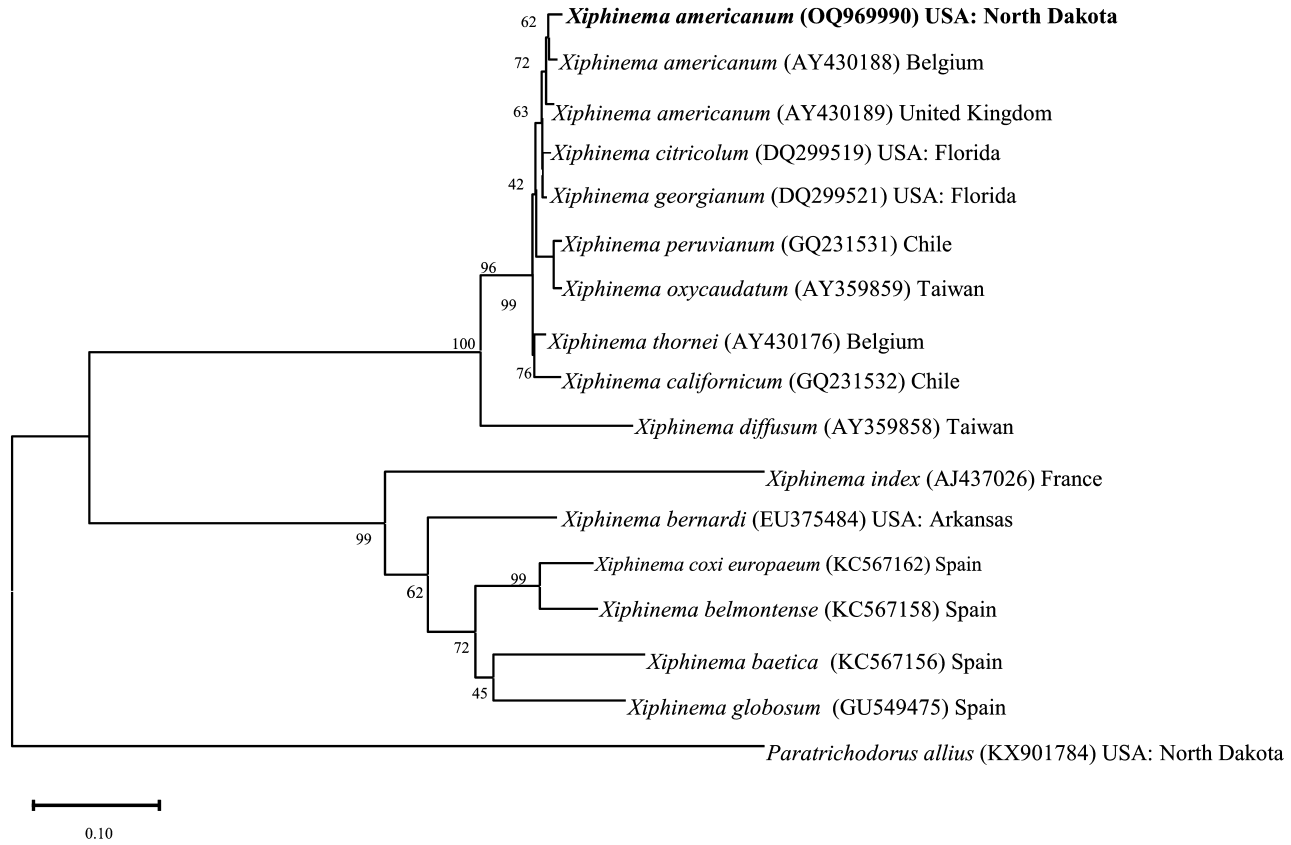

Fig. 3. Phylogenetic relations among the *Xiphinema* species based on the ITS ribosomal DNA (rDNA) sequences. The maximum likelihood (ML) tree was constructed using the MEGA11 software with alignment under a Kimura-2 parameter model using gamma-distributed invariant sites. The scale bar in the bottom left corner represents the nucleotide substitutions per position. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The ITS rDNA sequence of *Paratrichodorus allius* was used as outgroup. Newly obtained sequence in this study is in boldface.

Xiphinema americanum is one of the major plant-parasitic nematodes in the USA (Mitiku, 2018) with a wide host range (Gozel *et al.*, 2006; Wick, 2012; Mokrini *et al.*, 2014; Rosa *et al.*, 2014). To our knowledge, there has been no study that evaluated the reproduction of *X. americanum* on potato cultivars grown in the USA. In the current study, the reproduction of *X. americanum* on potato cultivars was demonstrated. Variable reproduction was observed for *X. americanum* in potato cultivars belonging to different market classes. Yellow type of potato cultivars had significantly high reproduction of dagger nematode as compared to cultivars of russet and white market classes in one trial and, hence, could be susceptible to *X. americanum.* 'Soraya', a yellow market class cultivar, showed higher RF in both the trials, suggesting it is a good host for reproduction of *X. americanum* and should not be grown in potato fields where *X. americanum* is a problem. Additionally, we observed that white type potato cultivars such as 'Manistee', 'Kennebec' and 'Cascade' did not favour the reproduction of *X. americanum*.

Although all the parameters were kept uniform as much as possible for all the replicates of the cultivars in both the trials, such as vigorously mixed soil for uniform distribution of nematodes, same temperature settings, and same planting as well as harvesting methods, yet within the replicates of the same cultivar, higher RF values were observed for some of the replicates. In our research data, 'Dark Red Chieftain', had one of the replicates with a very high RF value (RF = 5.6) as compared to other replicates. Similarly, 'Soraya' had one of the five replicates in the second trial that had a RF of 6.6.

A similar trend was observed by Dias *et al.* (2012) where, for the same cultivar, highly different RF values were obtained for the same *Meloidogyne* isolate. The presence of these variations or outliers might be due to variations in physiological, environmental, watering and sampling conditions (Dias *et al.*, 2012; Scanlan *et al.*, 2018).

In the current study, reproduction ability of *X. americanum* was observed to be influenced by the P_i. Since the soil used for planting potatoes was collected from a naturally infested field separately for two trials at different times of year, the initial nematode population was different for the two trials. It was observed that the reproduction of nematodes was higher in Trial 2 when the P_i was 350 nematodes (kg soil)$^{-1}$ as compared to Trial 1 when initial population was 700 nematodes (kg soil)$^{-1}$. Such variation could be explained by intraspecific competition due to feeding on same common site (Upadhaya *et al.*, 2019), which could cause a reduction in reproduction (Duncan & Ferris, 1983). In this regard, our results were similar to reproduction of pin nematode on field pea (Upadhaya *et al.*, 2019), tobacco (Coursen & Jenkins, 1958) and reproduction of *Rotylenchulus reniformis* on sweet potato (Clark & Wright, 1983), where higher initial population led to lower reproduction as compared to lower initial population. Apart from P_i, other factors that could also be responsible for variations in reproduction of first and second trials might be different planting time, soil moisture, direct sunlight or tuber health in the glasshouse (Lownsbery, 1975; Upadhaya *et al.*, 2019).

In conclusion, *X. americanum* is present in North Dakota and has the ability to reproduce in some of the potato cultivars grown in the region. This research provides critical information about the species identification of dagger nematode, which is important for phytosanitary and management measures. Since both the quarantine regulations and management approaches are put in place in accordance with the available information about the presence of species in an area (Allen *et al.*, 2017), our study provides essential baseline information by identifying *X. americanum* in a North Dakota potato field. The current study also demonstrates the reproductive ability of dagger nematode in 21 potato cultivars. In one of the trials, cultivars belonging to the yellow type of market classes favoured the reproduction of dagger nematode, whereas the white type of market classes lowered reproduction rate. Further study using higher number of cultivars in each market class could help to determine if the differences in RF of dagger nematode on market classes of potato cultivars persist. Since low dagger nematode population can cause crop losses (Evans *et al.*, 2007), further studies could be conducted to compare the yield losses of those potato cultivars that supported dagger nematode reproduction, and determine the economic threshold of dagger nematodes in potato fields of North Dakota.

In future, the information generated from this study could be used as an asset to manage the potato fields where *X. americanum* is a problem.

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References

Allen, E., Noseworthy, M. & Ormsby, M. (2017). Phytosanitary measures to reduce the movement of forest pests with the international trade of wood products. *Biological Invasions* 19, 3365-3376. DOI: 10.1007/s10530-017-1515-0

Andret-Link, P., Schmitt-Keichinger, C., Demangeat, G., Komar, V. & Fuchs, M. (2004). The specific transmission of grapevine fanleaf virus by its nematode vector *Xiphinema index* is solely determined by the viral coat protein. *Virology* 320, 12-22. DOI: 10.1016/j.virol.2003.11.022

Berry, S.D., Fargette, M., Spaull, V.W., Morand, S. & Cadet, P. (2008). Detection and quantification of root-knot nematode (*Meloidogyne javanica*), lesion nematode (*Pratylenchus zeae*) and dagger nematode (*Xiphinema elongatum*) parasites of sugarcane using real-time PCR. *Molecular and Cellular Probes* 22, 168-176.

Bird, G.W. & Melakeberhan, H. (1993). *Avoidance and management of nematode problems in tree fruit production in Michigan*. East Lansing, MI, USA, Michigan State University Extension Bulletin E-2419, pp. 1-20. Available online at https://www.canr.msu.edu/uploads/resources/pdfs/avoidance_and_management_of_nematode_problems_in_tree_fruit_production_in_michigan_(e2419).pdf

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M. *et al.* (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* 392, 71-75. DOI: 10.1038/32160

Brodie, B.B., Good, J.M. & Jaworski, C.A. (1970). Population dynamics of plant nematodes in cultivated soil: effect of

summer cover crops in newly cleared land. *Journal of Nematology* 2, 217-222.

Brown, D.J. & Topham, P.B. (1985). Morphometric variability between populations of *Xiphinema diversicaudaturn* (Nematoda: Dorylaimoidea). *Revue de Nématologie* 8, 15-26.

Brown, D.J., Halbrendt, J.M., Robbins, R.T. & Vrain, T.C. (1993). Transmission of nepoviruses *by Xiphinema americanum*-group nematodes. *Journal of Nematology* 25, 349-354.

Brown, D.J.F. & Topham, P.B. (1984). A comparison of reported variation in the morphometrics of *Xiphinema diversicaudatum* (Nematoda: Dorylaimida) and the effects of some methods of preparing specimens for examination by optical microscopy. *Nematologia Mediterranea* 12, 169-186. Available online at https://journals.flvc.org/nemamedi/article/view/85499

Clark, C.A. & Wright, V.L. (1983). Effect and reproduction of *Rotylenchulus reniformis* on sweet potato selections. *Journal of Nematology* 15, 197-203.

Coomans, A., Huys, R., Heyns, J. & Luc, M. (2001). Character analysis, phylogeny and biogeography of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae). *Annals of Zoological Sciences of the Royal Museum for Central Africa, Tervuren, Belgium* 287, 1-239.

Coursen, B.W. & Jenkins, W.R. (1958). Host-parasite relationships of the pin nematode, *Paratylenchus projectus*, on tobacco and tall fescue. *Plant Disease Reporter* 42, 865-872.

Daramola, F.Y., Knoetze, R., Swart, A. & Malan, A.P. (2019). First report and molecular characterization of the dagger nematode, *Xiphinema oxycaudatum* (Nematoda, Dorylaimidae) from South Africa. *ZooKeys* 894, 1-17. DOI: 10.3897/zookeys.894.35281

De Ley, I.T., Karssen, G., De Ley, P., Vierstraete, A., Waeyenberge, L., Moens, M. & Vanfleteren, J. (1999). Phylogenetic analyses of internal transcribed spacer region sequences within *Meloidogyne*. *Journal of Nematology* 31, 530-531.

Decraemer, W. & Geraert, E. (2006). Ectoparasitic nematodes. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology*. Wallingford, UK, CAB International, pp. 153-184. DOI: 10.1079/9781845930561.0153

Di Sanzo, C.P. & Rohde, R.A. (1969). *Xiphinema americanum* associated with maple decline in Massachusetts. *Phytopathology* 59, 279-284.

Dias, M.C., Conceição, I.L., Abrantes, I. & Cunha, M.J. (2012). *Solanum sisymbriifolium* – a new approach for the management of plant-parasitic nematodes. *European Journal of Plant Pathology* 133, 171-179. DOI: 10.1007/s10658-012-9945-0

Dickerson, O.J., Blake, J.H. & Lewis, S.A. (2000). *Nematode guidelines for South Carolina*. Clemson, SC, USA, Clemson University Cooperative Extension EC 703. Available online at https://www.clemson.edu/public/regulatory/plant-problem/pdfs/nematode-guidelines-for-south-carolina.pdf

Donald, P.A. & Hosford Jr, R.M. (1980). Plant parasitic nematodes of North Dakota. *Plant Disease* 64, 45-47.

Duncan, L.W. & Ferris, H. (1983). Validation of a model for prediction of host damage by two nematode species. *Journal of Nematology* 15, 227-234.

Evans, T.A., Miller, L.C., Vasilas, B.L., Taylor, R.W. & Mulrooney, R.P. (2007). Management of *Xiphinema americanum* and soybean severe stunt in soybean using crop rotation. *Plant Disease* 91, 216-219. DOI: 10.1094/PDIS-91-2-0216

Floyd, R., Abebe, E., Papert, A. & Blaxter, M. (2002). Molecular barcodes for soil nematode identification. *Molecular Ecology* 11, 839-850. DOI: 10.1046/j.1365-294X.2002.01485.x

Food and Agriculture Organization of the United Nations. Statistics Division (2020). *World food and agriculture statistical yearbook*. Rome, Italy, Food and Agriculture Organization of the United Nations.

Goodey, J.B., Peacock, F.C. & Pitcher, R.S. (1960). A redescription of *Xiphinema diversicaudatum* (Micoletzky, 1923 & 1927) Thorne, 1939 and observations on its larval stages. *Nematologica* 5, 127-135. DOI: 10.1163/187529260X00497

Gozel, U., Lamberti, F., Duncan, L., Agostinelli, A., Rosso, L., Nguyen, K. & Adams, B.J. (2006). Molecular and morphological consilience in the characterisation and delimitation of five nematode species from Florida belonging to the *Xiphinema americanum*-group. *Nematology* 8, 521-532. DOI: 10.1163/156854106778613958

Hall, T. (2011). BioEdit: an important software for molecular biology. *GERF Bulletin of Biosciences* 2, 60-61.

Handoo, Z.A. (1998). *Plant-parasitic nematodes*. Beltsville, MD, USA, USDA, ARS, Nematology Laboratory.

He, Y., Subbotin, S.A., Rubtsova, T.V., Lamberti, F., Brown, D.J. & Moens, M. (2005). A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology* 7, 111-124. DOI: 10.1163/1568541054192108

Heve, W.K., Crow, W.T. & Mengistu, T. (2015). *Dagger nematode* Xiphinema spp. *(Cobb, 1913) Inglis, 1983 (Nematoda: Enoplea: Dorylaimia: Dorylaimina: Xiphinematinae)*. Gainesville, FL, USA, EDIS, UF/IFAS Extension, EENY 626. Available online at https://edis.ifas.ufl.edu/publication/IN1097

Huang, D., Yan, G., Gudmestad, N. & Skantar, A. (2017). Quantification of *Paratrichodorus allius* in DNA extracted from soil using TaqMan probe and SYBR green real-time PCR assays. *Nematology* 19, 987-1001. DOI: 10.1163/15685411-00003101

Huang, D., Yan, G., Gudmestad, N., Whitworth, J., Frost, K., Brown, C., Ye, W., Agudelo, P. & Crow, W. (2018). Molecular characterization and identification of stubby root nematode species from multiple states in the United States. *Plant Disease* 102, 2101-2111. DOI: 10.1094/PDIS-10-17-1668-RE

Jenkins, W.R.B. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48, 692.

Jones, J.T., Haegeman, A., Danchin, E.G., Gaur, H.S., Helder, J., Jones, M.G., Kikuchi, T., Manzanilla-López, R., Palomares-

Rius, J.E., Wesemael, W.M.L. *et al.* (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14, 946-961. DOI: 10.1111/mpp.12057

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111-120. DOI: 10.1007/BF01731581

Koenning, S.R., Overstreet, C., Noling, J.W., Donald, P.A., Becker, J.O. & Fortnum, B.A. (1999). Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. *Journal of Nematology* 31, 587-618.

Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33, 1870-1874. DOI: 10.1093/molbev/msw054

Kumari, S. & Subbotin, S.A. (2012). Molecular characterization and diagnostics of stubby root and virus vector nematodes of the family Trichodoridae (Nematoda: Triplonchida) using ribosomal RNA genes. *Plant Pathology* 61, 1021-1031. DOI: 10.1111/j.1365-3059.2012.02598.x

Lamberti, F. & Bleve-Zacheo, T. (1979). Studies on *Xiphinema americanum sensu lato* with descriptions of fifteen new species (Nematoda, Longidoridae). *Nematologia Mediterranea* 7, 51-106.

Lamberti, F. & Carone, M. (1991). A dichotomous key for the identification of species of *Xiphinema* (Nematoda: Dorylaimida) within the *X. americanum*-group. *Nematologia Mediterranea* 19, 341-348.

Lima, F.S., Mattos, V.S., Silva, E.S., Carvalho, M.A., Teixeira, R.A., Silva, J.C. & Correa, V.R. (2018). Nematodes affecting potato and sustainable practices for their management. In: Yildiz, M. (Ed.). *Potato: from Incas to all over the World*, *6*, pp. 107-124. DOI: 10.5772/intechopen.73056

Lownsbery, B.F., Stoddard, E.M. & Lownsbery, J.W. (1952). *Paratylenchus hamatus* pathogenic to celery. *Phytopathology* 42, 651-653.

Mai, W.F., Mullin, P.G., Lyon, H.H. & Loeffler, K. (1996). *Plant-parasitic nematodes: a pictorial key to genera*. Ithaca, NY, USA, Cornell University Press.

Mitiku, M. (2018). Plant-parasitic nematodes and their management: a review. *Agricultural Research and Technology* 16, 555980. DOI: 10.19080/ARTOAJ.2018.16.555980

Neupane, K. & Yan, G. (2023). Host suitability of cover crops to the root-lesion nematode, *Pratylenchus penetrans* associated with potato. *Plant Disease* 107, 2096-2103. DOI: 10.1094/PDIS-08-22-2001-RE

Norton, D.C. (1979). Relationship of physical and chemical factors to populations of plant-parasitic nematodes. *Annual Review of Phytopathology* 17, 279-299. DOI: 10.1146/annurev.py.17.090179.001431

Nunn, G.B. (1992). *Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences*. Ph.D. Thesis, University of Nottingham, Nottingham, UK.

Oostenbrink, M. (1966). Major characteristics of the relation between nematodes and plants. *Mededelingen Van de Landbouwhogeschool te Wageningen* 66, 1-46.

Orlando, V., Chitambar, J.J., Dong, K., Chizhov, V.N., Mollov, D., Bert, W. & Subbotin, S.A. (2016). Molecular and morphological characterisation of *Xiphinema americanum*-group species (Nematoda: Dorylaimida) from California, USA, and other regions, and co-evolution of bacteria from the genus *Candidatus Xiphinematobacter* with nematodes. *Nematology* 18, 1015-1043. DOI: 10.1163/15685411-00003012

Perry, R.N., Subbotin, S.A. & Moens, M. (2007). Molecular diagnostics of plant-parasitic nematodes. In: Punja, Z.K., de Boer, S.H. & Sanfaçon, H. (Eds). *Biotechnology and plant disease management*. Wallingford, UK, CAB International, pp. 195-226.

Ravichandra, N.G. & Ravichandra, N.G. (2014). Nematode population threshold levels. In: *Horticultural nematology*, pp. 101-114. DOI: 10.1007/978-81-322-1841-8_6

Robbins, R.T. (1993). Distribution of *Xiphinema americanum* and related species in North America. *Journal of Nematology* 25, 344-348.

Rosa, J.M.O., Oliveira, S.A.D., Jordão, A.L., Siviero, A. & Oliveira, C.M.G.D. (2014). [Plant parasitic nematodes on cassava cultivated in the Brazilian Amazon]. *Acta Amazonica* 44, 271-275. DOI: 10.1590/S0044-59672014000200013

Rubtsova, T.V., Moens, M. & Subbotin, S.A. (2005). PCR amplification of a rRNA gene fragment from formalin-fixed and glycerine-embedded nematodes from permanent slides. *Russian Journal of Nematology* 13, 137-140.

Scanlan, L.D., Lund, S.P., Coskun, S.H., Hanna, S.K., Johnson, M.E., Sims, C.M., Brignoni, K., Lapasset, P., Petersen, E.J., Elliott, J.T. *et al.* (2018). Counting *Caenorhabditis elegans*: protocol optimization and applications for population growth and toxicity studies in liquid medium. *Scientific Reports* 8, 904. DOI: 10.1038/s41598-018-19187-3

Siddiqi, M.R. & Lenné, J.M. (1990). *Xiphinema llanosum* and *Trophurus vultus*, two new plant nematode species from pasture soils in Colombia. *Journal of Nematology* 22, 262-267.

Upadhaya, A., Yan, G. & Pasche, J. (2019). Reproduction ability and growth effect of pin nematode, *Paratylenchus nanus*, with selected field pea cultivars. *Plant Disease* 103, 2520-2526. DOI: 10.1094/PDIS-12-18-2136-RE

USDA-NASS (2020). *United States department of agriculture – National Agricultural Statistics*. Washington, DC, USA, USDA-NASS. Available online at https://quickstats.nass.usda.gov

Van Zyl, S., Vivier, M.A. & Walker, M.A. (2012). *Xiphinema index* and its relationship to grapevines: a review. *South African Journal of Enology and Viticulture* 33, 21-32. DOI: 10.21548/33-1-1302

Vrain, T.C., Wakarchuk, D.A., Levesque, A.C. & Hamilton, R.I. (1992). Intraspecific rDNA restriction fragment length poly-

morphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15, 563-573.

Wasilewska-Nascimento, B., Boguszewska-Mańkowska, D. & Zarzyńska, K. (2020). Challenges in the production of high-quality seed potatoes (*Solanum tuberosum* L.) in the tropics and subtropics. *Agronomy* 10, 260. DOI: 10.3390/agronomy10020260

Whitehead, A.G. & Hemming, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, 25-38. DOI: 10.1111/j.1744-7348.1965.tb07864.x

Wick, R. (2012). *Nematodes on golf greens*. Amherst, MA, USA, UMass Turf Extension Program, Center for Agriculture, Food and the Environment, University of Massachusetts. Available online at https://ag.umass.edu/turf/fact-sheets/nematodes-on-golf-greens

Yan, G. & Smiley, R.W. (2010). Distinguishing *Heterodera filipjevi* and *H. avenae* using polymerase chain reaction-restriction fragment length polymorphism and cyst morphology. *Phytopathology* 100, 216-224. DOI: 10.1094/PHYTO-100-3-0216