Reproduction Ability and Growth Effect of Pin Nematode, *Paratylenchus nanus*, With Selected Field Pea Cultivars

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Abstract

Greenhouse experiments were conducted to determine the reproductive ability and effect of the pin nematode *Paratylenchus nanus* from North Dakota on field pea cultivars. Reproduction of *P. nanus* was determined on seven field pea cultivars using naturally infested field soils at low (1,500/kg of soil) and high (4,500/kg soil) initial pin nematode densities. Nematode effect on plant growth and seed yield was evaluated at 4,500 *P. nanus* per 1 kg of soil by artificially inoculating *P. nanus* on six field pea cultivars. Reproductive factor (RF) of *P. nanus* was observed to be greater at the low density than the high density of the nematode. At the low population density, RF values ranged from 1.10 to 11.20, whereas at the high density, RF ranged from 1.20 to 2.50. In experiments evaluating *P. nanus* effects on cultivar growth, the nematode (4,500 *P. nanus*

per 1 kg soil) caused reduction ($P < 0.05$) of plant height in most cultivars tested, and it also significantly impacted dry shoot weight and dry seed weight in some experiments. Plant height and shoot weight reductions were the highest in the cultivar Arcadia (up to 37 and 53%, respectively), with a dry seed weight reduction up to 32%. This research demonstrated for the first time the negative impact of *P. nanus* on field peas in controlled greenhouse conditions, which is an important step toward developing effective management strategies to improve the productivity of this leguminous crop.

Keywords: cultivar/resistance, disease management, field crop, legume, nematode detection and reproduction, growth and yield impact

Pin nematodes, *Paratylenchus* spp., are among the smallest of the plant-parasitic nematodes (Geraert 1965; Raski 1975), and they are associated with diverse plant species worldwide (Van den Berg et al. 2014). They are migratory in nature and feed ectoparasitically on root epidermis and root hairs (Rhoades and Linford 1961), but they may be found as deep as the root cortex (Braun and Lownsbery 1975). *Paratylenchus* spp. can parasitize plant roots and cause detrimental effects on plant growth: *Paratylenchus hamatus* on wheat, pea (Riga et al. 2008), and celery (Lownsbery et al. 1952); *Paratylenchus neoambycephalus* on plum seedlings (Braun and Lownsbery 1975) as well as apple and apricot seedlings (Fisher 1967); *Paratylenchus projectus* on tobacco and tall fescue (Coursen and Jenkins 1958); and *Paratylenchus nanus* Cobb, 1923 on perennial rye grass (Bell 1999). Soil surveys in pea fields of North Dakota from 2014 to 2017 identified *Paratylenchus* spp. as the dominant plant-parasitic nematodes based on the frequency of occurrence and population densities (Upadhaya et al. 2019). The average population density of *Paratylenchus* spp. was 4,595/kg of soil, and the highest population density reached 35,570/kg of soil during that survey period. Pin nematode populations on three North Dakota farms were identified as *P. nanus* based on morphometric measurements and molecular examinations (Upadhaya et al. 2016).

P. nanus was reported in Europe (Brzeski 1995), North America (Raski 1975), Australia (Fisher 1966), Africa (Van den Berg et al. 2014), Asia (Esmaeili and Heydari 2017), and even Antarctica (Ryss et al. 2005). This nematode species has a strikingly different fourth-stage juvenile (J4), which is devoid of a prominent stylet, whereas other larval stages have a clear feeding stylet (Ghaderi et al. 2014). The J4 is a nonfeeding survival stage as found in some other *Paratylenchus* spp., which has been

explained as a form of self-regulation under unfavorable environmental conditions (Watson and Bell 2001; Rhoades and Linford 1961). *P. nanus* is known to be associated with grasses, fruits, vegetables, and cereals (Watson and Bell 2001; Esmaeili and Heydari 2017; Fisher 1965; Knight et al. 1997; Raski 1975). Grasses, such as Italian rye grass, perennial rye grass, and cocksfoot, are good hosts of *P. nanus* (Bell and Watson 2001). Moreover, apple and apricot seedlings were known to be hosts of *P. nanus* in Australia (Fisher 1965). Previous studies have shown the negative effect of *P. nanus* on grasses and fruits, but their impact on food legumes is largely unknown.

Field pea (*Pisum sativum* L.) is an important pulse crop with economic, nutritional, and agronomic benefits (McPhee 2003). The United States is the fourth largest producer of field pea after Canada, Russia, and China (FAOSTAT 2018). North Dakota is one of the major producers of field peas in the United States; North Dakota together with Montana contributed 79 to 87% to the total U.S. field pea production during 2014 to 2017 (USDA-NASS 2017). Many plant-parasitic nematodes are associated with field peas in North Dakota, and pin nematodes are the most prevalent nematodes among seven plant-parasitic nematode genera, including the genera *Paratylenchus*, *Tylenchorhynchus*, *Pratylenchus*, *Xiphinema*, *Helicotylenchus*, *Hoplolaimus*, and *Paratrichodorus*, detected in North Dakota pea fields (Upadhaya et al. 2016, 2019). However, the effect of pin nematode populations on field peas in North Dakota is not known. Hence, the objectives of this study were to (i) evaluate reproduction ability of pin nematode in field pea cultivars using naturally infested pea field soil in greenhouse conditions and (ii) determine the effect of pin nematode detected on plant growth and yield of field pea cultivars through artificial inoculation studies in the greenhouse. This research is important to assess the impact of pin nematode in pea fields and develop effective strategies for managing this nematode disease.

Materials and Methods

Pin nematode species identification and confirmation. *Morphological identification.* Twenty-seven soil samples were collected arbitrarily from 11 pea fields in western region of North Dakota, including McHenry, McLean, Mountrail, Sheridan, Ward, and Wells Counties, in the fall of 2015. One to three samples were collected from a field depending on field size, and normally, one sample was collected from an area of ~1.2 ha. Soil sample collection

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techniques, instrument used, postcollection processing, and storage were done as described in Upadhyaya et al. (2019); 63% (17 of 27) of these soil samples had pin nematode as the only plant-parasitic nematode present, whereas the rest of the samples had a mixture including *Helicotylenchus*, *Tylenchorhynchus*, and *Xiphinema* nematodes (Upadhyaya et al. 2019). Morphometric measurements of pin nematode adult females (total: $n = 32$) from four pea fields with greater pin nematode densities and absence of other genera of plant-parasitic nematodes were taken. Morphometric measurements included total body length (micrometers), stylet length (micrometers), esophagus length (micrometers), pharynx length (micrometers), body diameter (micrometers), tail length (micrometers), V% (vulva position from anterior end in percentage of body length), a (body length/greatest body width), b (body length/distance from anterior end to junction of esophagus and intestine), and c (body length/tail length) (Raski 1975; Thorne and Smolik 1971). Temporary nematode slides were prepared by transferring freshly heat-killed nematodes to a drop of water on a glass slide, and cover glass was placed slowly on the top of the water droplet from one side to prevent air bubbles and sealed with clear fingernail polish. Nematode slides were placed under a modular microscope (Zeiss Axio Scope.A1; Zeiss) fitted with a camera (Zeiss Axiocam 105 color; Carl Zeiss Microscopy), and images were taken at three magnifications (200 $\times$, 400 $\times$, and 800 $\times$) depending on the target body part. Measurements were made on images using a software program, ZEN 2 lite (Carl Zeiss Microscopy). These measurements were then compared with those found in the literature (Raski 1975; Thorne and Smolik 1971).

Molecular confirmation. To confirm the species identity of the pin nematodes from four fields, molecular examination was also performed. Adult female pin nematodes were chosen based on their morphological features (Raski 1975). A single nematode was chopped in a concave glass slide, and a nematode suspension (10 μ l) was pipetted into a 0.5-ml sterile Eppendorf tube containing 10 μ l of DNA extraction buffer solution (2 μ l of 10 $\times$ PCR buffer, 2 μ l of Proteinase K [600 μ g/ml], and 6 μ l of double-distilled water) (Huang and Yan 2017). Tubes containing chopped nematodes and lysis solution were incubated at -20°C for 30 min followed by 65°C for 1 h and then, 95°C for 10 min (Yan et al. 2008). DNA was extracted individually from chopped pieces of single nematodes ($n = 4$) per field.

Direct sequencing was done after amplifying nematode DNA from two genomic regions. The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified by a universal primer set, rDNA1 (5'-ACGAGCCGAGTGATCCACCG-3') and rDNA2 (5'-TTGATTACGTTCCCTGCCCTT-3') (Cherry et al. 1997). The D2 to D3 expansion region of 28S rRNA was amplified using primers D2A (5'-ACAAGTACCGTGAGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Courtright et al. 2000). Template DNA (2 μ l) was transferred into 18 μ l of the PCR mixture consisting of 0.8 μ l of each primer (10 μ M), 0.4 μ l dNTP, 1.2 μ l MgCl_2 , 4.0 μ l 5 $\times$ PCR buffer, and 0.15 U of Taq DNA Polymerase (Promega Corp.). PCR cycle conditions consisted of initial denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 1 min, and extension at 72°C for 1 min and a final extension at 72°C for 10 min. After completion of PCR cycles, 2 μ l of PCR product was mixed with 3 μ l of 2 $\times$ loading dye, and a total of 5 μ l of the mixture was loaded in 2% agarose gel. Gel electrophoresis was done at 100 V for 25 min. The gel was visualized under ultraviolet light, and images were taken using an AlphaImager Gel Documentation System (Proteinsimple Inc.). After confirmation of the PCR amplification, amplified DNA was purified from the remaining PCR product using the E.Z.N.A. Cycle Pure Kit (Omega BIO-TEK). Purified DNA was sent for DNA sequencing by GenScript (GenScript). DNA sequences were aligned using the sequence alignment tool ClustalX, and the BLAST tool in NCBI (<https://www.ncbi.nlm.nih.gov/>) was used to compare and identify similarity with the known nematode species sequences in the GenBank database.

Reproduction of *P. nanus* using infested field soil. *Soil collection and processing.* Naturally *P. nanus* infested soil was collected

from a pea field in McLean County in western North Dakota to determine the reproduction ability of this nematode using field pea cultivars in greenhouse conditions. Infested soil was mixed thoroughly so that uniformity for initial nematode densities could be maintained in each pot (experimental unit). Three subsamples of 0.2 kg were removed from the mixed soil. The average of nematode densities in those subsamples was determined after nematode extraction using the sieving and decanting and the sugar centrifugal floatation techniques (Jenkins 1964). The average nematode densities per kilogram of soil were calculated and used as the initial nematode densities for reproduction ability experiments.

Greenhouse experiments. Two greenhouse experiments were conducted using naturally infested soils at different starting densities of *P. nanus*. In the first experiment, the initial density of *P. nanus* was low (1,500/kg of soil), whereas in the second experiment, it was high (4,500/kg of soil). Nematodes in both experiments included all vermiform life stages. Both experiments were repeated (Table 1). Seven field pea cultivars were used in these experiments: cultivars Columbia, Cooper, Bridger, Arcadia, Cruiser, Aragorn, and Salamanca (Table 2). Seeds of these cultivars were pregerminated for 4 to 5 days by placing them in petri dishes with wet paper. Clay pots (15-cm diameter $\times$ 15-cm height) were filled with naturally infested soil with the initial nematode populations as explained earlier after mixing the naturally infested soil with sterilized soil to maintain required nematode populations. Each pot with soil was fertilized once at the time of planting with one teaspoon (~ 5 g) of controlled release formulation 14-14-16 N-P-K Multicote 4 (Haifa Chemicals) and mixed thoroughly. After fertilizer application, a single pregerminated seed of each cultivar was placed at a depth of 3 to 4 cm in each of the filled pots. Watering of the plants was done once every day as needed. Each cultivar was replicated four times during both experiments. Experiments were completely randomized in blocks and placed on greenhouse benches. Plants were kept at 16 h daylight with an average temperature of 22°C ($22 \pm 5^{\circ}\text{C}$). Plants were allowed to grow in the greenhouse for 12 weeks, and the experiments were terminated on the 13th week. Plant tops were cut near the soil surface, and the soils with roots were placed in plastic bags and stored at 4°C until nematode extractions occurred within one week.

Nematode extraction. Each soil plus root sample collected from a single pot was placed in a tray (36 $\times$ 27 cm). Soil was then mixed thoroughly after roots were removed from soil. A 0.2-kg subsample was taken from each sample for nematode extraction using the method described above. During the extraction process, roots were

Table 1. Experimental details (experiment type, soil, initial population densities, and experimental period) for studies of *Paratylenchus nanus* nematode reproduction and its effects on pea cultivar growth in greenhouse experiments

Experiment type ^a soil ^b and nematode density	Pi/kg of soil ^c	Repetition (trial)	Planting date	Harvesting date
Reproduction ability				
Naturally infested soil				
Low	1,500	1	23/4/2016	19/7/2016
Low	1,500	2	29/12/2016	24/3/2017
High	4,500	1	23/4/2016	19/7/2016
High	4,500	2	29/12/2016	24/3/2017
Nematode effect				
Sterilized soil				
High	4,500	1	18/3/2016	31/5/2016
High	4,500	2	5/12/2016	19/2/2017

^a In reproduction ability experiments, the reproductive factor (final population/initial population per 1 kg of soil) of *P. nanus* was calculated. In experiments looking at nematode impacts on plant growth parameters, plant height, dry root weights, dry shoot weights, dry seed weight, and final nematode populations were recorded.

^b Naturally infested soil indicates the use of field soil positive for *P. nanus* in reproduction experiments. For pea growth studies, nematodes were artificially inoculated in sterilized soil, which was then used as inoculum.

^c Pi refers to the initial population of *P. nanus* at the time of planting.

rinsed with water to get all of the nematodes from the remaining small amount of attached soil around the roots. Nematodes were collected in 20 to 25 ml of tap water in a 50-ml suspension tube. All of the *P. nanus* J4s (those without a prominent stylet) and other life stages with a stylet were counted based on the nematode morphology together under an inverted transmitted light microscope at 100× magnification (Zeiss Axiovert 25; Carl Zeiss Microscopy). The number of *P. nanus* nematodes was expressed as total number of individuals in 1 kg of soil per pot.

Reproductive factor and ratings. A nematode reproductive factor (RF) was calculated for each individual experimental pot by dividing the final population of *P. nanus* by its initial population in 1 kg of soil. The average RF of nematodes per treatment was a mean RF of four replications of each cultivar. To delineate the response of pulse crops, including peas, to vermiform nematodes (*Ditylenchus* spp.), Hajihassani et al. (2016) described four host groups: nonhost (RF < 1), poor host (RF = 1 to 2), good host (RF = 2 to 4), and excellent host (RF > 4). In this study, four susceptible/resistant groups were designated to describe the reproduction ability of pin nematode on different cultivars: resistant (RF = 0.0 to 1.0), moderately resistant (RF = 1.0 to 2.0), moderately susceptible (RF = 2.0 to 4.0), and susceptible (RF > 4.0).

Data analysis. Levene's test of homogeneity of variance was performed using PROC GLM of SAS 9.4 to determine whether the data from repeated trials could be combined for additional analysis (SAS Institute Inc.). Repeated trials for both low and high nematode densities were confirmed to be homogenous ($P > 0.05$), and therefore, combined analyses on RFs of *P. nanus* were performed using PROC GLM of SAS 9.4. Mean separations were performed using the *F*-protected least significant difference at $P < 0.05$ to determine differences in RFs of nematodes in the tested pea cultivars. To determine if the average RF of nematodes is statistically different between two experiments with different initial population densities, a *t* test at 95% confidence interval was performed using SAS 9.4.

Effect of *P. nanus* on plant growth and seed weight of field pea. *Inoculum preparation and experimental setup.* The population of *P. nanus*, collected from the same field as described earlier in the reproduction experiments, was reared on field pea Columbia and Cooper in the greenhouse. After an 11-week incubation period, *P. nanus* was extracted from soil with plants using the Whitehead tray method (Whitehead and Hemming 1965). In the Whitehead tray method, 200 g of soil was spread on two-ply facial tissue paper placed over mesh in a metallic tray. The metallic tray was placed in a plastic tray with water just deep enough to submerge the soil and incubated for

48 h at $22 \pm 2^\circ\text{C}$. The Whitehead tray method was preferred for extracting nematodes to be used as inoculum, because the sugar centrifugal flotation method used above put more stress on the nematodes during the sugar extraction and multiple centrifugation steps. After nematode extraction, the *P. nanus* population was concentrated to 300 nematodes per 1 ml, which included all vermiform life stages. These nematodes were stored at 4°C for 2 days until used as inoculum.

Greenhouse experiments to determine the effect of *P. nanus* on growth and yield of field pea cultivars (Table 2) were done twice. Five pea cultivars, including Arcadia, Cruiser, Bridger, Aragorn, and Salamanca, were used in the first round of the experiment, and the Columbia cultivar was added in the second round. Clay pots were filled with 1 kg of sterilized soil, and one teaspoon (~5 g) of the slow-release fertilizer 14-14-16 N-P-K was put in each pot and mixed well. A single pregerminated seed of each field pea cultivar was placed in the center of a filled pot at 3- to 4-cm depth. Nematode suspensions of 15 ml, ~4,500 *P. nanus* per 1 kg of soil, were pipetted into three holes at equal distances around the pregerminated seed to facilitate nematode invasion. Holes were covered with ample sterilized soil to result in an even soil surface after inoculation. Pea plants were staked using bamboo sticks 3 weeks after planting to prevent plant lodging, and watering was done once every day to maintain plant growth. Both nematode-inoculated and noninoculated controls of all cultivars were replicated four times, and pots for all cultivars were completely randomized in blocks on a greenhouse bench. Plants grew in the greenhouse for 10 weeks, and the experiments were terminated on the 11th week.

Data collection. Data on plant parameters and final nematode populations were collected to determine the effect of *P. nanus* on field pea cultivars. Plant height was measured at the R6 stage (yellow or dry seeds fill the pod cavity at one or more nodes). Other plant parameters, including shoot weight, root weight, and seed weight, were determined after drying these parts at 80°C for 48 h in an incubator (VWR International). Nematodes were extracted from a 0.2-kg subsample from each pot using the sieving and decanting and the sugar centrifugal flotation methods (Jenkins 1964). *P. nanus* was collected and counted using the same method described in the reproduction experiments. Final population of *P. nanus* nematodes was expressed as the total number of individuals in 1 kg of soil.

Data analysis. Data obtained from the first and second rounds of nematode effect experiments were analyzed separately, because they were found to be heterogeneous after Levene's test of homogeneity of variance ($P < 0.05$). Mean comparison of average plant parameters (height, dry shoot weight, dry root weight, and dry seed weight) between nematode-inoculated and noninoculated controls of each cultivar was performed using a two-factor analysis of variance with slicing on cultivar (Proc Mixed with Slicediff option) at $P < 0.05$ using SAS 9.4.

Table 2. Field pea cultivars used in this study

Genotypes	Market class ^a	Days to maturity	Originator ^b	Growing regions ^c
Columbia	Green	80–90	Campbell Soup Co.	PNW (ID, WA)
Cooper	Green	90–100	Innoseeds B.V. Co.	ND
Arcadia	Green	80–90	Lantmannen SW Seed Co.	MT, ND
Cruiser	Green	80–90	ProGene	PNW (WA), MT, ND
Aragorn	Green	80–90	ProGene	PNW (ID, WA), MT, ND
Bridger	Yellow	80–90	Legume Logic	MT, ND, SD
Salamanca	Yellow	80–90	Legume Logic	ND

^a Green and yellow field pea cultivars are categorized based on seed cotyledon color.

^b Originator refers to the developer of the field pea cultivars.

^c PNW refers to the states of the Pacific Northwest region (Washington, Idaho, and Oregon). ND, MT, and SD indicate the states of North Dakota, Montana, and South Dakota, respectively. These data were obtained from field pea varietal trial extension bulletins from North Dakota State University (Kandel et al. 2016), South Dakota State University (Graham et al. 2017), Montana State University (Mohammed and Chen 2016), University of Idaho, Washington State University, and U.S. Department of Agriculture–Agricultural Research Service Pullman, Washington (USA Dry Pea and Lentil Council 2012).

Results

***P. nanus* identification and confirmation.** Morphometric measurements of adult female individuals ($n = 32$) included body length (range = 300 to 395 μm , average = 350 μm), stylet (range = 24.80 to 31.30 μm , average = 27.50 μm), esophagus length (range = 63.90 to 82.10 μm , average = 74.06 μm), pharynx length (range = 60.66 to 75.65 μm , average = 66.64 μm), body diameter (range = 14.30 to 23.40 μm , average = 17.90 μm), tail length (range = 20.10 to 28.20 μm , average = 24.70 μm), a (range = 16.80 to 23.50, average = 19.75), b (range = 3.13 to 4.52, average = 3.64), c (range = 13.17 to 15.43, average = 14.16), and V% (range = 83.30 to 86, average = 84.80). The head region was round, and the tail terminus was subacute and blunt but not digitate. These characters are important to separate *P. nanus* from the morphologically closely related species *P. projectus*, which has a truncated head and often, a digitate tail (Ghaderi et al. 2014). Males were not observed in our populations. Males for this species have been rarely observed (Raski 1975). These morphometrics are within the range of those described for *P. nanus* by Thorne and Smolik (1971). Molecular analysis of the ITS region of rDNA showed that the sequence (MH236098, 828 bp) was 100%

identical with one population of *P. nanus* from South Africa (KF242264) and 99% identical with two populations of *P. nanus* from South Africa (KF242263 and KF242266) and two populations of *P. nanus* from South Korea (KY468906 and KY468910). The highest similarity of the sequence with other pin nematode species was only 87%. Molecular analysis of the D2 to D3 expansion region of 28S rRNA revealed that the sequence (MH237651, 770 bp) was 100% similar to one population of *P. nanus* from South Korea (KY468901) and one population of *P. nanus* from USA (KF242199) and 99% identical with four populations of *P. nanus* from South Korea (KY468900, KY468902, KY468903, and KY468899). The molecular examinations and morphological measurements confirmed the species of pin nematode from the North Dakota pea fields as *P. nanus*.

Reproduction ability of *P. nanus* on field pea cultivars using naturally infested soil. *Reproduction ability with low initial *P. nanus* density.* With an initial *P. nanus* density of 1,500 nematodes per 1 kg of soil, reproduction was the highest (RF = 11.20) on Columbia, with significantly higher RFs ($P < 0.05$) than other tested field pea cultivars (Fig. 1A). At low initial nematode density, *P. nanus* reproduction on Arcadia (RF = 4.80) and Cooper (4.30) was >4.0 , indicating that these two cultivars were susceptible. Other cultivars had RF values between 2.0 and 4.0, suggesting moderately susceptible reactions (Fig. 1A). Among all of the tested cultivars, *P. nanus* reproduced the least on Salamanca (RF = 1.10), a moderately resistant reaction. RF values of *P. nanus* were not significantly ($P > 0.05$) different among Arcadia, Cooper, Cruiser, and Bridger (Fig. 1A). Similarly, RF values of *P. nanus* were not significantly ($P > 0.05$) different between Aragorn and Salamanca (Fig. 1A).

*Reproductive ability with high initial *P. nanus* density.* The RF of *P. nanus* averaged across cultivars was statistically lower ($P = 0.0001$) at the high initial inoculum density (RF = 1.94) than that at the low inoculum density (RF = 4.44). *P. nanus* reproduction was reduced by fivefold in Columbia at the high initial inoculum density of 4,500 *P. nanus* per 1 kg of soil compared with the low initial density of 1,500 *P. nanus* per 1 kg of soil (Fig. 1). Similarly, an approximately twofold reduction was observed in Arcadia, Cooper, Bridger, and Cruiser. Salamanca favored the lowest reproduction at both initial densities. At the high initial *P. nanus* inoculum density, RF values were from 2.0 to 4.0 for Arcadia (2.50), Cruiser (2.50), and Columbia (2.00), all moderately susceptible reactions (Fig. 1B). Other cultivars tested had RF values from 1.0 to 2.0, indicating moderately resistant reactions, including Cooper (1.90), Bridger (1.90), Aragorn (1.60), and Salamanca (1.20) (Fig. 1B). RF values of Arcadia, Cruiser, Columbia, Cooper, and Bridger were significantly higher ($P < 0.05$) than Salamanca, whereas significant differences were not observed in RF values of Aragorn and Salamanca (Fig. 1B).

Effect of *P. nanus* on plant growth and yield using artificially infested soil. In the first repetition of this experiment, plant height and dry seed weight were reduced for some field pea cultivars at the inoculation density of 4,500 *P. nanus* per 1 kg of soil. Both plant height and dry seed yield were significantly lower ($P < 0.05$) in Arcadia and Cruiser, whereas only plant height in Bridger was reduced (Table 3). The highest reduction of plant height and dry seed yield was in Arcadia (reduced by 18 and 28%, respectively). No significant differences were observed between inoculated and control plants for dry shoot or root weight. The average final population of *P. nanus* reached as high as 9,656/kg of soil in Cruiser, and it was the least in Aragorn (2,938/kg of soil). The highest RF was slightly >2.0 for Cruiser (RF = 2.14), and the lowest was <1.0 for Aragorn (Fig. 2).

In the repeat of this experiment, nematode inoculation resulted in statistically lower ($P < 0.05$) plant height, dry shoot weight, dry root weight, and dry seed weight for Columbia and Bridger compared with their controls, whereas all parameters mentioned above except dry root weight were reduced for Arcadia (Table 4). Two parameters (plant height and seed weight) were also reduced ($P < 0.05$) for Cruiser, but only plant height for Salamanca and seed weight for Aragorn were reduced (Table 4). Across all cultivars, the greatest

reductions of plant height, stem weight, root weight, and seed weight were by 37, 53, 50, and 40%, respectively. Arcadia cultivar had the greatest reductions in both plant height and dry shoot weight. The average RF of *P. nanus* was the highest for Columbia (RF = 2.09) and the least for Aragorn (RF = 0.86) (Fig. 2).

Discussion

This study describes the reproductive ability of pin nematode, *P. nanus*, on field pea cultivars commonly grown in major field pea-producing states of the United States. *P. nanus* was demonstrated for the first time to reproduce well on some field pea cultivars, and it may have a negative impact on plant growth and seed weight in controlled greenhouse conditions.

Experiments using naturally infested soils indicated that *P. nanus* could survive and propagate in field pea cultivars. Previously, association of *P. nanus* with cereals, grasses, and fruits was reported (Raski 1975). Bell and Watson (2001) also indicated the reproduction of *P. nanus* on grasses, such as *Dactylis glomerata*, *Lolium perenne*, and *Lolium multiflorum*, in pot experiments. Other species of pin nematodes, such as *P. projectus* and *P. hamatus*, reproduced on cereals (Niblack 1992; Riga et al. 2008), and *P. neoambycephalus* reproduced on apple (*Malus domestica*) and myrobalan plum (*Prunus cerasifera*) (Braun and Lownsbey 1975; Fisher 1967). Based on the results presented here, *P. nanus* reproduction potential varied with cultivars. Variable responses

Fig. 1. Average reproductive factor (RF) values (final nematode density divided by initial density) of *Paratylenchus nanus* on field pea cultivars grown in naturally infested field soil. RF values are the mean of two trials for each cultivar ($n = 8$). Mean RF values with the same letters are not significantly different according to the *F*-protected least significant difference test ($P < 0.05$). Final nematode density in each pot with a single plant was determined after harvesting the trial on the 13th week after planting: **A**, 1,500 *P. nanus* per 1 kg of soil at the time of planting in greenhouse conditions and **B**, 14,500 *P. nanus* per 1 kg of soil at the time of planting in greenhouse conditions.

of field pea cultivars to *P. nanus* infection could be due to differences in cultivar genotypes. In line with these results, differential hosting abilities of field pea cultivars to the stem and bulb nematode *Ditylenchus dipsaci* were observed in greenhouse studies in Canada (Hajihassani et al. 2016).

RF of *P. nanus* was observed to be influenced by the initial nematode density at the time of planting. In this study, RF values at the low starting density (1,500 *P. nanus* per 1 kg of naturally infested soil) were generally higher than those at the high density (4,500 *P. nanus* per 1 kg of same soil). Such variation in reproduction rate could be owing to more intraspecific competition for food at the high starting density than at the low density. Nematode competition for feeding sites/food could limit their reproduction (Duncan and Ferris 1983). van der Putten et al. (2005) observed the intraspecific competition of *Pratylenchus penetrans*, leading to a lower RF, because of fewer females than males at high inoculation densities. Our results agreed with Coursen and Jenkins (1958), who reported different reproduction rates and rapid multiplication rates of *P. projectus* at a lower initial density (1,000 per plant) compared with a higher initial density (5,000 or 10,000 per plant) on tobacco. Similarly, Braun and Lownsbery (1975) detected statistically higher RF of *P. neoambycephalus* on myrobalan plum seedlings at lower starting densities compared with higher densities.

In our study, *P. nanus* caused detrimental effects on plant growth and seed yield of pea cultivars during artificial inoculation. However, variation in reduction of growth parameters and seed weight in two sets of repeated experiments may be due to the different times of planting shown in Table 1, which may affect the temperature, direct sunlight, and soil moisture content for plant growth and nematode reproduction in the greenhouse. Pea plant stunting was obvious at the R6 stage in the *P. nanus*-inoculated plants of some cultivars. Similar stunting was caused by *P. projectus*, a pin nematode on tobacco and tall fescue plants (Coursen and Jenkins 1958). *P. nanus* caused reduction of dry shoot weight of some pea cultivars in our study. Similarly, in a greenhouse study in Australia, *P. nanus* caused decrease in shoot dry matter of perennial rye grass at an initial inoculation density of 950 *P. nanus* per tube of 20 × 2.5 cm (Bell 1999). Other researchers have reported reductions in seed yield owing to pin nematodes, reductions similar to those observed in our study. Niblack (1992) reported yield reduction of soybean cultivars owing to pin nematode *P. projectus* at an initial nematode density of 2,000/kg of soil in Missouri. In Idaho, pin nematode *P. hamatus* was able to negatively impact plant growth and yield of Columbia and Small Sieve cultivars of field pea under greenhouse conditions at an initial density of 400/200 g of soil (Riga et al. 2008).

In our study, plant growth and seed weight were observed to be reduced during artificial inoculation even when the nematode RF values in both trials generally were around 2.0. Similar observations were made in Canada, where the RF of *D. dipsaci* in field pea cultivars (Admiral and Bronco) was ≤2.5 and the RF in bean cultivars (Windbreaker and Envoy) was <2.0 (Hajihassani et al. 2016). However, aboveground biomass of both crops was significantly reduced by *D. dipsaci*. The authors did not offer a possible reason for such observations. The lower RF values of *P. nanus* at harvest in our studies could be because of decline of nematode population

at the end of growth season. In the experiments evaluating nematode effect on plant development, a high number of *P. nanus* (4,500/kg of soil) was inoculated directly around the root zone of seedlings. Based on the images of pea plants at R3 growth stage in Figure 3, nematode damage started from an early growth stage and was visible in the late growth stage (R6) as well. Generation time of pin nematodes in general has been reported to be 30 to 31 days at 25 to 28°C (Rhoades and Linford 1961). Pin nematodes might feed on roots and start to multiply, but by the time that populations start to accelerate, a single older pea plant might not be able to support the increased nematode numbers. In the latter one-half of a pea cultivar's growing period, poor plant growth limits food for the nematodes, which contributes to nematode population decline. This assumption was supported by the increase of *P. projectus* during the midgrowing season but reduction by more than one-half at the end of harvest (Niblack 1992).

In conclusion, this study provides evidence that the *P. nanus* population from a pea field in McLean County, North Dakota can reproduce well on some field pea cultivars, such as Columbia, Arcadia, Cruiser, and Cruiser. However, the reproduction rate of *P. nanus* was dependent on initial population density at the time of planting. Hence, additional research may be required to confirm this differential rate of reproduction of *P. nanus* in field pea cultivars. Moreover, *P. nanus* was able to negatively impact both plant growth and seed yield of Columbia, Arcadia, Bridger, and Cruiser cultivars of field pea. In the future, screening of more diverse field pea cultivars is recommended considering the effect of *P. nanus* on plant growth, even with slow reproduction of *P. nanus* at the high inoculation density used in this study.

Fig. 2. Average reproductive factors (RFs; final nematode density divided by initial density) of *Paratylenchus nanus* on field pea cultivars grown in artificially infested soil with 4,500 *P. nanus* per 1 kg of soil at the time of planting in greenhouse conditions for trial 1 and trial 2. RF values are the mean of four replicates of each nematode-inoculated cultivar within a trial. Bars with the same letters within a trial are not significantly different according to the *F*-protected least significant test ($P < 0.05$). Both greenhouse trials were harvested at the 11th week after planting and inoculation. The Columbia cultivar was added in trial 2.

Table 3. Average plant growth (plant height, dry shoot weight, and dry root weight) and average dry seed weight of five field pea cultivars inoculated with *Paratylenchus nanus* (4,500/kg of soil) and the corresponding noninoculated control—trial 1^a

Pea cultivars	Height (cm)		Dry shoot weight (g)		Dry root weight (g)		Dry seed weight (g)	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
Arcadia	39.81 ^b	48.57	2.16	2.54	0.23	0.23	2.82 ^b	3.90
Cruiser	38.54 ^b	45.33	2.43	2.38	0.21	0.23	2.74 ^b	3.53
Bridger	40.95 ^b	46.79	1.62	2.02	0.18	0.20	3.32	3.03
Aragorn	30.16	32.82	1.51	2.02	0.14	0.13	1.87	2.56
Salamanca	42.22	45.40	1.55	1.86	0.20	0.21	2.41	3.02

^a Mean comparison between nematode-inoculated and noninoculated treatments within a specific cultivar ($n = 4$) was performed using a two-factor analysis of variance (proc mixed model) with slicing on cultivar.

^b Significant difference for plant parameters between nematode-inoculated and noninoculated treatments of each cultivar at $P < 0.05$.

Table 4. Average plant growth (plant height, dry shoot weight, and dry root weight) and average dry seed weight of six field pea cultivars inoculated with *Paratylenchus nanus* (4,500/kg of soil) and the corresponding noninoculated control—trial 2^a

Pea cultivars	Height (cm)		Dry shoot weight (g)		Dry root weight (g)		Dry seed weight (g)	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
Columbia	57.78 ^b	90.17	1.06 ^b	1.83	0.18 ^b	0.26	2.34 ^b	3.91
Arcadia	26.67 ^b	42.54	0.99 ^b	2.12	0.07	0.10	2.06 ^b	3.01
Cruiser	32.38 ^b	41.27	1.34	1.83	0.08	0.11	2.98 ^b	4.14
Bridger	31.11 ^b	43.81	1.17 ^b	1.91	0.10 ^b	0.20	2.24 ^b	3.39
Aragorn	30.48	37.46	1.28	1.86	0.08	0.14	2.17 ^b	3.29
Salamanca	42.54 ^b	53.34	2.08	2.68	0.11	0.17	3.24	3.48

^a Mean comparison between inoculated and noninoculated treatments within a specific cultivar ($n = 4$) was performed using a two-factor analysis of variance (proc mixed model) with slicing on cultivar.

^b Significant difference for plant parameters between nematode-inoculated and noninoculated treatments of each cultivar at $P < 0.05$.

A

B

Fig. 3. Plant growth comparison of field pea cultivar Arcadia at 40 days after planting and pin nematode (*Paratylenchus nanus*) inoculation. Growth stage was R3. **A**, Pea plants without *P. nanus* inoculation; **B**, pea plants were artificially inoculated with 4,500 *P. nanus* per 1 kg of soil.

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