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A Canister Assay for Evaluating Host Status of Potato to *Meloidogyne Chitwoodi*

Michelle Soulé[1] · Megan Kitner[1] · Gabrielle Studebaker[1] · Max J. Feldman[2] · Vidyasagar Sathuvalli[3] · Inga Zasada[1]

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Abstract
New, reliable strategies are needed to control *Meloidogyne chitwoodi* in potato; plant host resistance is central to this effort. While efforts to breed potato for resistance to *M. chitwoodi* are underway, a major bottleneck in this process is phenotyping plant genotypes for nematode resistance. Currently, time and resource consuming phenotyping takes place in the greenhouse or field. The objective of this study was to establish a high throughput methodology for screening potatoes against *M. chitwoodi* and quantify nematode egg densities at the end ofscreening using qPCR. Various parameters were evaluated for a canister assay where soil was added to a small container, planted with potato seed tuber, inoculated with nematode eggs, and incubated at a constant temperature in the dark. To obtain maximum reproduction factor (RF = final population density/initial population density) values, a minimum of 6 weeks after inoculation was required. Timing of inoculation was also important, with higher RF values when inoculation with eggs occurred at planting compared to 2 weeks after planting. The volume of water in which inoculum was delivered to soil did not impact RF values, nor did inoculation density (0.5, 1, or 5 eggs/g soil). The canister assay was evaluated using genotypes from a breeding population with varying levels of resistance to *M. chitwoodi*. Egg enumeration by qPCR was more sensitive than by microscopy, however, this increased sensitivity did not result in a significant difference in RF values nor the designation of a genotype being a good or poor host for *M. chitwoodi*. This method has the potential to greatly decrease the amount of time and resources needed to phenotype potato against *M. chitwoodi* and can allow for multiple screenings throughout the year, regardless of the season.

Keywords Phenotyping · Columbia root-knot nematode · Resistance screening · Reproduction factor · *Solanum tuberosum*

Introduction

Plant-parasitic nematodes are economically important pests and a major threat to the production of potato (King and Taberna 2013). With a global estimate of an average 8% loss in crop productivity due to nematodes, the U.S. potato industry stands to lose $368 million annually to plant-parasitic nematodes. One of the most significant nematode threats to potato (*Solanum tuberosum* L.) in the Pacific Northwest (PNW) is the Columbia root-knot nematode, *Meloidogyne chitwoodi* (Nyczepir et al. 1982; Zasada et al. 2018). In the PNW, *M. chitwoodi* can overwinter at the egg stage and has a relatively low temperature threshold for development (Pinkerton et al. 1991; Santo and O'Bannon 1981). This means the life cycle can begin early, complete multiple reproductive generations during the growing season, and even continue through harvest while the tubers are in storage (Pinkerton et al. 1991). The processing market has a very low tolerance for the damage caused by *M. chitwoodi*. For example, if just 6% of the potato tubers in a field are infected with *M. chitwoodi*, the entire crop may be a total loss (King and Taberna 2013). Currently, soil fumigation is the most effective means of controlling *M.*

✉ Inga Zasada
inga.zasada@usda.gov

[1] Horticultural Crops Disease and Pest Management Research Unit, USDA Agriculture Research Service, 3420 NW Orchard Ave, Corvallis, OR 97330, USA

[2] Temperate Tree Fruit and Vegetable Research Unit, USDA Agriculture Research Service, Prosser, WA 99350, USA

[3] Hermiston Agricultural Research & Extension Center, Oregon State University, Hermiston, OR 97838, USA

chitwoodi. However, plant-parasitic nematode management is difficult because many long-relied upon nematicides are being banned, or phased out, or heavily restricted, and are expensive (Zasada et al. 2010). New, reliable strategies are needed to control plant-parasitic nematodes in potato, and plant host resistance is central to this effort and a highly desirable and easy to deploy alternative control.

Efforts are being made to develop improved selections with resistance to *M. chitwoodi* (Graebner et al. 2018). Resistance to *M. chitwoodi* has been identified in *Solanum bulbocastanum, S. hougasii, S. stenophyllidium* and *S. fendleri* (Brown et al. 1995, 2006; Graebner et al. 2018; Janssen et al. 1997). The resistance identified from clone 22 of diploid *S. bulbocastanum* (SB22) was hybridized with cultivated tetraploid *S. tuberosum* using protoplast fusion. The somatic hybrid obtained by fusion was subsequently backcrossed five times with various tetraploid *S. tuberosum* genotypes resulting in nematode resistant advanced breeding selection, PA99N82–4 (Brown et al. 1999). The effort to introgress resistance into advanced breeding selections, and the subsequent development of selections into new potato varieties takes years if not decades. Phenotyping material for nematode resistance is a major bottleneck in this process. Currently, time and resource consuming phenotyping takes place in the greenhouse or field (Graebner et al. 2018). A greenhouse assay can take up to 83 days and a field trial the entire growing season. Both greenhouse and field screenings must be repeated at least twice and require a lot of resources and even under ideal conditions, it is challenging not to find nematode escapes, which can be an impediment to determining if material is resistant or susceptible to *M. chitwoodi*. It would be advantageous to develop an assay that requires less space and resources and can be run in a constant environment to ensure consistency across experiments.

A high throughput canister-based laboratory method for screening potatoes against potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) has been developed (Foot 1977). In this canister method, tubers were planted in sand in a transparent container. After root initiation, each canister was inoculated with eggs and at 17.5 °C the nematode's life cycle was completed in 10–12 weeks after inoculation. The canister method was demonstrated to have the advantage over greenhouse or field methods because light is not required and temperature can be regulated. This canister method was subsequently used to address research questions with potato cyst nematodes such as the impact of climate change (Skelsey et al. 2018) and nematode virulence (Gartner et al. 2021). This method has also been used to screen potato and wild *Solanum* spp. against *M. chitwoodi* and *M. hapla* (Janssen et al. 1995). Potatoes in containers were inoculated with *Meloidogyne* spp., incubated at 20 °C, and then harvested after 7 weeks. In this study a single set of experimental parameters were used to compare several types of experimental systems for evaluating potatoes for *Meloidogyne* spp. resistance.

The goal of our study was two-fold. First, several parameters of the canister method were evaluated to determine the optimal methodology to obtain repeatable and reliable data on *M. chitwoodi* reproduction on potato. Second, a qPCR method was compared to traditional microscopic counts for the quantification of eggs at the end of the experiment. Combined, the optimized canister method for *M. chitwoodi* strives to reduce the time for phenotyping of potato germplasm.

Materials and Methods

Nematode Inoculum

Meloidogyne chitwoodi race 1 was collected from a potato field in Prosser, WA. Single female lines were established by adding nematode infested soil to a pot and planting 'Rutgers' tomato (*Solanum lycopersicum*). The species identity was confirmed by molecular analysis by the North Carolina Department of Agriculture & Consumer Services (Raleigh, NC) and race identity by Washington State University (Hu et al., 2023). After approximately 8 weeks, the plants were destructively harvested and single egg masses were transferred to a new tomato plant. Cultures were then continuously maintained on 'Rutgers' tomato. To extract eggs for use in experiments, tomato plants were destructively harvested and the roots were rinsed free of soil. Eggs were extracted by shaking the root system in a 0.3% NaClO solution for 3 min and then passing the solution over a 500 mesh sieve to collect eggs. *Meloidogyne chitwoodi* egg densities were adjusted in water to achieve desired inoculation densities needed for each experiment.

Optimization of Canister Assay Methodology

Tubers of potato cultivar 'Ranger Russet' were washed with a 0.6% NaClO solution and then placed at room temperature for 1–2 weeks to allow for sprouting before adding to canisters. The size of the canister was a 237 cc deli cup 4 cm in height with a dimension of 11.4 cm (ULINE, Pleasant Prairie, WI, USA). To each canister approximately 110 g of a dry 1:1 sand:Willamette loam pasteurized soil mix was added. Soil was then wetted with 30 ml of water and mixed thoroughly to ensure uniform wetting. A tuber piece (30 to 60 g) with at least 2 or 3 eyes was then pushed into the moistened soil with the eye side down and the canister was closed with a lid and placed in a 24 °C incubator (Thermo

Fisher Scientific, Waltham, MA, USA) (Fig. 1). This experimental set-up was used for all experiments that were conducted to identify the optimal conditions for the assay.

In Experiment 1, the effect of inoculation density and assay duration on *M. chitwoodi* reproduction was evaluated. The experiment consisted of 80 experimental units (canisters) and was a factorial design with the following factors: 4 inoculation densities (0.5, 1, 2, and 5 *M. chitwoodi* eggs/g soil) and 4 take-down dates (5, 6, 7, and 8 weeks post inoculation). All factor combinations were replicated five times and canisters were arranged in a completely randomized design; the experiment was repeated twice. Tubers were planted in canisters and inoculated with *M. chitwoodi* eggs suspended in 2 ml water two weeks after planting. At each take-down date the canisters were removed from the incubator and roots separated from soil. Collected roots were rinsed free of soil and eggs extracted by shaking the root system in 0.6% NaClO solution for 3 min and then passing the solution over a 500 mesh sieve to collect eggs in a 50 ml tube. After extraction, roots were placed in a drying oven at 65 °C for a week, and then weighed. The solution containing collected *M. chitwoodi* eggs was adjusted to 20 ml and 1 ml of the solution was placed on a counting slide and eggs were enumerated using a Leica DM IL inverted microscope (Leica Microsystems, Wetzlar, Germany).

In Experiment 2, the effect of inoculum volume on *M. chitwoodi* reproduction was evaluated. The experiment consisted of 15 experimental units (canisters) with inoculation volume (2, 5 or 10 ml) as the treatment. Treatments were replicated five times, arranged in completely randomized design in the incubator, and the experiment was conducted twice. The soil was initially wetted with 30 ml of water, placed in a canister, and then planted with potato. Two weeks after planting the canisters were opened and inoculated with 2 *M. chitwoodi* eggs/g soil in 2, 5 or 10 ml of water. The experiment was terminated at 6 weeks and nematode eggs were extracted and counted as described for Exp. 1.

In Experiment 3, the effect of inoculation timing on *M. chitwoodi* reproduction was considered. The experiment consisted of 15 experimental units (canisters) with timing of inoculation (0, 1 and 2 weeks post planting) as the treatment. Treatments were replicated five times, arranged in a completely randomized design in an incubator, and the experiment was conducted twice. Canisters were inoculated with 2 *M. chitwoodi* eggs/g soil in 2 ml water at the described times at or after planting. The experiment was terminated at 6 weeks and nematode eggs were extracted and counted as described for Exp. 1.

For all of the optimization experiments, data were analyzed using JMP vs. 9.1 (SAS, Cary, NC). Data are presented as eggs/g root and as reproduction factor (RF) values, where RF = final population density/initial population density. A RF value > 1 indicates that the plant is a host, while an RF value < 1 indicates the plant is a poor host and a RF = 0 is a non host (Oostenbrink, 1966). In all analyses, trial was considered a random factor while all other treatments were fixed factors. When the trial × treatment interaction was significant ($P < 0.001$), the trials were analyzed separately.

Fig. 1 The canister assay used to screen potato against *Meloidogyne chitwoodi*

To meet ANOVA assumptions, nematode data were log10 (x + 1)-transformed prior to analysis. Statistically significant differences among treatments were computed by Tukey's honest significant difference test with significance at $P < 0.05$.

Phenotyping of a Breeding Population against *Meloidogyne Chitwoodi* Using the Canister Assay

To evaluate the utility of the canister method for phenotyping, potato germplasm with varying levels of susceptibility to *M. chitwoodi* from a breeding population was tested. The breeding population included progeny from OR170115 (PA99N82-4 x AOR13260-3adg). PA99N82-4 is the *M. chitwoodi* resistant parent with introgression from *S. bulbocastanum*. The seedlings segregate for resistance to *M. chitwoodi* 1:1 (Brown et al. 1995; Sathuvalli, personal commuication). Two experiments were conducted. In one of the experiments, 60 accessions were evaluated along with 'Ranger Russet' as a susceptible control; each accession/control was replicated four times. At the end of the experiment, 66 samples (out of 244) were randomly selected for qPCR analysis. These samples varied in final *M. chitwoodi* egg densities as determined by microscopy. In another experiment, 15 accessions were evaluated along with 'Shepody' as a susceptible control; each accession/control was replicated four times. At the end of the experiment, egg densities were determined by microscopy and by qPCR.

In both experiments, tuber pieces with 2 to 3 eyes of each accession were added to canisters as described above. Canisters were inoculated with 5 *M. chitwoodi* eggs/g soil at planting. After 6 weeks, the canisters were destructively harvested, eggs extracted from roots and then enumerated as described for Exp. 1 (see above). After enumeration of eggs, the aliquot was returned to the tube and stored at 4 °C until DNA extraction. Eggs were concentrated into DNeasy PowerSoil Pro kit bead beating tube (QIAGEN, Hilden, Germany) and DNA was extracted according to manufacturer instructions with one modification: after the bead beating step samples were incubated at 4 °C for 24 h before completing the rest of the manufacturer protocol.

The primers and probe for quantitative PCR were adapted from a hydrolysis probe qPCR assay to detect *M. chitwoodi* (Soulé et al. submitted). The primers target the gene *Hsp90* of *M. chitwoodi*. The qPCR was performed in 20 µl reaction volumes consisting of 2x TaqMan™ Universal Master Mix II, no UNG (Life Technologies Corporation, Carlsbad, CA, USA), 500 nm forward and reverse primers (Sigma-Aldrich, Inc. St. Louis, MO, U.S.A.), 250 nM of FAM TaqMan™ (Life Technologies Corporation), 2 µl of template DNA, and water. Cycling conditions were 95 °C for 10 min and 50 cycles at 95 °C for 15 s and 60 °C for 1 min using the Applied Biosystems StepOne Plus (Applied Biosystems, Waltham, MA, USA). A five-point standard curve was prepared by extracting DNA from 30,000 *M. chitwoodi* eggs. The purified DNA extract was serially diluted 1:10 so that the five points represented 30,000, 3,000, 300, 30, and 3 eggs. Each sample was run in triplicate and generated Ct-values were compared to those on the standard curve to estimate starting sample quantity.

Data from the genotype experiments were analyzed using R Statistical Software (v4.2.0; R Core Team 2022). For the experiment with 60 accessions, qPCR egg density and RF estimates were plotted against microscope egg densities and RF estimates and were analyzed by ANOVA with repeated measures using the package rstatix (Kassambara 2021) with significance at $P < 0.05$. The same dataset was subset to only include data with egg densities < 200 estimated by microscopy and the same analysis conducted to determine correlation with qPCR estimates. In the experiment with15 accessions, RF estimates from microscopy and qPCR were compared with a t-test with significance at $P < 0.05$. Data from the second experiment was further considered by assigning host status to the genotypes based upon RF values with RF 0.5 to 1 = poor host; RF 1 to 5 = moderate host; and, RF > 5 = good host. Host status based on RF values calculated by qPCR were compared to host status as previously determined by resistance linked marker status (Bali et al. 2022).

Results

Optimization of the Canister Assay

In Experiment 1, there was no difference in final dry root weight among treatments or treatment combinations ($P > 0.1$; data not shown). Dry root weights ranged from 0.01 to 0.18 g. For RF values, there was no interaction of inoculation density and takedown date ($P = 0.2975$), therefore, the factors of inoculation density and takedown date were considered independently. There was no significant effect of inoculation density on RF values ($P = 0.8710$; Fig. 2A), with RF values ranging from 26 to 41. Takedown date had a significant effect on RF values ($P = 0.0009$; Fig. 2B). The RF value at 5 weeks was 49 to 67% lower than for RF values at 6, 7, and 8 weeks. When final *M. chitwoodi* eggs/g root was considered, there was no interaction of inoculation density and takedown date ($P = 0.5752$), therefore, the factors of inoculation density and takedown date were considered independently. There was no significant effect of takedown date on eggs/g root ($P = 0.0556$; Fig. 2C) with a mean of 239,647 ± 26,004 eggs/g root. There was a significant effect of inoculation density on *M. chitwoodi* eggs/g root

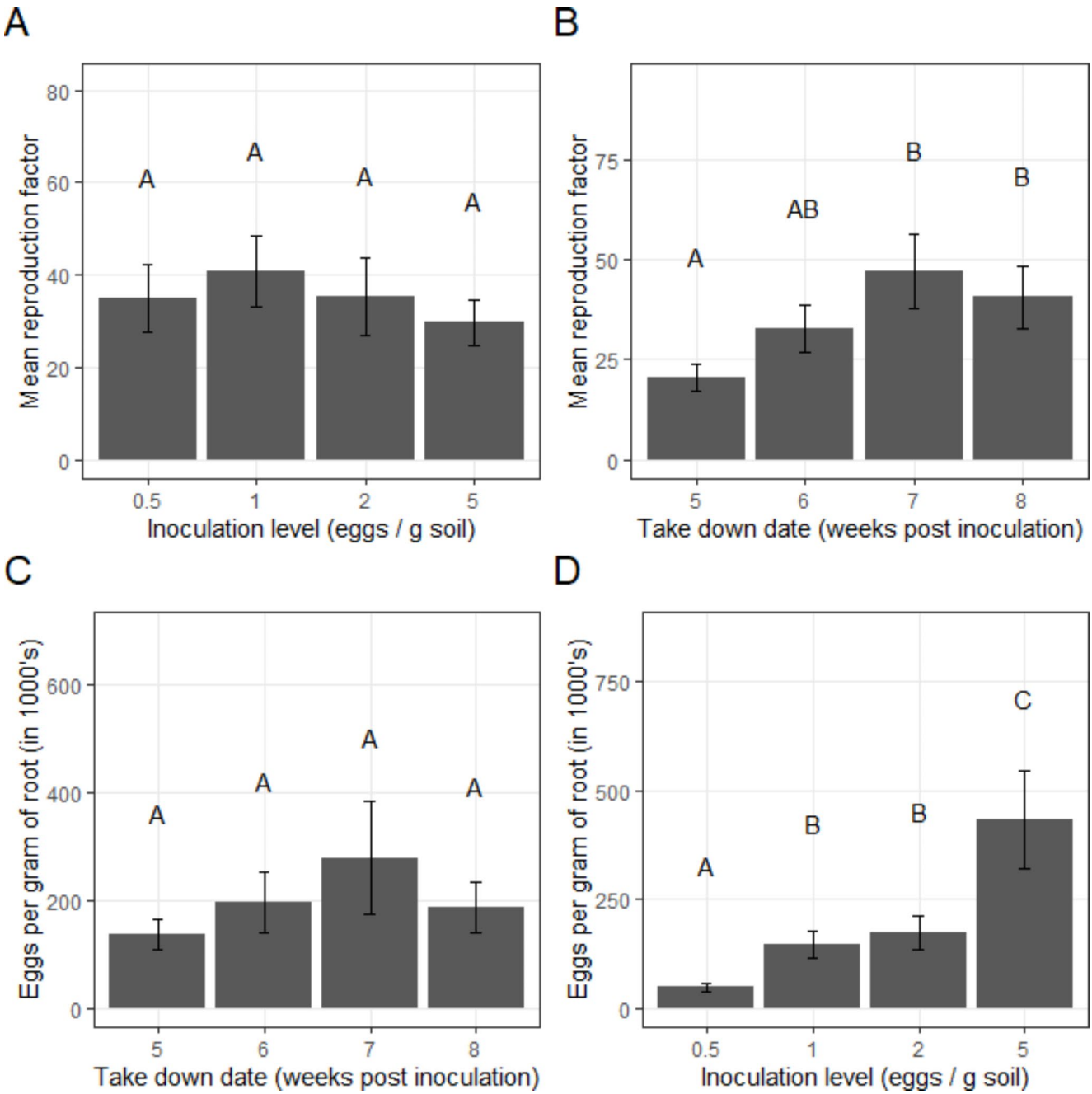

Fig. 2 Effect of inoculation density of *Meloidogyne chitwoodi* (eggs/g soil) (**A** and **B**) and take down date (**C** and **D**) on final reproduction factor (final population density/initial population density) values and final eggs/g root. The interaction of these factors was not significant ($P > 0.01$); therefore, they are presented separately. Bars within a panel with the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($p > 0.05$). Bars are the mean ± standard error of N = 10

($P < 0.0003$; Fig. 2D). As Pi increased so did the number of eggs/g root extracted at the end of the experiment. At Pis of 1 and 2 eggs/g soil there were 1.3 and 1.4 fewer eggs/g root than at Pi of 5 eggs/g soil, respectively. The highest number of eggs/g root (356,962 ± 53,141) eggs/g root was recovered from a Pi of 5 eggs/g soil.

In Experiment 2, there was no effect of inoculation volume on RF values, eggs/g root, or dry root weights ($P = 0.7665$; data not shown). Dry root weights ranged from 0.01 to 0.08 g. The mean RF value across treatments was 118.0 ± 20.4 and there were 25,952 ± 4,498 *M. chitwoodi* eggs/g root.

In Experiment. 3, dry root weight averaged 0.3 ± 0.003 g across treatments and trials. There was a significant effect of inoculation timing on RF values and *M. chitwoodi* eggs/g root ($P = 0.0001$; Table 1). When *M. chitwoodi* was

Table 1 Effect of inoculation timing on reproduction factor (final population density/initial population density) values and eggs/g root of *Meloidogyne chitwoodi*. Values with the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($p > 0.05$). Values are the mean ± standard error of N = 10

Inoculation timing	RF value	Eggs/g root
0	27.7 ± 3.9	189,561 ± 25,292
1	19.1 ± 4.8	126,591 ± 22,158
2	4.3 ± 0.8	29,863 ± 7,722

inoculated 2 weeks after planting the tuber piece, RF values were significantly lower by 85 and 78% compared to inoculation at planting (0 week) and 1 week after planting. There was no difference in RF values when inoculation occurred at 0 and 1 week after planting. There was a similar trend for *M. chitwoodi* eggs/g root with 6.3 and 4.2 times more eggs/g root when inoculation occurred at planting (0 week) and 1 week vs. 2 weeks, respectively (Table 1).

Phenotyping of a breeding population against *Meloidogyne chitwoodi* using the canister assay

There was a strong correlation between microscope counts and qPCR estimates of *M. chitwoodi* egg density when all data (n = 66) were considered ($R^2 = 0.97$; Fig. 3A). There was also no significant difference in egg density estimates between microscope counts and qPCR estimate ($P = 0.06$; Fig. 3B). For the 46 samples in which there were > 200 eggs/g root based on microscope counts, the data was not highly correlated ($R^2 = 0.33$; Fig. 4A), and there was a significant difference between counting methods ($p < 0.0001$; Fig. 4B) with 21 microscope assessments of zero generating amplification that resulted in qPCR estimates ranging from 1 to 658 egg/g root. Reproduction factor values for all samples were not significantly different between microscope and qPCR assessment methods ($p < 0.33$; Fig. 5A). Further, when samples were analyzed with RF values less than one, the difference between methods was also not significant ($p = 0.19$; Fig. 5B).

Microscope counts and qPCR estimates of *M. chitwoodi* egg density and resulting RF values resulted in similar characterization of the host status of the genotypes, from nonhost to good host (Table 2). When the RF values/host status of the genotypes was compared to the marker data, genotypes that contained resistant makers were also determined to be non- or poor hosts for *M. chitwoodi.* The exception was sample 45 where the results from the canister assay contradicted the marker data.

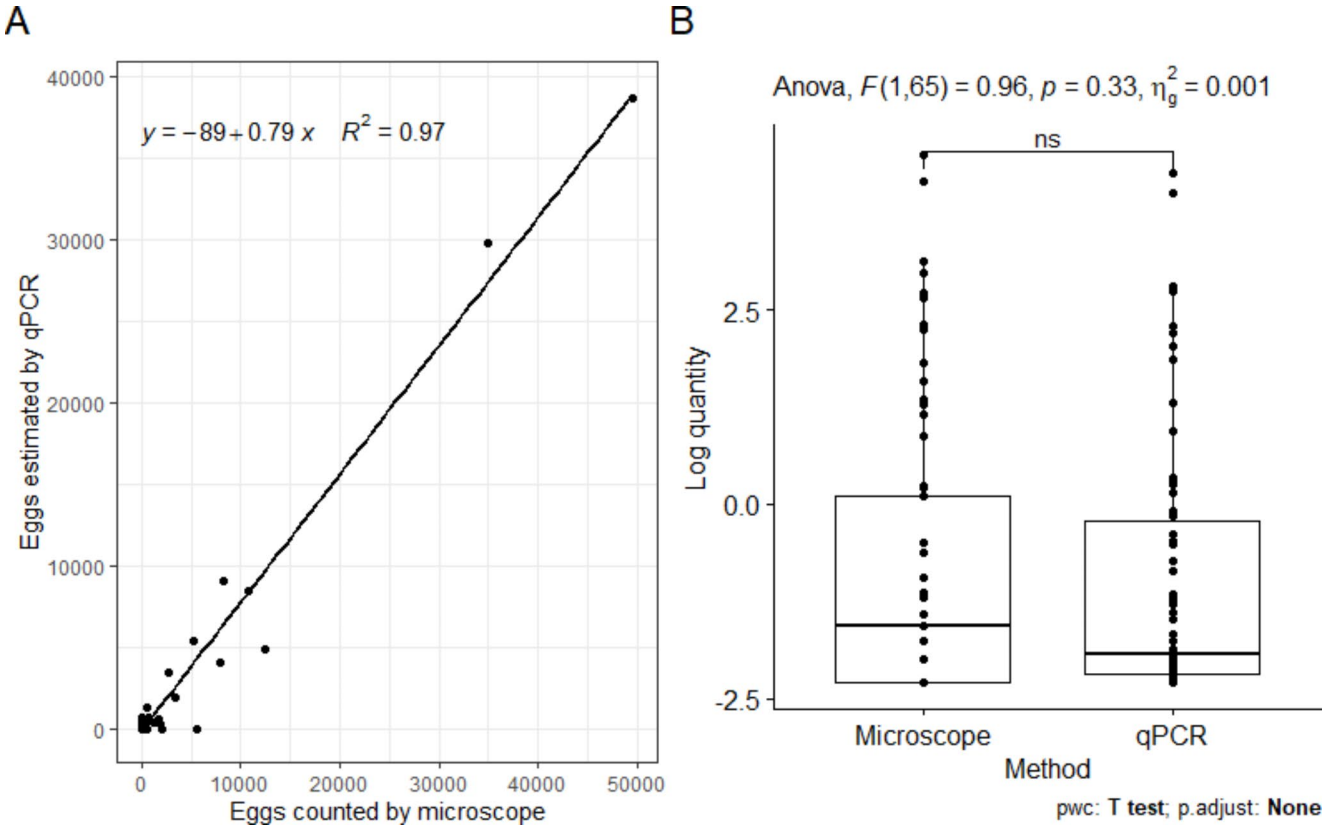

Fig. 3 Comparison between *Meloidogyne chitwoodi* egg densities for all data estimated by microscopy and the associated qPCR estimates. Correlation (**A**) and ANOVA (**B**) with repeated measures. Data were ln(x + 0.1)-transformed prior to ANOVA and significant differences were determined at $p = 0.05$. (N = 66)

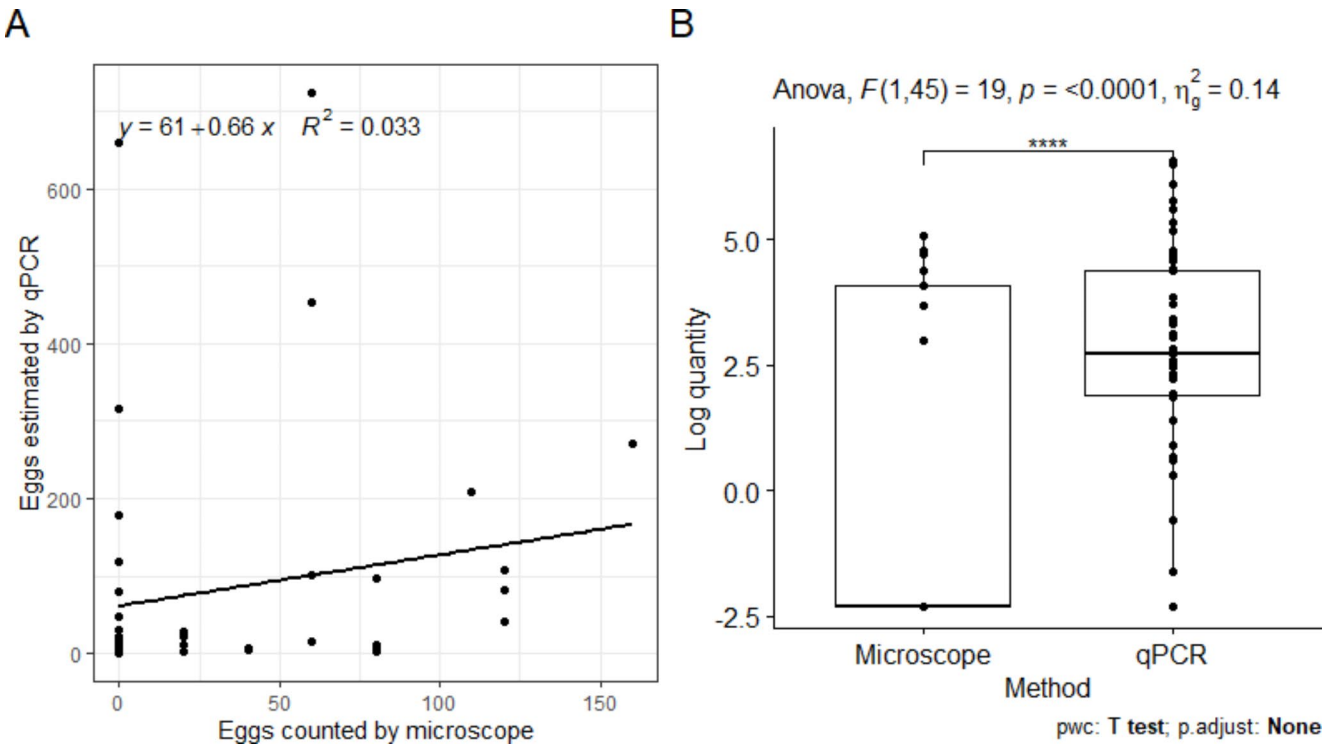

Fig. 4 Comparison between *Meloidogyne chitwoodi* egg densities of less than 200 estimated by microscopy and the associated qPCR estimates. Correlation (**A**) and ANOVA (**B**) with repeated measures. Data were ln(x + 0.1)-transformed prior to ANOVA and significant differences were determined at $p = 0.05$. (N = 66)

Discussion

While we are not the first to adopt a canister assay to screen potatoes against plant-parasitic nematodes (Foot 1977; Janssen et al. 1995; Phillips et al. 1980), it is important for labs to validate and fine tune methodologies prior to implementation. In this study, we explored several methodological aspects of a canister assay for rearing of *M. chitwoodi* to enable high throughput phenotyping and biological studies in potato. We found that length of assay and inoculation timing were both important factors to consider. We also, for the first time, deployed a molecular quantification method to determine *M. chitwoodi* egg densities to further improve the high-throughput nature of the assay for rapid screening of potatoes against *M. chitwoodi*.

The first published report of a container assay to rear and conduct experiments with plant-parasitic nematodes, specifically *Globodera* spp., was by Foot (1977). The two major advantages of this method presented by Foot (1977) were that light was not required and that temperature could be regulated. This method was further refined by Phillips et al. (1980). This assay with various modifications has been used with *Globodera* spp. to evaluate quantitative resistance (Mugniéry et al. 1989), single locus immunity (Gartner et al. 2021; Moloney et al. 2010), climate change (Skelsey et al. 2018), biological control (LaMondia and Brodie 1984), and influence of temperature on development (Kaczmarek et al. 2014).

To the best of our knowledge, there is only one published report on utilizing a closed canister assay to evaluate potatoes or *Solanum* spp. for resistance to *Meloidogyne* spp. (Janssen et al. 1995). In that study, the canister assay was compared to other experimental methods to evaluate a seedling population of *Solanum* spp. against *M. chitwoodi* and *M. hapla.* The container, 125 ml, was filled with moist silver sand containing slow release NPK fertilizer. Sprouted tuber pieces, approximately 20 to 30 g, were planted and one week later inoculated with 260 *Meloidogyne* spp. juveniles. The assay ran at 20 ± 1°C for 7 weeks and egg masses were counted and fresh roots weighed. While the closed canister assay resulted in lower reproduction of *Meloidogyne* spp. than two other methods, clay pots and plastic tubes, the canister assay still indicated that the 10 potato cultivars evaluated were hosts for *M. chitwoodi* and *M. hapla* (Janssen et al. 1995). In our optimization experiments we used the susceptible potato ' Ranger Russet'. The canister assay resulted in RF values > 1 95% (N = 234) of the time, indicating that *M. chitwoodi* reproduced very well in this assay.

Several parameters were evaluated to optimize the performance of the canister assay for *M. chitwoodi.* Comparable experiments were conducted by Phillips et al. (1980). The moisture level in our experiments was adjusted at

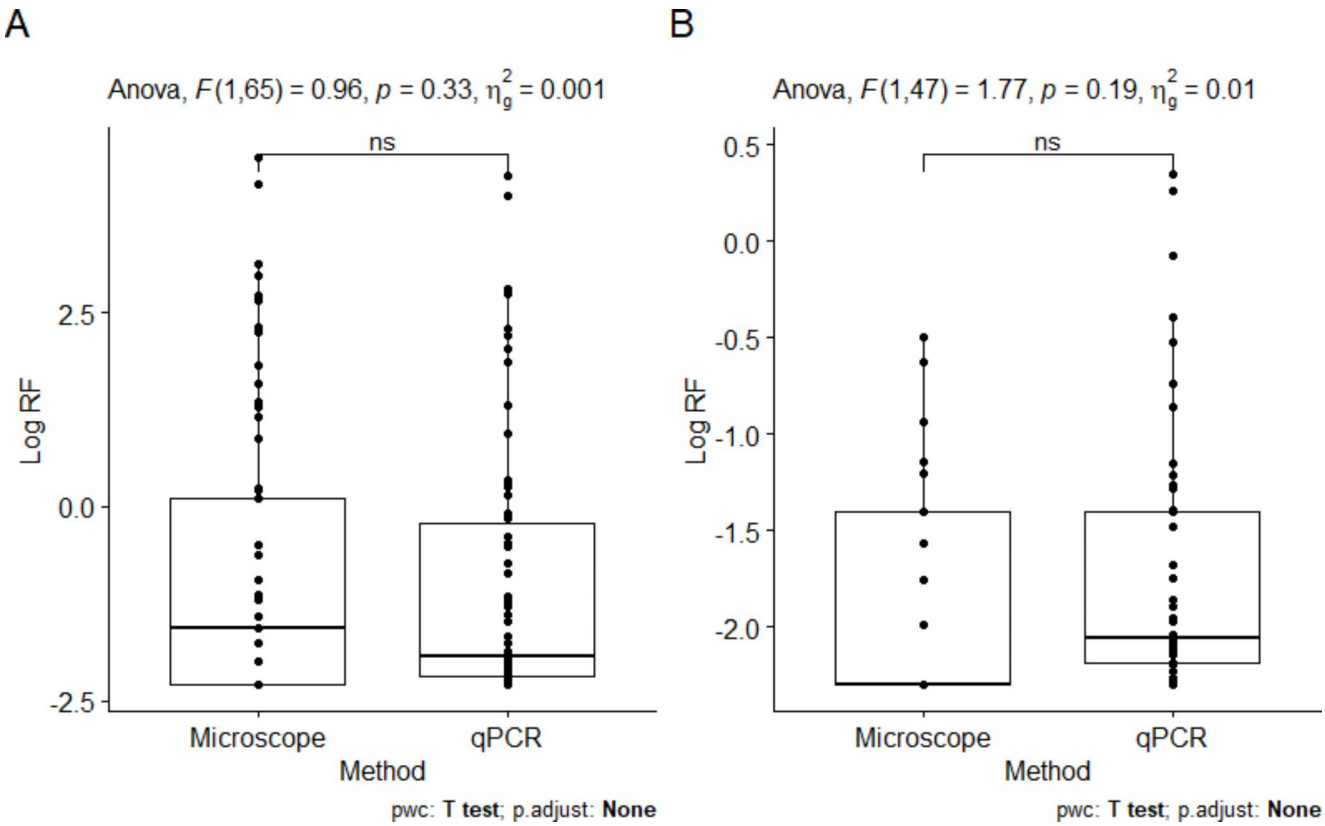

Fig. 5 Comparison of *Meloidogyne chitwoodi* reproduction factor (RF = final population density/initial inoculation density) calculated from estimates made by microscopy versus qPCR. All data (N = 66; **A**) and data RF < 1 (N = 47; **B**) were ln(x + 0.1)-transformed prior to ANOVA and significant differences were determined at $p = 0.05$

Table 2 Reproduction factor value and host status designation of a potato breeding line for *Meloidogyne chitwoodi* based upon microscopic counts and qPCR as well as marker designations (n = 4 for microscope and 3–4 for qPCR). Reproduction factor (RF) = final egg density/initial egg density; values are mean ± standard error. Host status is defined as: RF < 0.5 = nonhost; RF 0.5 to 1 = poor host; RF 1 to 5 = moderate host; and, RF > 5 = good host. Markers details are available at Bali et al. (2022)

Sample	Microscope		qPCR		Markers				
	RF	Host status	RF	Host status	SNP4	Indel4	SSR4	SSR8	SSR20
OR170115-18	37.6 (± 10.7)	Good	11.5 (± 5.4) [a]	Good	-	-	-	-	-
OR170115-22	78.4 (± 9.2)	Good	49.3 (± 7.1)	Good	-	-	-	-	-
OR170115-25	0.5 (± 0.5)	Poor	0.4 (± 0.4)	Non	+	+	+	+	+
OR170115-26	0.3 (± 0.3)	Non	0.4 (± 0.4)	Non	+	+	+	+	+
OR170115-45	8.7 (± 6.6)	Good	1.5 (± 0.1)	Moderate	+	+	+	+	+
OR170115-46	21.7 (± 12.4)	Good	7.8 (± 3.5)	Good	-	-	-	-	-
OR170115-56	0.2 (± 0.1)	Non	0.1 (± 0.1)	Non	+	+	+	+	+
OR170115-57	5.1 (± 5.1)	Good	5.8 (± 5.6)	Good	-	-	-	-	-
OR170115-86	0.1 (± 0.1)	Non	0.2 (± 0.2)	Non	+	+	+	+	+
OR170115-87	0.7 (± 0.4)	Poor	0.6 (± 0.4)	Poor	+	+	+	+	+
OR170115-114	62.6 (± 45.8)	Good	21.9 (± 12.0)	Good	-	-	-	-	-
OR170115-138	112.4 (± 28.3)	Good	22.3 (± 5.8)	Good	-	-	-	-	-
OR170115-139	132.2 (± 18.2)	Good	39.5 (± 18.0)	Good	-	-	-	-	-
Shepody	17.9 (± 8.0)	Good	6.4 (± 3.2)	Good					

inoculation with *M. chitwoodi* eggs being inoculated in 2, 5 or 10 ml water (equivalent to approximately 22, 24 and 26% moisture levels); there was no difference in final egg densities or RF values due to modifying this variable. Contrary to our findings, with *G. pallida* there was a reduction in cyst numbers when moisture level of sand was increased from 10 to 17% (Phillips et al. 1980). A difference in our study might have been more apparent if a wider range of percentage soil moistures were evaluated.

In Janssen et al. (1995), the experiment was inoculated with 2 *M. hapla* or *M. chitwoodi*/ml soil. We varied initial inoculation density and inoculated with 0.5 to 5 *M. chitwoodi*/g soil. There was an effect of varying initial inoculation density on final eggs/g root, with more eggs produced when more *M. chitwoodi* were added to the assay, however, this initial inoculation density did not impact RF values. The duration of the assay was also evaluated with take-down time after inoculation occurring at 5, 6, 7, and 8 weeks. Similar to a greenhouse study (Filialuna et al. 2022), higher RF values were observed for *M. chitwoodi* at later harvest dates. In both the canister assay and aforementioned greenhouse study, five weeks was insufficient time for the assay. In the canister assay, it is possible to run the experiment for six weeks and achieve similar RF values as later dates. The difference between this study and the greenhouse study is that temperature was held constant at 24 °C in the canister assay while temperature varied in the greenhouse study. A temperature of 24 °C was chosen for this experiment based upon findings that *M. chitwoodi* egg densities were the highest six weeks after inoculation at an incubation temperature of 25 °C (O'Bannon and Santo 1984).

This canister assay has great utility in a potato breeding program that needs to rapidly phenotype hundreds of individuals in a breeding population. Currently, it takes 12 and 14 weeks to phenotype potatoes against *M. chitwoodi* in the greenhouse and field, respectively (Brown et al. 2006; Graebner et al. 2018). With the canister assay, only 6 weeks are required. With greenhouse and field experiments only one experiment can be run per year. With the canister assay and access to an incubator, many assays can be run in a year so long as viable tubers are available. In comparison to a greenhouse experiment there is also a space and resource saving advantage with the canister assay. Greenhouse studies with *M. chitwoodi* are conducted in 10 cm pots containing 1 L of soil. Per experimental unit, only one tenth of the amount of soil is required for the canister assay compared to a greenhouse pot assay. Additionally, the footprint of the experiment is greatly reduced using the canister assay because the experimental units can be stacked. This is not to say that the canister assay will replace greenhouse or field experiments; rather, it can be used to complement them. Initially, hundreds of progeny from a breeding population can be rapidly phenotyped to eliminate highly susceptible material. Those that are found to be resistant or partially resistant can then be further evaluated to confirm this finding in greenhouse and field experiments.

When molecular quantification of eggs was compared to quantification by microscope there was no difference between the two methods in egg densities or RF value. However, considering the data as a whole masks the effect that egg density has on density estimates. That is, at high densities, egg counts by microscope become more accurate. When we compared egg density estimates by microscope that estimated under 200 *M. chitwoodi* eggs in the sample, we found a significant difference between qPCR estimates and microscope estimates. This is especially relevant for the 21 samples which had a microscope estimation of zero and a positive qPCR estimate. Complete resistance is the goal in a breeding program because of the zero tolerance for *M. chitwoodi* in international potato shipments (King and Taberna 2013), therefore, accuracy is needed for screening progeny for immunity to *M. chitwoodi*. That said, our comparison of host status as evaluated by RF value generated by microscope versus qPCR resulted in majority agreement between the two methods demonstrating the reliability of qPCR data for making host status designations.

The potato canister assay presents the opportunity to significantly increase the volume of progeny tested for *M. chitwoodi* resistance. In this study we validated and optimized the potato canister assay and demonstrated its use in screening a breeding population for *M. chitwoodi* resistance. The results obtained from the container assay matched marker data for the genotypes evaluated. Combined with high-throughput molecular diagnostics like qPCR, the canister assay is a much more sensitive and time efficient process than is currently in use. Decreasing the amount of time required to bring a resistant potato cultivar to market is central to reducing pesticide use and imperative in the age of increasing pesticide restrictions. The increased sensitivity is also important for breeding potatoes with immunity to *M. chitwoodi* because of the low tolerance for the presence of *M. chitwoodi* in the international export market. Future research efforts with the potato canister project should focus on the validation of the potato canister assay for the evaluation of other plant-parasitic nematodes.

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Data Availability Data will be shared as per request to the corresponding author.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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