Journal of Nematology
Volume 58 Issue 1 · pp. 591–597 · DOI: 10.2478/jofnem-2026-0039

JOURNAL OF NEMATOLOGY

Comparisons of nematode assay efficiencies and recommendations across 10 state nematode assay labs utilizing turfgrass

Research Article

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Received 09 July 2025; Accepted 19 May 2026

Abstract: Nematode assay extraction methods vary among state laboratories for quantifying plant-parasitic nematodes (PPNs) from turfgrass samples, which can lead to differences in nematode population estimates and management recommendations for turfgrass. This study compared the extraction efficiencies of 10 state nematode assay labs in the eastern United States using homogenous turfgrass soil samples. Random samples were collected from three locations; homogenized; and distributed to each lab. Three labs used semi-automatic elutriation, while seven employed hand sieving with centrifugation and sugar floatation. Variability in population estimates among state labs was evident in five of six recorded PPN genera by state and four of six by hand-sieving. *Hoplolaimus* spp. were different when compared by semi-automatic elutriation with all other genera recorded being similar. Despite variations in counts, recommendations for nematode management were generally consistent within each lab. These findings highlight the importance of using the same assay lab over time for reliable nematode monitoring and suggest that automation may reduce the variability in nematode extraction.

Keywords: *Assay • Centrifugation • Efficiency • Elutriation • Extraction • Golf • Nematodes • Plant-parasitic • Population • Recommendations • Sample • Sieving • Sugar-floatation*

1. Introduction

Nematode advisory programs were established in various states to assess the damage caused by plant-parasitic nematodes to diverse crops including turfgrass used for golf (Barker & Imbriani, 1984). In the eastern United States, nematode assay labs use one of two extraction methods: the N.C. State Model semi-automatic elutriator (Byrd et al., 1976) or hand sieving with centrifugation–floatation (Jenkins, 1964). The elutriator is more effective with a larger soil sample (250–500 cm^3), and hand sieving is limited to a smaller sample size (100 cm^3). However, the elutriator is expensive ($70,000) (Weimin Yi, North Carolina Department of Agriculture, personal comm.) compared to the lower cost of materials needed for hand sieving ($500) (Jenkins, 1964). Both methods use similar sieves, centrifuges, and sugar solutions, but the elutriator processes more samples per hour with less manual labor, though it requires substantially more water. Processing fees vary among labs, ranging from $0 to $75 per sample, with greater charges ($10–$75) for out-of-state submissions.

Assessing the damage caused by the plant-parasitic nematodes (PPNs) on different crops across different states is costly and has rarely been conducted beyond the early years of plant nematology. As a result, each state has its own recommendations based on research identifying the harmful nematodes in turfgrass used for golf. Some states adapt data from neighboring states, adjusting for climate and environmental differences, while others rely on a state lab nematologist to refine recommendations for diagnosing nematode-related damage specific to their conditions (Jagdale, 2013; Mehl and Langston, 2024; Strayer-Scherer et al., 2025).

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This study aimed to evaluate the efficiency of 10 nematode assay labs in the eastern United States for quantifying nematode populations and to compare the turfgrass management recommendations for golf greens. Three locations were selected including the green at the Virginia Tech Turfgrass Research Center (VTTRC); Augustine Golf Course (AGC) in Stafford, VA; and the Philadelphia Country Club Golf Course (PCCGC) in Gladwyne, PA. The VTTRC was selected because it was known to have an abundant population of root-knot nematodes (*Meloidogyne naasi* Franklin, 1965). AGC and PCCGC were selected based on previous sampling that identified elevated PPN populations and recommendations indicating potential turf damage.

2. Materials and methods

This study included nematode assay laboratories from 10 states: Alabama, Florida, Georgia, Louisiana, Massachusetts, Michigan, Mississippi, North Carolina, South Carolina, and Virginia were used in this study. Soil samples were collected from three locations – two in Virginia and one in Pennsylvania. Due to greater processing costs, only samples from the Philadelphia Country Club were analyzed by the Massachusetts lab.

Nineteen liters of soil were collected from each of three sites by raking fall aerification soil cores of the bentgrass greens. The soil from each site was thoroughly mixed for 5 min using a concrete mixer, then divided into 30 samples of 500 cm^3 each, and placed into 1-L plastic bags. Three 500 cm^3 soil samples from the three turfgrass sites with varying levels of nematode populations were sent to 10 nematode assay labs. Samples were shipped in Styrofoam coolers overnight although the previous research indicates that shipping has minimal impact on extraction efficiency (Riggs et al., 1997).

Each lab processed the samples using its standard protocols and provided recommendations based on assay results and state guidelines. Standard extraction protocols for Virginia, North Carolina, and Louisiana involved the use of semi-automatic elutriation followed by sucrose centrifugation, while the remaining seven labs extracted nematodes through hand sieving followed by sucrose centrifugation.

Population means of nematode genera per 100 cm^3 of soil by state lab and by extraction methods were compared using an analysis of variance with means separated using Tukey's Honestly Significant Difference *post hoc* test (α = 0.05) in JMP® Pro 18 (SAS Institute Inc., Cary, NC) (Table 2). Virginia and South Carolina labs further subdivided samples to assess assay efficiency. South Carolina, which typically uses 100 cm^3 of soil, divided each sample into three subsamples, while Virginia, using 250 cm^3, divided each sample into two. To account for differences in soil volume used across labs, all results were standardized to nematodes per 100 cm^3 of soil. State-specific recommendations were also compared.

3. Results

The study investigated the nematode extraction efficiencies and compared recommendations from 10 state nematode assay labs in the eastern United States using soil samples from three locations.

3.1 Virginia tech turfgrass research center

Total PPN counts were greatest in South Carolina (567, 533, 483 per 500 cm^3 of soil) and lowest in Louisiana (13 per 500 cm^3 of soil), which detected only *Mesocriconema* spp. (Figure 1). *Tylenchorhynchus* spp. were found in low numbers by SC1, VA1, VA2, FL, GA, MA, and MS (<20 per 500 cm^3 of soil) but absent in SC2, SC3, MI, AL, NC, and LA. *Hoplolaimus* spp. were reported in all states except for Louisiana, while *Meloidogyne* spp. were found in all states except for VA1, AL, and LA. The greatest number of *Meloidogyne* spp. were recorded in SC1, SC2, and SC3 (267, 233, 233 per 500 cm^3 of soil). *Mesocriconema* spp. were present in all states, but varied ranging from 267 per 500 cm^3 of soil in MI, to as low as 13 and 7 per 500 cm^3 of soil in LA and NC, respectively. Only AL and NC identified a possible turf issue due to *Meloidogyne* spp. (40 and 156 per 500 cm^3 of soil, respectively) despite greater counts in SC1, SC2, SC3, and FL (267, 233, 233, 122 per 500 cm^3 of soil, respectively) (Table 1).

3.2 Augustine golf course

Michigan recorded the greatest total plant-parasitic nematode counts (317 per 500 cm^3 of soil) while Louisiana had the lowest detecting only *Mesocriconema* spp. (13 per 500 cm^3 of soil) (Figure 2). North Carolina did not find *Mesocriconema* spp. but reported *Tylenchoryhnchus* spp. (3 per 500 cm^3 of soil) and *Meloidogyne* spp. (7 per 500 cm^3 of soil). *Tylenchoryhnchus* spp. were detected only in SC2, SC3, VA1, VA2, and NC. *Meloidogyne* spp. were found by SC1, SC2, SC3, VA1, GA, FL, MS, and NC. *Hoplolaimus* spp. were reported by all labs except for MI, LA, and NC with

Table 1. Comparisons of nematode assay lab recommendations for three locations

State	Sampling site	Nematode	Technique	Soil	Rec.*
Alabama	TRC	Root-knot	Hand sieve	100 cm^3	B, B, B
Florida	TRC		Hand sieve	100 cm^3	A, A, A
Georgia	TRC		Hand sieve	100 cm^3	A, A, A
Louisiana	TRC		Elutriator	500 cm^3	A, A, A
Michigan	TRC		Hand sieve	100 cm^3	A, A, A
Mississippi	VTRC		Hand sieve	100 cm^3	A, A, A
North Carolina	VTRC	Root-knot	Elutriator	500 cm^3	B, B, B
South Carolina 1	VTRC		Hand sieve	100 cm^3	A, A, A
South Carolina 2	VTRC		Hand sieve	100 cm^3	A, A, A
South Carolina 3	VTRC		Hand sieve	100 cm^3	A, A, A
Virginia 1	VTRC		Elutriator	500 cm^3	A, A, A
Virginia 2	VTRC		Elutriator	500 cm^3	A, A, A
Alabama	AGC		Hand sieve	100 cm^3	A, A, A
Florida	AGC		Hand sieve	100 cm^3	A, A, A
Georgia	AGC		Hand sieve	100 cm^3	A, A, A
Louisiana	AGC		Elutriator	500 cm^3	A, A, A
Michigan	AGC		Hand sieve	100 cm^3	A, A, A
Mississippi	AGC		Hand sieve	100 cm^3	A, A, A
North Carolina	AGC		Elutriator	500 cm^3	A, A, A
South Carolina 1	AGC		Hand sieve	100 cm^3	A, A, A
South Carolina 2	AGC		Hand sieve	100 cm^3	A, A, A
South Carolina 3	AGC		Hand sieve	100 cm^3	A, A, A
Virginia 1	AGC		Elutriator	500 cm^3	A, A, A
Virginia 2	AGC		Elutriator	500 cm^3	A, A, A
Alabama	PCCGC	Ring	Hand sieve	100 cm^3	C, C, C
Florida	PCCGC		Hand sieve	100 cm^3	A, A, A
Georgia	PCCGC		Hand sieve	100 cm^3	A, A, A
Louisiana	PCCGC		Elutriator	500 cm^3	A, A, A
Massachusetts	PCCGC	Ring, Stunt	Hand sieve	100 cm^3	B, C, C
Michigan	PCCGC	Ring, Stunt	Hand sieve	100 cm^3	B, B, C
Mississippi	PCCGC		Hand sieve	100 cm^3	A, A, A
North Carolina	PCCGC		Elutriator	500 cm^3	A, A, A
South Carolina 1	PCCGC	Ring	Hand sieve	100 cm^3	B, C, C
South Carolina 2	PCCGC	Ring	Hand sieve	100 cm^3	C, C, C
South Carolina 3	PCCGC	Ring	Hand sieve	100 cm^3	C, C, C
Virginia 1	PCCGC	Ring	Elutriator	500 cm^3	B, C, C
Virginia 2	PCCGC	Ring	Elutriator	500 cm^3	B, C, C

This table presents the recommendations of 10 nematode assay labs for three turfgrass locations: Virginia Tech Turfgrass Research Center (TRC), Blacksburg, VA.; Augustine Golf Course (AGC), Stafford, VA.; and Philadelphia Country Club Golf Course (PCCGC), Gladwyne, PA. **A** = nematodes are not likely to cause a problem, **B** = nematodes may cause a problem, and **C** = nematodes are likely to cause a problem. Cells that are shaded indicate a **B** or **C** recommendation. *Rec. = recommendation.

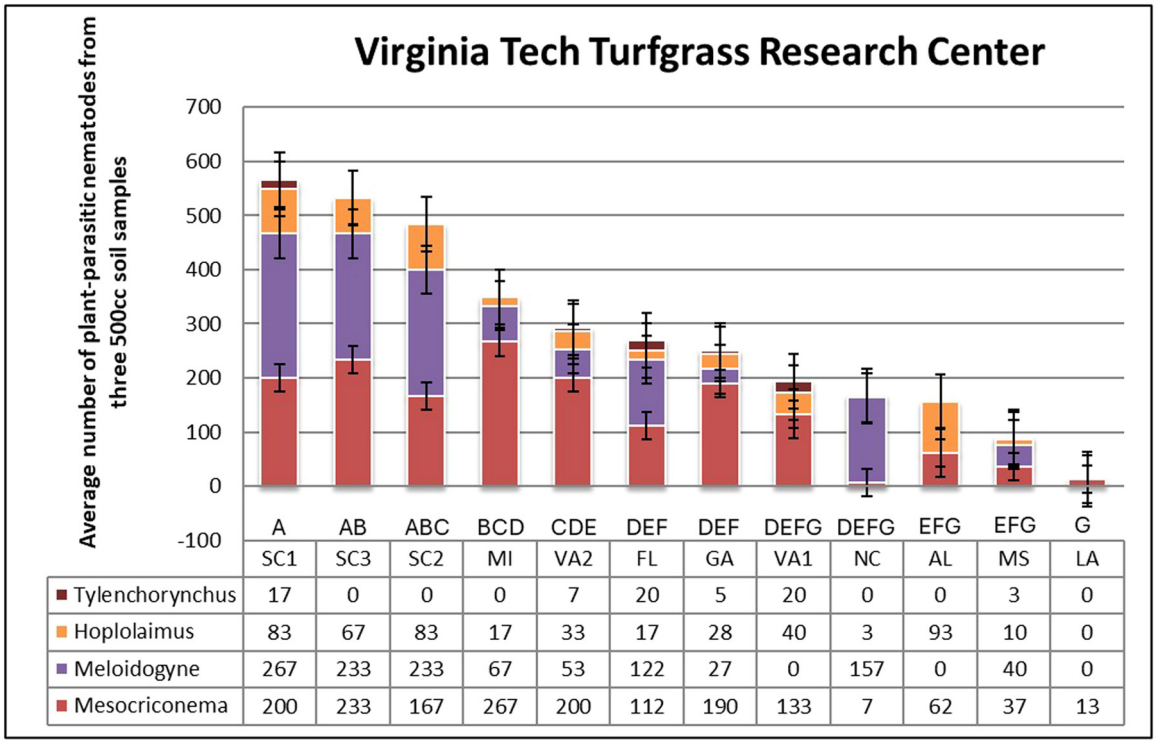


Figure 1. Comparison of nematode extraction efficiencies among 10 state nematode assay labs for the four most common genera of plant-parasitic nematodes at the Virginia Tech Turfgrass Research Center, Blacksburg, VA. Total means in each column followed by the same letters are not significantly different according to Tukey's Honestly significant difference ($P < 0.05$).

the greatest counts recorded in SC. *Mesocriconema* spp. were present in all labs except NC, with counts ranging from 317 (MI) to 13 per 500 cm^3 of soil (LA). None of the labs identified nematodes as a significant problem on this green (Table 1).

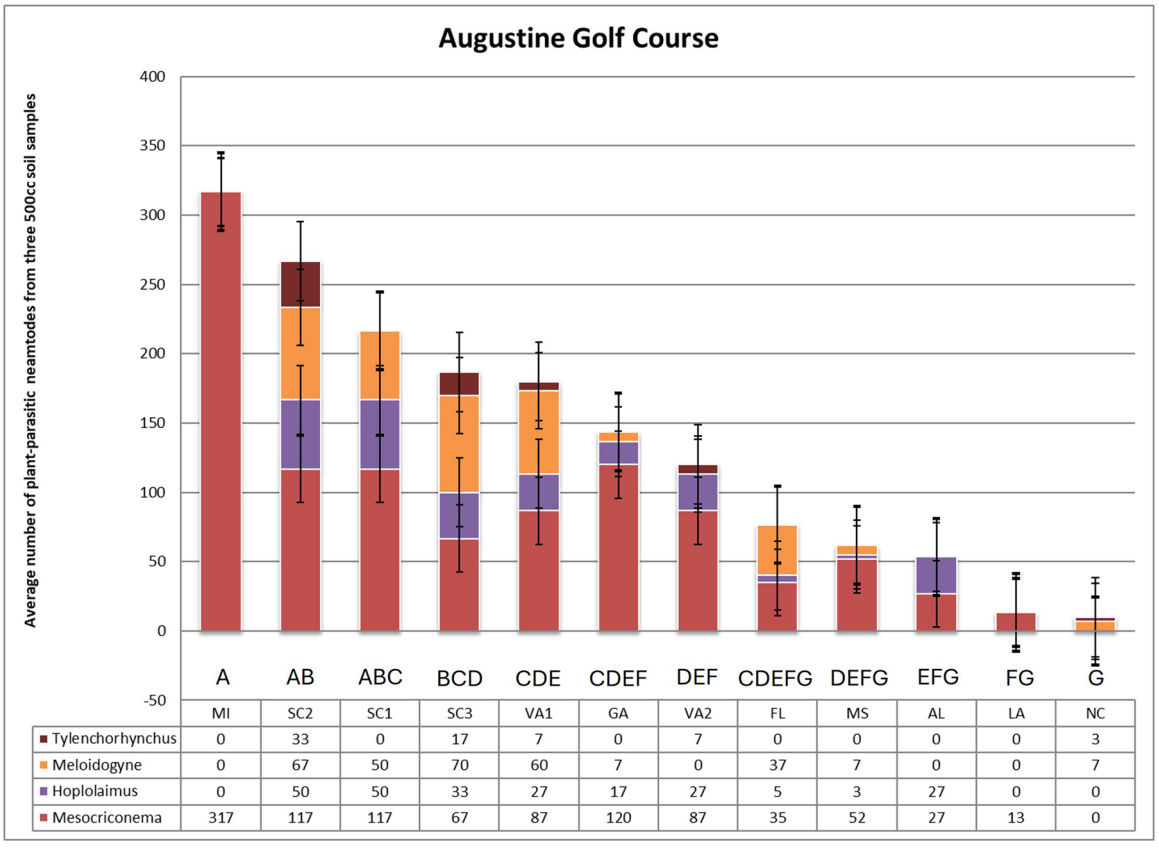
3.3 Philadelphia country club golf course

Massachusetts and Michigan reported the greatest total PPN counts for this green (14,239, 11,800 per 500 cm^3 of soil, respectively), while Mississippi and North Carolina

Table 2. Comparison of nematode extraction efficiencies among six genera of plant-parasitic nematode by state and by extraction method

	Comparison by state		Comparison by semi-automatic elutriation		Comparison by hand-sieving	
	P value	*F* ratio	*P* value	*F* ratio	*P* value	*F* ratio
Hoplolaimus spp.	0.0001*	4.2557*	0.0021*	7.5056*	0.0027*	3.8647*
Mesocriconema spp.	0.0385*	2.0908*	0.1487	2.0200	0.1030	1.8655
Tylenchorhynchus spp.	0.0051*	2.8722*	0.1001	2.4703	0.0344*	2.4698*
Meloidogyne spp.	0.0329*	2.1530*	0.0639	2.9928	0.0802	2.0054
Helicotylenchus spp.	0.443	1.0023	0.3678	1.0313	0.0066*	3.3684*
Paratrichodorus spp.	0.0191*	2.3648*	0.5025	0.7027	0.0462*	2.3093*

*Statistical significance ($\alpha = 0.05$). *P*-values and *F* ratios were determined using analysis of variance in JMP® Pro 18 (SAS Institute, Cary, NC) and Tukey's Honestly significant difference *post hoc* test were used to compare means.



	MI	SC2	SC1	SC3	VA1	GA	VA2	FL	MS	AL	LA	NC
Tylenchorhynchus	0	33	0	17	7	0	7	0	0	0	0	3
Meloidogyne	0	67	50	70	60	7	0	37	7	0	0	7
Hoplolaimus	0	50	50	33	27	17	27	5	3	27	0	0
Mesocriconema	317	117	117	67	87	120	87	35	52	27	13	0

Figure 2. Comparison of nematode extraction efficiencies among 10 state nematode assay labs for the four most common genera of plant-parasitic nematodes from the Augustine Golf Course, Stafford, VA. Total means in each column followed by the same letters are not significantly different according to Tukey's Honestly significant difference ($P < 0.05$).

recorded the lowest (849, 260 per 500 cm^3 of soil, respectively) (Figure 3). *Paratrichodorus* spp. were found in MI, VA1, VA2, SC2, SC3, GA, MS, and NC, with MI reporting the greatest count (50 per 500 cm^3 of soil) and NC the lowest (10 per 500 cm^3 of soil). *Meloidogyne* spp. were detected in MI, VA1, SC2, SC3, GA, MS, and NC with MI having the greatest count (50 per 500 cm^3 of soil). *Mesocriconema* spp. were present in all labs, with MI and MA (6,900, 4,553 per 500 cm^3 of soil, respectively) reporting the greatest counts, and the lowest counts in LA, MS, and NC (1,413, 477,170 per 500 cm^3 of soil, respectively). *Tylenchorhynchus* spp. were extracted from all labs, most abundant in MA (9,686 per 500 cm^3 of soil) and MI (4,800 per 500 cm^3 of soil), and least common in MS, LA, and NC (336, 173, 67 per 500 cm^3 of soil, respectively). Half of the labs (AL, MA, MI, SC, and VA) provided recommendations indicating potential nematode damage, while the other half (FL, GA, LA, MS, and NC) did not consider the population levels great enough to cause harm (Table 1).

3.4 Comparing nematode lab methods

Two nematode extraction methods were used across the 10 labs: (1) semi-automatic elutriation with modified sugar flotation and (2) hand-sieving with modified sugar floatation. Three labs (Virginia, North Carolina, and Louisiana) used an elutriator, while the remaining seven labs used hand-sieving. Nematode counts varied by state for five of the six PPN genera (Table 2). *Helicotylenchus* spp. were the genera that all labs reported occurring at similar population levels. Among the labs using elutriation, only *Hoplolaimus* spp. showed significant ($P < 0.05$) differences in counts across all locations (Table 2). The elutriation extraction reported similar ($P > 0.05$) numbers for *Helicotylenchus* spp., *Meloidogyne* spp., *Mesocriconema* spp., *Paratrichodorus* spp., and *Tylenchorhynchus* spp. In contrast, four of six PPN genera exhibited differences in nematode population levels among labs using the hand-sieving method (Table 2). *Hoplolaimus* spp., *Helicotylenchus* spp., *Paratrichodorus* spp., and *Tylenchorhynchus*

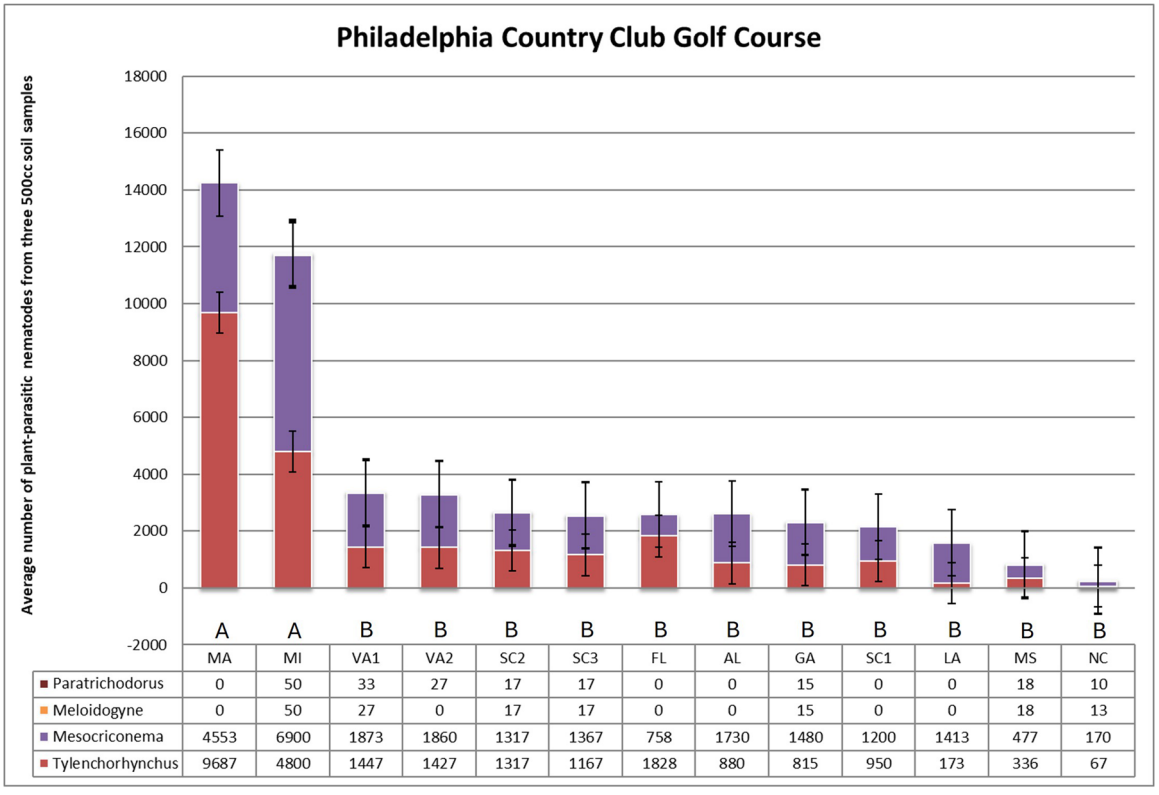


Figure 3. Comparison of nematode extraction efficiencies among 10 state nematode assay labs of the four most common genera of plant-parasitic nematodes at the Philadelphia Country Club Golf Course, Gladwyne, PA. Total means in each column followed by the same letters are not significantly different based on Tukey's Honestly significant difference ($P < 0.05$).

spp. were found at significantly different ($P < 0.05$) different levels in four labs.

4. Discussion

Nematode assay results varied across labs. Alabama and North Carolina identified a nematode problem at VTTRC. Alabama used hand sieving and a 100 cm^3 sample, whereas NC employed an elutriator with a 500 cm^3 sample. Virginia did not detect a significant root-knot population despite a previous finding indicating a problem (personal comm.). *Meloidogyne* spp. was found in one sample, but not in the another. The South Carolina lab (SC1, SC2, SC3) reported the greatest root-knot nematode counts, but did not consider them to be damaging. Assessing *Meloidogyne* spp. nematodes in golf greens is challenging due to their patchy distribution and the fact that the J2 stage infects the root, and all following life stages are completely contained within the roots (Crow, 2021; Karssen et al., 2013). Juveniles of *M. naasi*, one of the most common PPN on turf, have very long tails, which inexperienced counters, may misidentify as *Tylenchus* spp. Most labs agreed that plant-parasitic nematodes were not a problem on this green.

The labs agreed that plant-parasitic nematode populations at the AGC were too low to cause damage that would warrant a nematicide application. While the genera and counts varied among nematode assay labs, the previously reported damaging level of lance nematode was not detected (personal comm.). Discussions with the assistant superintendent revealed that the original sample had been collected as a single cup-cutter plug, which is inadequate for accurate nematodes assessment. Standard recommendations call for at least 20 soil cores (2.54 cm in diameter) taken at root depth, mixed, and submitted as a 500 cm^3 sample per green (Jagdale, 2011; Galle and Kerns, 2024). Since nematodes are unevenly distributed in soil, multiple subsamples are necessary to account for their patchy distribution. Submitting a single cup-cutter plug to one or more labs may yield inconsistent results.

Half of the labs recommended action for the elevated plant-parasitic nematode levels on the Philadelphia golf course green. Southern labs (VA, SC, AL) attributed turf damage to *Mesocriconema* spp., while northern labs (MA, MI) identified both the *Mesocriconema* spp. and

Tylenchorhynchus spp. as the cause. Although SC, FL, and GA found similar *Mesocriconema* spp. nematode counts, they did not consider the levels great enough to harm turfgrass. Interestingly, the northern labs detected significantly greater populations of both *Mesocriconema* spp. and *Tylenchorhynchus* spp., though the reason for this discrepancy is unclear. Both used hand-sieving for extraction, yet the nematode counts for the VTTRC green and AGC were not significantly different from the other labs.

Results showed significant variability in counts across state labs though this had little impact on management recommendations for each location. Nematode recommendations are typically based on population ranges. Despite differences in counts, most labs provided similar recommendations. Notably, *Hoplolaimus* spp. was the only genus with differences among labs using elutriation with modified sugar floatation method. This may be due to its biology as a migratory endoparasite which is not well studied in turfgrass systems, particularly in relation to its movement in and out of the root based on biological rhythms (Settle et al., 2006; Martin, 2017). In contrast, the hand-sieving with modified sugar floatation resulted in counts for four of six genera, indicating greater variability. These findings suggest that while hand-sieving introduces more variability, semi-automatic elutriation may provide more consistent data.

To ensure reliable results, golf course superintendent should choose a single nematode assay lab to send samples. Using multiple labs can be time consuming, costly, and lead to inconsistent results. More importantly, proper sample collection plays a greater role in accuracy than lab selection. Additionally, state nematode assay labs should regularly evaluate their extraction methods to maintain efficiency and accuracy, ensuring that recommendations are supported by field trials and proven nematode management practices.

Funding information

Authors state no funding involved.

Author contributions

M. Aaron Tucker: Writing, editing, data analysis. David McCall: editing. Jonathan D. Esisenback: data collection, writing, and editing.

Conflict of interest statement

Authors state no conflict of interest.

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