OTRF Project No.

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# OTRF Funded Research Project

☐ Interim Report ■ Final Report

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| **Title** | Plant-Parasitic Nematodes in Managed Golf Course Greens throughout Canada |
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| **Date of Submission to OTRF** | |
| **OTRF Funding Period** | January 2013 to December 2014 |

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| **Executive Summary** | Summary of the entire report that includes the intended goal, a brief summary of the results and the conclusion. Interpret the results and explain how the research and results will benefit the turfgrass industry. It is intended to encourage the reader to further examine the full project report. The executive summary should be no more than ½ page in length and written in a manner appropriate for the target audience (generally non-scientific) |


Plant-parasitic nematodes (PPN) feed on the roots of plants, reducing the uptake of nutrients and water. These pests are often found in golf greens due to several factors: a) golf green soils have good pore spacing which helps oxygen infiltration and allows for movement, b) the greens are irrigated frequently, providing adequate moisture for this aquatic organism, and c) the turfgrass is perennial which allows for constant reproduction and feeding of PPN when temperatures are above 10°C. Accurate identification and quantification of PPN populations and understanding the population dynamics are key to managing these destructive pests in golf greens.

This research was conducted to compare two nematode extraction techniques, identify the genera of PPN present on golf greens throughout Canada, determine conditions that influence PPN populations, and identify effective low-risk nematicides. Golf courses in Ontario, Quebec, Nova Scotia, New Brunswick, Alberta and British Columbia participated in a soil sampling survey to help identify the predominant genera of nematodes present. Samples were taken from courses in Ontario three times per year for two years and courses outside of Ontario had samples collected three times per year for one year. Sites were selected so that the research team could compare old (over 20 years of age) and young (under 20 years of age) greens as well as determine the regional influence on PPN populations.

Summary of results:

- Five predominant nematode genera were present in Canadian golf greens: *Meloidogyne, Heterodera*, *Tylenchorhynchus, Helicotylenchus* and *Criconemoides*, all of which were more effectively extracted using the sugar centrifugation technique.
- Approximately 25% of the sites tested had PPN levels that were at or near threshold values.

- Golf courses in coastal cities had higher populations than those that were inland and greens established over 20 years ago had more PPN than newer greens.
- Some of the variation in PPN populations could be explained by soil properties and management techniques.
- None of the nematicides tested effectively reduced nematode populations.

Understanding more about PPN population dynamics will help turfgrass managers target the management efforts more effectively.

| **Background** | Description of the rationale of the project including references to a literature review |
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Nematodes are worm-like, aquatic organisms that are ubiquitous throughout the world. They have a variety of feeding strategies which allow them to fill almost every ecological niche. They live in salt water, freshwater, in the films of water in soil, in plant tissue and even in the bodies of higher organisms (Shurtleff and Averre 2000). These unsegmented round worms belong to one of the largest phyla, Nematoda, in the kingdom Animalia and are the most abundant multicellular organisms on the planet (Schumann and D'Arcy 2010; Shurtleff and Averre 2000). Their abundance in the landscape makes them an integral part of every ecosystem as they are important in many nutrient cycling processes (Schumann and D'Arcy 2010). The majority of nematodes are microscopic but some, which infect vertebrate animals and humans, can be more than 8 m in length (Gubanov 1951). There are over 25,000 species identified worldwide, approximately 4,000 of which parasitize plants (Hugot et al. 2001).

Plant parasitic nematodes (PPN) are of particular interest because they injure economically important plants, costing approximately USD $90 billion of damage per year in the United States alone (Dong and Zhang 2006; Koenning et al. 1999). These pathogens have a wide host range, making them a problem for almost every plant grown for commercial production (Shurtleff and Averre 2000). The damage from an individual nematode is minor but as the number of nematodes increases, the extent of the damage does as well (Dropkin 1980).

Plant-parasitic nematodes are obligate, biotrophic parasites that feed on plant tissues using a mouthpiece called a stylet (Dropkin 1980). The stylet functions as a hypodermic needle that is used to suck out the contents of cells (Shurtleff and Averre 2000). Plant-parasitic nematodes are predominantly found near the root zone of plants in the top 10-15 cm of soil, feeding on the tissues of host plants (Miller 1978). The body structure of nematodes is simple: a set of tubes is enclosed in a long, slender body that contains a nervous system, digestive system, muscular system, secretory-excretory system, and reproductive organs (Dropkin 1980). Nematodes are so small that respiratory or circulatory systems are not needed because gases, nutrients, and excrement can move around the bodies through simple diffusion (Dropkin 1980; Shurtleff and Averre 2000). The bodies are usually transparent with the exception of the digestive tract which is opaque when full.

There are two feeding strategies which PPN can adopt: ectoparasitism and endoparasitism (Shurtleff and Averre 2000). Ectoparasites feed from the outside of the plant, thrusting the long stylet into a plant cell without entering the plant tissue. Endoparasites feed from inside the plant and their entire body is within the plant for part of their lifecycle. Because of this, they often have a shorter stylet than ectoparasites. Semi-endoparasites enter the plant tissue with just the head. PPN can also be categorized based on motility (Schumann and D'Arcy 2010). Migratory PPN move around in the soil and host, feeding on multiple plants or multiple sites of the same plant. Sedentary PPN feed in the same location, sometimes creating specialized feeding sites.

Common sedentary ectoparasitic nematodes are: ring (*Criconemoides* spp.) and pin (*Paratylenchus* spp.) (Shurtleff and Averre 2000). Common migratory ectoparasites are: sting (*Belonolaimus* spp.), dagger (*Xiphinema* spp.), needle (*Longidorus* spp.), and stubby root nematodes (*Paratrichodorus* and *Trichodorus* spp.) (Shurtleff and Averre 2000). The common sedentary semi-endoparasites are: reniform (*Rotylenchulus* spp.) and citrus nematodes (*Tylenchulus* spp.) (Shurtleff

and Averre 2000). The common migratory semi-endoparasites are: spiral (*Helicotylenchus* spp.), lance (*Hoplolaimus* spp.), and stunt nematodes (*Tylenchorhynchus* spp.) (Shurtleff and Averre 2000). The sedentary endoparasitic nematodes often get more attention as they frequently cause extensive damage due to their high reproductive rates and large host range. The common sedentary endoparasites are: potato cyst (*Globodera* spp.), cyst (*Heterodera* spp.), and root-knot nematodes (*Meloidogyne* spp.) (Shurtleff and Averre 2000). Finally, the common migratory endoparasitic nematodes are: foliar (*Aphelenchoides* spp.), pine wilt (*Bursaphelenchus* spp.), lesion (*Pratylenchus* spp.) and bulb and stem (*Ditylenchus* spp.) (Shurtleff and Averre 2000).

Plant-parasitic nematodes cause disease through three mechanisms: a) mechanical injury to the cells and tissue through use of the stylet to puncture the tissues, b) interference with the plant's physiology through secretions that contain enzymes and other toxic substances, and c) disruption of the host's vascular tissues through the formation of specialized feeding structures known as giant cells and syncytia (Dropkin 1969; Jones 1981; Schumann and D'Arcy 2010). These forms of damage leave behind non-functioning cells that do not have the ability to transport nutrients and water to the upper parts of the plant, resulting in reduced fitness and sometimes death.

The symptoms of PPN feeding observed in plants are difficult to diagnose as they can often be attributed to the influence of other pathogens or abiotic issues. The aboveground symptoms include stunting, unthrifty growth, chlorosis, early decline, wilting, and symptoms associated with nutrient and water deficiency (Shurtleff and Averre 2000). The belowground symptoms associated with the feeding of the PPN are excessive root branching, suppression of root growth, root pruning, and lesions, galls and rots on the roots (Shurtleff and Averre 2000).

PPN damage roots but also affect the metabolic functions of the whole plant. Root damage results in inhibition in nutrient and water uptake which makes the plant more susceptible to abiotic stresses such as drought and nutrient deficiency (Dropkin 1980). There is a clear correlation between nematode damage and the invasion of other plant pathogens (Fushtey and McElroy 1977; Yu et al. 1998). The openings made by PPN allow for other pathogens to enter, causing secondary illnesses in the plant (Dropkin 1980).

Nematodes can only move approximately 1 m in the soil per year (McCarty 2001). Due to limited ability to travel long distances in the soil, nematode damage in a field is patchy and lacks the sharp boundaries that symptoms from other pathogens may exhibit. They rely on surface water runoff, irrigation water, and soil clinging to equipment or to transplanted plants to move long distances (McCarty 2001).

Plant parasitic nematodes are common in many types of cultivated crops but can be a major issue in managed turfgrass. Both esthetics and functionality are important in turfgrass stands and although the feeding of PPN may not kill the afflicted plant it can reduce the quality and resiliency of the plant.

Turfgrass is grown throughout Canada for both esthetic and functional purposes. Turfgrass is an essential part of Ontario's economy, contributing $2.6 billion dollars annually in gross revenue (Tsiplova et al. 2008). The turfgrass industry employs over 30,000 people in the sectors of lawn care, sod production, golf course management, athletic field maintenance, and other areas of turfgrass management (Tsiplova et al. 2008). This revenue stimulates the Canadian economy and provides jobs for professionals as well as people looking for transitional, seasonal jobs. The industry also provides important environmental benefits.

Ground cover plants, such as turfgrass, planted as home lawns, at the side of roads, or in parks, help to reduce soil erosion by anchoring the soil with their roots (Gyssels et al. 2005). Water from precipitation percolates though the soil profile rather than running off, as it does on hard surfaces like cement. This process helps to purify water before it reaches streams and rivers (Beard and Green 1994). Using grass as a ground cover rather than rocks or cement can help to control the local climate by absorbing heat, rather than reflecting it. It can also trap dust particles in the air and sequester carbon dioxide, improving air quality (Beard and Green 1994). These qualities illustrate why grasses are the primary ground cover in urbanized areas.

Not only is turfgrass important for the environment, it is integral for exercise. Turfgrass is

an essential part of recreation in Canada, providing playing surfaces for many different sports (Crow 2005a). Any field sports like soccer, football, lacrosse, and even golf, require healthy turf that is free of inconsistencies, to prevent injuries and to improve conditions for playing. Maintaining a healthy stand of turfgrass can be difficult when there are pressures from constant use, diseases, weeds, insects, and adverse environmental conditions.

The losses of golf greens to nematode damage cannot be measured the same way that yield losses are measured for an agricultural crop, which makes quantifying the amount of damage difficult. Koenning et al. (1999), determined that in the United States in 1994 alone, over 100,000 hectares were lost to PPN damage. Golf course greens are an ideal habitat for PPN as many of them are constructed on a sand-based rootzone. The sandy soil is well aerated, the grass is watered frequently, and it is a perennial system meaning there is a host constantly present. When a green is established it is sometimes done with sod, and PPN can be transferred from the sod site, infesting the newly planted site. In Ontario there are 806 golf courses (as of 2007), many of which deal with nematodes (Simard et al. 2008; Tsiplova et al. 2008; Yu et al. 1998).

Current management methods for PPN in most crops focus on crop rotation, host-plant resistance, and the use of nematicides (Dropkin 1980). Crop rotation is not an option in turfgrass systems and host-plant resistance is not available in the common cultivars of turfgrass grown on golf courses. The application of nematicides is the only viable solution to reduce populations of PPN but the only registered products are labeled for pre-plant use and most of these are phytotoxic. In Canada there are currently no post-plant nematicidal products registered for use on turfgrass, making these pests particularly difficult to manage once they have become established.

| **Objectives** | Using the outline of expected deliverables from the project proposal indicate the completion or progress from each objective and milestone. Also indicate if objectives or milestones were revised and the reason for revision. |
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The objectives of this project were

a) to identify the best method for extracting PPN from golf course soil,

b) to understand the population dynamics of PPN found in golf course soils throughout Canada,

c) to determine which soil properties and golf course management techniques impact nematode populations, and

d) to determine the efficacy of five low-risk nematicides.

| **Methods & Results** | Include as much of the methodology and results to fully explain the goals and objectives of the project. Graphs, pictures, tables, etc are encouraged to easily relay the study's findings. |
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**Methods Comparison**

Soil samples were collected from four golf courses around Toronto and Guelph, Ontario. Two courses were sampled June 24th, 2014 and the other two were sampled on September 23rd, 2014. This represented two different points in the season to ensure that the seasonal variation in the nematode populations was accounted for. The courses were also chosen based on their previously estimated nematode populations so that the effect of population size on nematodes extracted from soil could be determined.

Three greens at each site were sampled using a soil probe with an internal diameter of 16 mm and taken to a depth of 10 cm. The soil probe was used to collect approximately 30 soil cores from each green in a grid pattern that represented the entire surface. When the plug was removed the hole was filled with sand provided by the golf course and the turf (specifically, the top 2 cm that contained the turf and thatch layer) was carefully replaced to minimize the damage to the greens.

All soil cores were placed into a plastic freezer bag and mixed together to create a composite sample for each green. Once collected, the samples were placed in a cooler with ice and transported back to the University of Guelph. Soil collected for the sugar centrifugal flotation nematode extraction that was not extracted on the day it was collected was placed in a refrigerator at 4°C for no more than 3 weeks to reduce nematode death and reproduction. The Baermann pan nematode extractions were set up on the day the soil was collected. These methods are common practice for diagnosing nematode presence in turf (Shurtleff and Averre 2000).

Soil Sample Preparation

Composite samples representing each green were placed on a sieve with a 6 mm pore size that had brown kraft paper underneath and the soil was pressed through to minimize clumps. Once soil was homogenized the paper was used to mix the samples by picking up the edges and rolling the soil on it. The soil was mixed in this fashion for approximately 30 seconds. The mixed, composite sample was then separated into six 50-cc subsamples in order to obtain a representative but small portion of soil. Three greens at each of the four golf courses were sampled and the soil from each green was subsampled three times for a total of 36 samples per extraction technique.

Baermann Pan Extraction Method

Six 1-ply Kleenex® tissues were stacked and staggered to make the receptacle for the soil. Plastic screening was cut to fit a petri dish (150 mm diameter) and placed in the bottom to increase the structural integrity of the tissues and allow for space between the dish and the soil for the nematodes to move into the water. The tissues were placed on top of the screen in the dish and the soil was placed in the center. The soil was spread out to increase the surface area in contact with the water as seen in Figure Error**! No text of specified style in document.**-1. The tissues were folded in to cover the soil. Enough tap water was added to the dish to saturate the tissues and fill the remaining space between the soil and dish. The water was added carefully so as not to disturb the tissues or damage them. Lids were placed on the dishes and labeled.

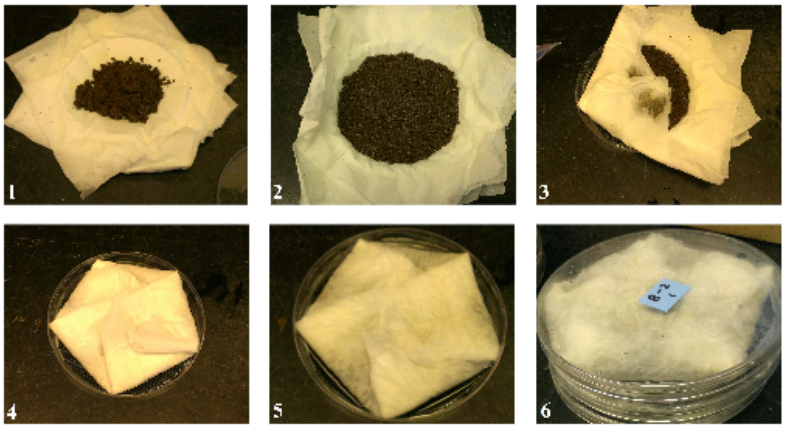


Figure **Error! No text of specified style in document.**-1: Nematode extraction set up using the Baermann pan method. 1) 50 cc of soil was placed in the tissue, 2) the soil was spread out and flattened, 3) the tissue corner was folded in over the soil, 4) the rest of the corners of the tissues were folded in, 5) tap water was added, 6) the lids were places on the dishes, and they were labelled and stacked.

Baermann pan nematode extractions were left for seven days and tap water was added to the dishes as needed to ensure they stayed wet. Once the samples had incubated the tissue and screen were picked up and very lightly squeezed into the dish. The bottom of the screen was carefully rinsed into the dish and the soil and tissues discarded.

The samples were concentrated by pouring them into a disposable, Fisher 50 mL centrifuge tube using a funnel which was rinsed into the tube after the collection water was poured in. Two tubes were necessary as there was often more than 50 mL of water in the dishes. The nematodes were given 24 hours to settle to the bottom and then the top portion of the liquid was decanted. The

remaining 5 mL were poured into a 60 mm diameter counting dish which had a grid scored on the bottom. Nematodes were identified to genus and counted using an Olympus SZX12 microscope. All data were reported as number of nematodes per 100 cc soil.

Sugar Centrifugal Flotation Extraction Method

The soil was prepared as described above and a 50 cc subsample was placed on a #40 sieve (0.42 mm pore size) and washed thoroughly with tap water at medium pressure over a 9 L bucket until the water reached the 3 L line in the bucket. The water and soil in the bucket were given 30 seconds to settle then poured through a #400 sieve (0.037 mm pore size), taking care not to include sediment. The sieve was agitated as the water was poured through to ensure there was no overflow. The sieve was rinsed again with water at low pressure or with a wash bottle and then poured into a round bottom 50 mL centrifuge tube. Two tubes were sometimes required depending on the amount of soil and water that were present.

Tubes were balanced to within 0.2 g of each other and spun at 3400 rpm for 6 minutes in a centrifuge (Thermo Scientific™ Sorvall™ Legend™ RT Plus Centrifuge). Once the cycle was complete the supernatant was decanted and the heavy sugar solution (454 g sugar/L water) was added. The following steps were done as quickly as possible (within approximately 15 minutes) as the osmotic pressure of the sugar solution can cause the nematodes to implode. Once the sugar solution was added a scoopula was used to break up the soil pellet to ensure the nematodes were in solution. The tubes were again balanced to within 0.2 g of each other and the samples were spun for 1 minute at 3400 rpm.

When the cycle was complete the samples were removed from the centrifuge and the supernatant was poured into a #500 sieve (0.025 mm pore size) without disturbing the soil pellet at the bottom. The sieve was rinsed thoroughly (approximately 1 min) at low water pressure to remove the sugar solution from the nematodes. The nematodes were rinsed into a counting dish (60 mm diameter) that had a scored grid on it with as little water as possible. Nematodes were identified to genus and counted using the microscope described above and reported as nematodes per 100 cc soil.

Results

More total PPN were extracted from the soil using the sugar centrifugal flotation method than with the Baermann pan method ($p < 0.0001$). There was no significant difference between the extraction methods for free-living nematodes ($p < 0.0001$, Table Error! No text of specified style in document.-1). All genera of PPN were extracted in significantly higher numbers by the sugar centrifugal flotation method than the Baermann pan method, and this was also the case when the PPN were divided into endoparasitic nematodes and a second group of semi-endoparasitic nematodes plus ectoparasitic nematodes (**Error! Reference source not found.**). No ring nematodes were extracted using the Baermann pan method.

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-1: LEAST SQUARED MEANS (LS MEANS) OF NEMATODES EXTRACTED FROM SOIL SAMPLES BY BAERMANN PAN (BP) AND SUGAR CENTRIFUGAL FLOTATION (SCF) METHODS.

| Nematode Genus | Extraction Method | LS Mean (nematodes/100 cc soil) |
|---|---|---|
| Cyst | BP | 2 b[1] ± 4.92[2] |
| | SCF | 46 a ± 4.92 |
| Root-knot | BP | 18 b ± 4.20 |
| | SCF | 59 a ± 4.20 |
| Ring | BP | 0 b ± 9.63 |
| | SCF | 135 a ± 9.63 |
| Spiral | BP | 23 b ± 15.84 |

| | | |
|---|---|---|
| Stunt | SCF | 106 a ± 15.84 |
| | BP | 57 b ± 105.52 |
| | SCF | 1031 a ± 105.52 |
| Free Living | BP | 383 a ± 21.59 |
| | SCF | 409 a ± 21.59 |

[1] Extraction method pairs for each nematode genus followed by the same letter are not significantly different P< 0,05, Tukey-Kramer's adjustment. Samples from all sites and dates were used in this analysis (n=72).
[2] Value after the ± is the standard error of the mean

**Canadian Plant-Parasitic Nematode Survey**

Regions were selected within Ontario which represented four different climates. The regions samples were: London/Windsor, the greater Toronto area (GTA) and Guelph, the Niagara region, and Ottawa/Cornwall (Figure Error! No text of specified style in document.-2). The Ottawa/Cornwall area is further north and is not as close to the Great Lakes as the other regions, which results in a cooler climate. Toronto/Guelph and Niagara are on the edge of Lake Ontario while London and Windsor are closer to Lake Erie and Lake Huron. These large bodies of water affect the temperature and precipitation in these areas. Golf course managers within each region in Southern Ontario were contacted in 2013 to request their participation in the survey. Six golf courses were chosen in each region, three old and three young, for a total of 24 courses. An additional course was added in the Toronto/GTA region due to the turfgrass manager's interest in the project.

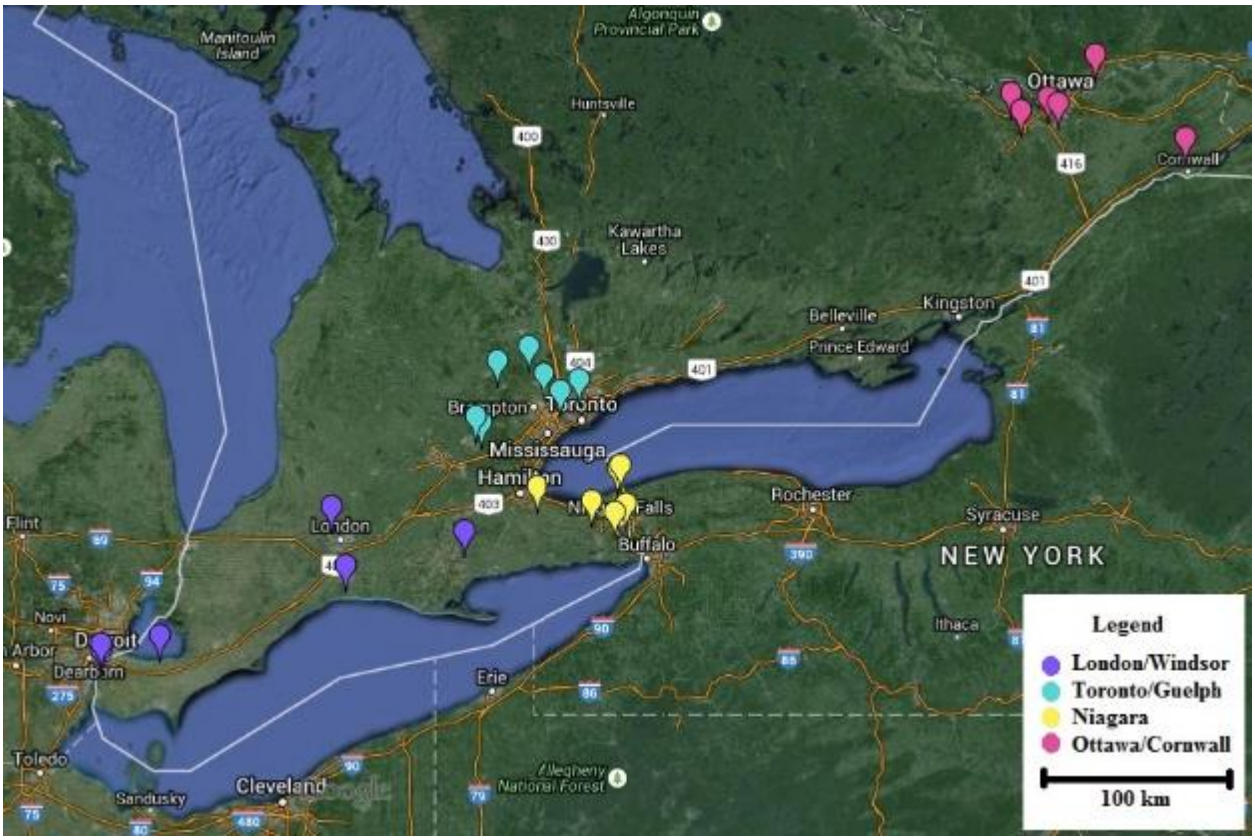


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-2: MAP OF SITES PARTICIPATING IN THE SURVEY IN ONTARIO. CREATED USING GOOGLE EARTH™.

Three greens were selected by the superintendents on each course for sampling based on one or some of the following characteristics: known issues with nematodes, ongoing issues with

disease management, drainage problems, excess shading, and poor turf quality. These characteristics were chosen because they are often linked to moderate to high levels of PPN (Crow 2001).

Soil samples were collected as outlined in Chapter 3 in May, July and September of 2013. Golf courses within a region were sampled on the same day to minimize variability in sampling conditions. All courses were sampled over a two-week span during each sampling period. Soil samples awaiting extraction were stored for no longer than three weeks in a large walk-in refrigerator at 4˚C as described in Chapter 3.

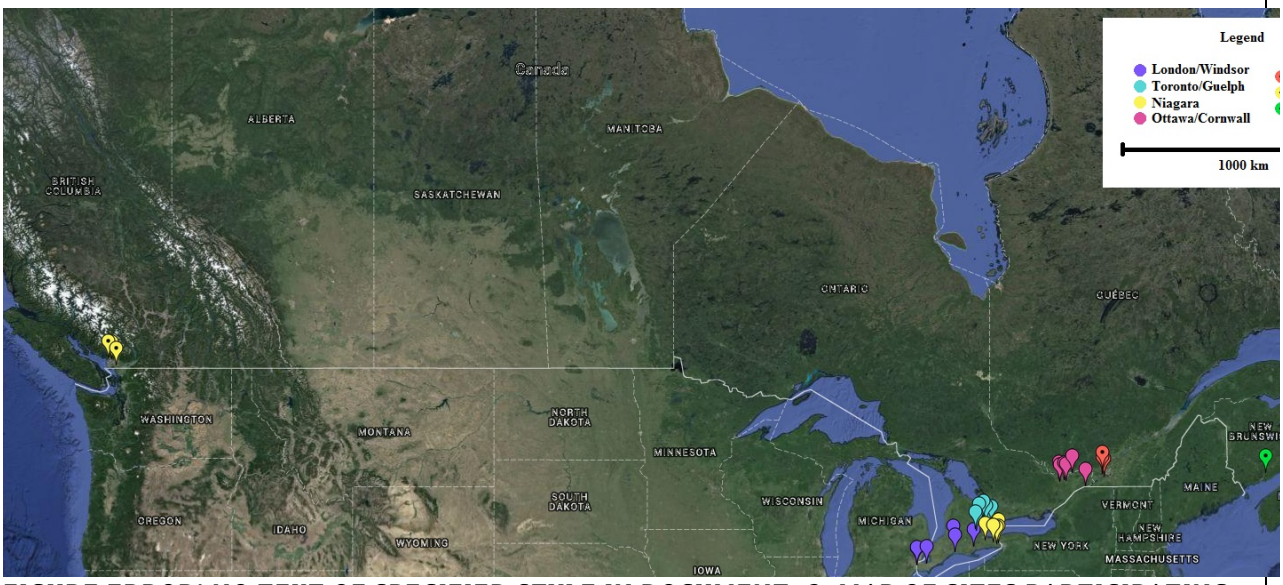


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-3: MAP OF SITES PARTICIPATING IN THE SURVEY ACROSS CANADA. CREATED USING GOOGLE EARTH™.

In February 2014, superintendents of golf courses from, British Columbia, Alberta, Quebec, Nova Scotia, and New Brunswick were contacted. Confirmation was received from three courses in British Columbia and Quebec, two courses in Nova Scotia and New Brunswick, and one course in Alberta (Figure Error! No text of specified style in document.-3). Unfortunately, one course in New Brunswick dropped out of the study after one sample collection and the course in Alberta was left out of the analysis due to the small sample size in that region (data not included). Superintendents of participating golf courses in British Columbia, Alberta, Nova Scotia, and New Brunswick were asked to send soil samples to our laboratory in May, July and September via overnight shipping. These samples were placed in a refrigerator at 4˚C while awaiting extraction to ensure nematode integrity. The research team sampled the courses in Quebec during the trip to sample courses in Ottawa and Cornwall.

The courses in Ontario were sampled in 2014 as they were in 2013 in May, July and September. All samples from across the country were collected within a two-week span for each sampling period. Soil sample preparation, nematode extraction using the sugar centrifugal flotation method, identification and quantification were conducted as outlined in Chapter 3 with two modifications. In these samples 25 cc of soil was used instead of 50 cc, and the composite samples were only subsampled once. All nematode counts were reported as nematodes per 100 cc soil.

Results

The predominant genera of PPN found in the soil samples were ring, spiral, stunt, cyst, and root-knot nematodes. Lance, lesion, and pin nematodes were found in only a small number of samples (Figure Error! No text of specified style in document.-4). The data were pooled across all three seasons to show the general distribution of the population. The sample size for Ontario was smaller in 2014

than 2013 because a site in Toronto dropped from the study due to renovation of their greens.

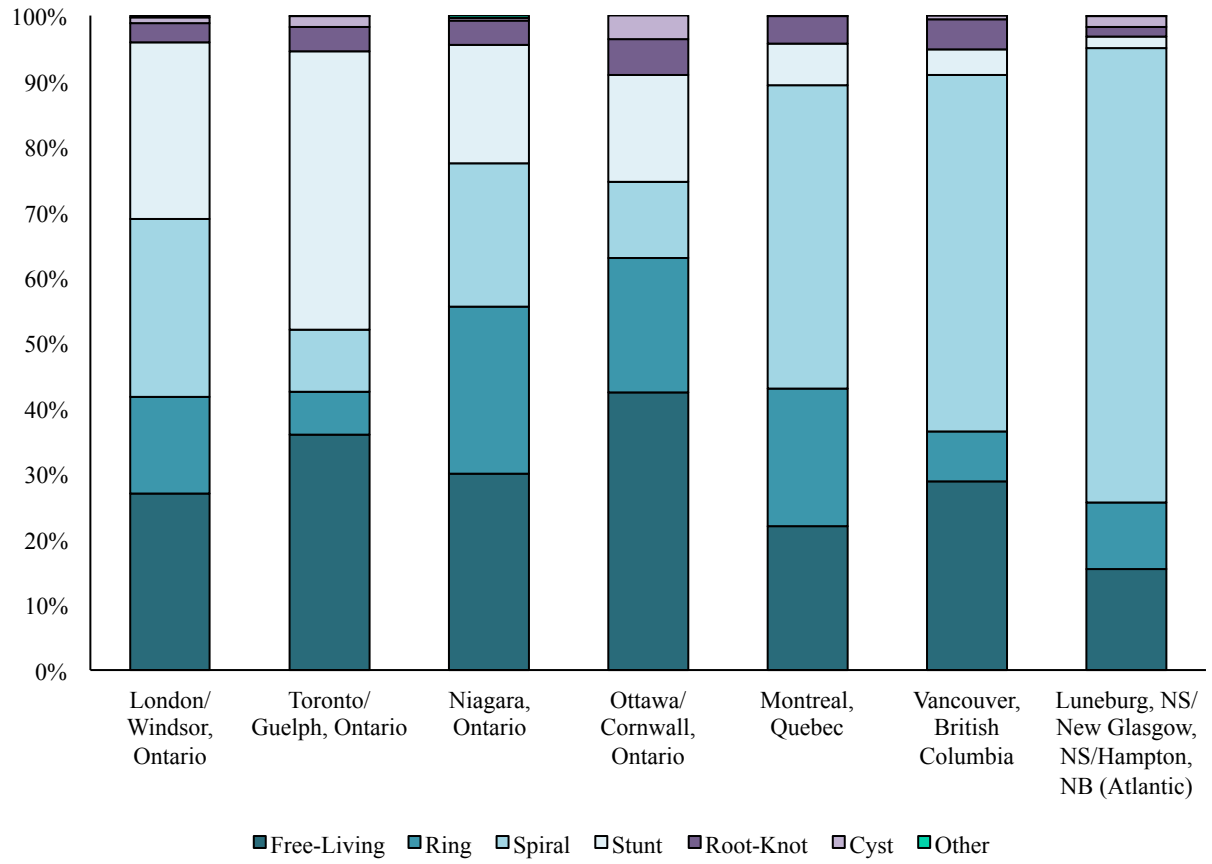


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-4: MEAN CANADIAN NEMATODE POPULATION PROPORTIONS BY CITY/REGION IN 2014 (N = 297). OTHER CATEGORY INCLUDES LANCE, LESION, AND PIN NEMATODES. NOVA SCOTIA (NS), NEW BRUNSWICK (NB).

Different genera of nematodes were found in the highest numbers in each region. In Ontario, only 1% of the greens did not have PPN over the course of the two year study. The population of each nematode genus was relatively consistent in Ontario from year to year during the survey with the exception of root-knot nematode which was found in 54% more greens in 2014 than in 2013 (Table Error**! No text of specified style in document.**-2). Stunt nematodes were the most prevalent genus of PPN followed by ring, spiral and root-knot (Figure Error! No text of specified style in document.-4). In the Montreal area of Quebec, all samples contained spiral, ring, and stunt nematodes. In the Vancouver area of British Columbia, the most prevalent genus of PPN was spiral, followed by ring, root-knot and then stunt. In the Atlantic region, spiral nematodes were found in the greatest proportion, followed by ring nematodes. Stunt, root-knot and cyst nematodes were also observed in the Atlantic provinces but in lower proportions than were seen in the other provinces. Free-living nematodes were found in all samples analyzed (Table Error**! No text of specified style in document.**-2).

Table **Error! No text of specified style in document.**-2: Percentage of golf course greens with genera of plant-parasitic nematode across Canada.

| Percentage of sites with each nematode genus across Canada | | | | | |
|---|---|---|---|---|---|
| **Province** | **Ontario** | | **Quebec** | **British Columbia** | **Atlantic[1]** |
| **Year** | 2013 | 2014 | 2014 | 2014 | 2014 |
| **Ring** | 81 | 74 | 100 | 70 | 11 |
| **Spiral** | 68 | 69 | 100 | 100 | 96 |
| **Stunt** | 87 | 91 | 100 | 93 | 74 |
| **Cyst** | 29 | 31 | 22 | 26 | 59 |
| **Root-Knot** | 18 | 72 | 85 | 93 | 56 |
| **Lance** | 21 | 11 | 0 | 4 | 4 |
| **Lesion** | 5 | 0 | 0 | 0 | 0 |
| **Pin** | 0 | 1 | 0 | 0 | 4 |
| **Free-living** | 100 | 100 | 100 | 100 | 100 |
| **Sample Size** | 225 | 216 | 27 | 27 | 27 |

[1] Atlantic region includes Nova Scotia and New Brunswick

Data were separated based on region of collection as the factors in the Ontario data set were age category of the green, sampling year, region, and season while the factors in the Canada-wide data set were city/region and season. Where interactions were observed among factors, the interactions (simple effects) are presented instead of the main effects.

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-3: PERCENTAGE OF GOLF COURSE GREENS WITH GENERA OF PLANT-PARASITIC NEMATODE ACROSS ONTARIO

| Percentage of sites tested positive for each nematode genus within Ontario | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nematode Genus** | **London/Windsor** | | **Toronto/Guelph** | | **Niagara** | | **Ottawa/Cornwall** | |
| **Year** | 2013 | 2014 | 2013 | 2014 | 2013 | 2014 | 2013 | 2014 |
| **Ring** | 87 | 89 | 65 | 50 | 81 | 74 | 94 | 81 |
| **Spiral** | 96 | 94 | 52 | 61 | 91 | 81 | 37 | 39 |
| **Stunt** | 100 | 100 | 78 | 83 | 91 | 91 | 81 | 89 |
| **Root-Knot** | 28 | 72 | 27 | 69 | 9 | 72 | 6 | 74 |
| **Cyst** | 31 | 41 | 41 | 35 | 19 | 15 | 22 | 31 |
| **Lance** | 30 | 19 | 5 | 7 | 44 | 17 | 9 | 0 |
| **Lesion** | 13 | 0 | 2 | 0 | 2 | 0 | 4 | 0 |
| **Pin** | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 0 |
| **Free-Living** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **Sample Size** | 54 | 54 | 63 | 54 | 54 | 54 | 54 | 54 |

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-4: MINIMUM (MIN), MAXIMUM (MAX), AND AVERAGE (AVE) NEMATODE COUNTS FROM GOLF COURSE GREENS BY PROVINCE. ONTARIO DATA WAS POOLED OVER BOTH YEARS.

| Nematode counts per 100 cc soil | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Province** | **Ontario** | | | **Quebec** | | | **British Columbia** | | | **Atlantic[1]** | | |
| **Statistic** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** |
| **Ring** | 0 | 2184 | 164 | 80 | 620 | 288 | 0 | 1292 | 219 | 0 | 1208 | 217 |
| **Spiral** | 0 | 2952 | 176 | 24 | 2200 | 636 | 100 | 11168 | 1563 | 0 | 11376 | 1485 |
| **Stunt** | 0 | 4752 | 239 | 4 | 376 | 87 | 0 | 640 | 112 | 0 | 172 | 37 |
| **Cyst** | 0 | 320 | 16 | 0 | 16 | 2 | 0 | 276 | 17 | 0 | 172 | 36 |
| **Root-Knot** | 0 | 616 | 23 | 0 | 204 | 57 | 0 | 400 | 131 | 0 | 220 | 33 |
| **Lance** | 0 | 120 | 4 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 8 | 0 |
| **Lesion** | 0 | 72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Pin** | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 |
| **Free-living** | 12 | 1856 | 338 | 72 | 636 | 302 | 56 | 2664 | 826 | 72 | 840 | 331 |
| **Sample Size** | | 441 | | | 27 | | | 27 | | | 27 | |

[1] Atlantic region includes Nova Scotia and New Brunswick

All Plant-Parasitic Nematodes

The factors which influenced the PPN populations as a whole in Ontario were region, age of the green, season, and the year in which the green was sampled. Within Ontario, the Ottawa/Cornwall area had the lowest number of PPN. All other areas of Ontario had average PPN counts of 600 – 800 nematodes per 100 cc soil (Table Error! No text of specified style in document.-5). Greens that were over the age of 20 years had significantly more PPN than those that were under 20 (1 LS Mean (least squared mean) of nematodes per 100 cc of soil (n = 441; P <0.0001)

[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT

[3] Value after the ± is the standard error of the mean

Table Error! No text of specified style in document.-6).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-5: TOTAL PLANT-PARASITIC NEMATODE (PPN) POPULATIONS BY REGION IN ONTARIO (2013 AND 2014 COMBINED).

| **City** | **LS Means[1]** |
|---|---|
| London/Windsor | 812 a[2] ± 60.48[3] |
| Toronto/Guelph | 674 a ± 58.51 |
| Niagara | 730 a ± 64.15 |
| Ottawa/Cornwall | 401 b ± 60.48 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P <0.0001)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT

[3] Value after the ± is the standard error of the mean

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-6: TOTAL PLANT-PARASITIC NEMATODE (PPN) POPULATIONS (NEMATODES PER 100 CC OF SOIL) BY AGE CATEGORY IN ONTARIO (2013 AND 2014 COMBINED).

| Age Category | LS Mean[1] |
|---|---|
| Old (< 20 years) | 914 a[2] ± 41.25[3] |
| Young (> 20 years) | 395 b ± 44.86 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P <0.0001)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

The number of PPN increases with age of the green until approximately 75 years of age (Figure Error**! No text of specified style in document.**-5). After 75 the PPN population levels plateau and drop off as greens reach the age of 90 years. There was only one green that was over the age of 100 while all others were between the ages of 2 years and 92 years of age.

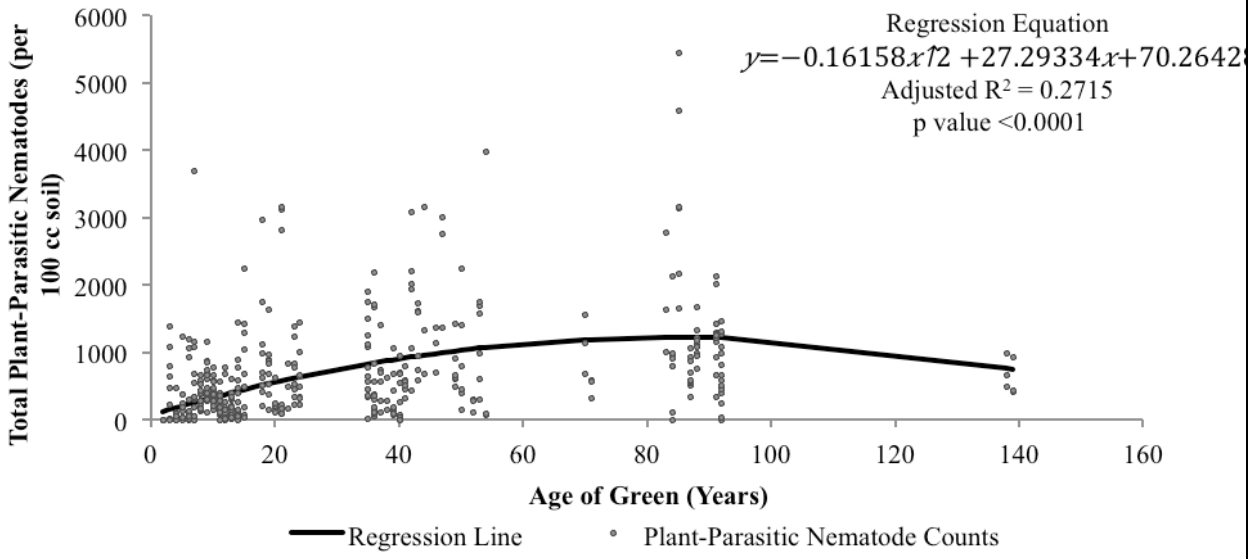


Figure **Error! No text of specified style in document.**-5: Influence of the age of the green on total plant-parasitic nematodes in Ontario golf courses. The data were pooled over both years (2013 and 2014) all seasons and regions (n = 441).

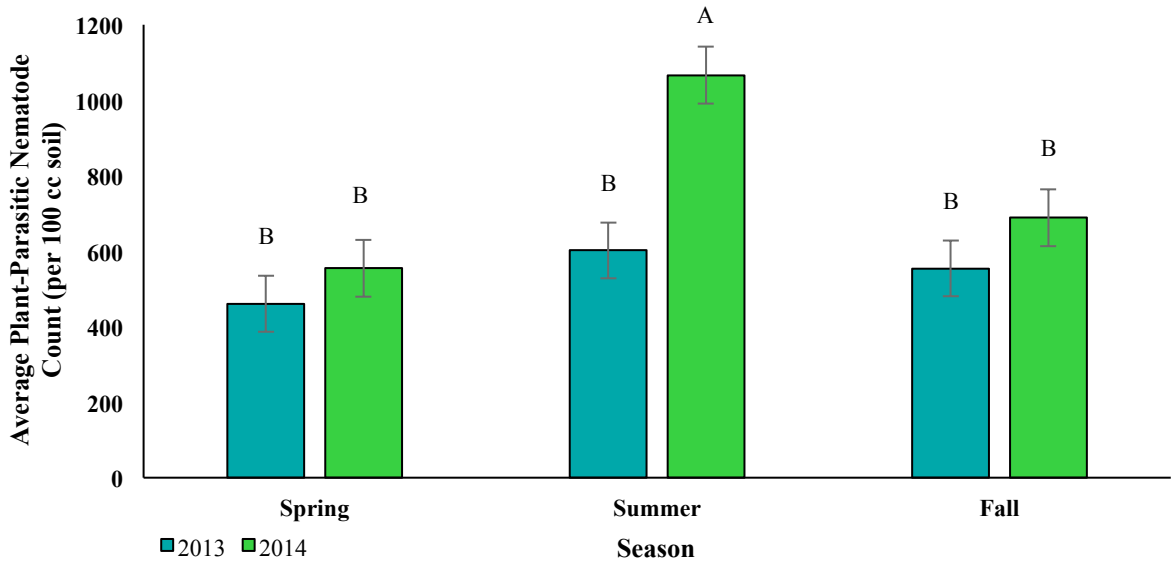


Figure **Error! No text of specified style in document.**-6: Seasonal variation of total plant-parasitic nematode populations in 2013 and 2014 in Ontario (n = 441; P = 0.0259). Bars with the same letter are not significantly different at P > 0.05, Tukey's adjustment. The error bars represent the standard error of the mean.

Both sampling year and season within each year had a significant effect on the estimated total PPN population in golf course greens in Ontario. An interaction was found between season and year due to the high nematode population found in the summer of 2014. These samples had significantly more PPN than all other time points sampled (

Figure **Error! No text of specified style in document.**-6).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-7 TOTAL PLANT-PARASITIC NEMATODE (PPN) POPULATION IN GOLF COURSE GREENS IN CANADA (2014) BY REGION.

| City | LS Mean[1] |
|---|---|
| London/Windsor, ON[2] | 921 c[3] ± 176.15[4] |
| Toronto/Guelph, ON | 839 c ± 176.15 |
| Niagara, ON | 859 c ± 176.15 |
| Ottawa/Cornwall, ON | 493 c ± 176.15 |
| Montreal, QC | 1123 bc ± 249.11 |
| Vancouver, BC | 2215 a ± 249.11 |
| Luneburg, NS/New Glasgow, NS/Hampton, NB (Atlantic) | 1886 ab ± 249.11 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P <0.0001)
[2] ON – Ontario, QC – Quebec, BC – British Columbia, NS – Nova Scotia, NB – New Brunswick
[3] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[4] VALUE AFTER THE ± IS THE STANDARD ERROR OF THE MEAN

Across Canada both region and season were significant factors influencing the total PPN population in golf course greens. The average population of PPN estimated from greens was highest in British Columbia and lowest in Ontario (Table Error! No text of specified style in document.-7). The

population of total PPN were significantly higher in the summer than in the spring, while fall was not different from either of the other two seasons (Table Error! No text of specified style in document.-8).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-8: TOTAL PLANT-PARASITIC NEMATODE (PPN) POPULATIONS BY SEASON IN CANADA (2014).

| **Season** | **LS Mean[1]** |
|---|---|
| Spring | 811 b[2] ± 137.83[3] |
| Summer | 1494 a ± 137.83 |
| Fall | 1269 ab ± 137.83 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P = 0.002)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

Ring Nematodes

In Ontario, the growing season, age of the green, and region were significant factors affecting ring nematode populations. The populations of ring nematodes were lowest in the spring and higher but similar in the summer and fall (Table Error! No text of specified style in document.-9).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-9: RING NEMATODE POPULATIONS BY SEASON IN ONTARIO (2013 AND 2014 COMBINED).

| **Season** | **LS Mean[1]** |
|---|---|
| Spring | 101 b[2] ± 18.81[3] |
| Summer | 178 a ± 18.81 |
| Fall | 172 a ± 18.81 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P = 0.0062)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

There was a significant region by green age interaction for ring nematode. Populations of ring nematodes did not differ between old and young greens except in Niagara, where the old greens had very high populations (Figure Error! No text of specified style in document.-7). There were no differences among the other regions between old and young greens.

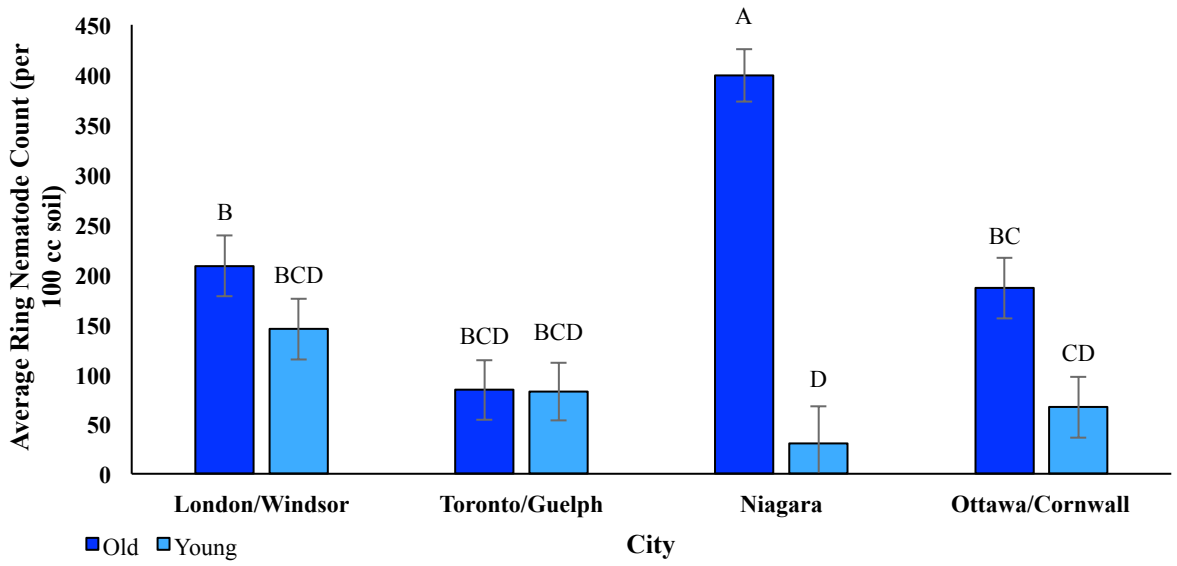


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-7: RING NEMATODE POPULATION BY REGION ON OLD (OVER 20 YEARS OF AGE) AND YOUNG (UNDER 20 YEARS OF AGE) GREENS IN ONTARIO (N = 441; P <0.0001). BARS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT P > 0.05, TUKEY'S ADJUSTMENT. THE ERROR BARS REPRESENT THE STANDARD ERROR OF THE MEAN.

Canada-wide, there were differences among the populations of ring nematodes. Montreal and the Niagara region had higher mean populations than Toronto/Guelph region. Ring nematode populations, on average, were much lower than the threshold of 1500 nematodes per 100 cc soil for primary damage as determined by the University of Massachusetts (Wick 2012).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-10: RING NEMATODE POPULATION IN CANADA (2014) BY REGION.

| City/Region | LS Mean[1] |
|---|---|
| London/Windsor, ON[2] | 175 ab[3] ± 37.44[4] |
| Toronto/Guelph, ON | 74 b ± 37.44 |
| Niagara, ON | 292 a ± 37.44 |
| Ottawa/Cornwall, ON | 152 ab ± 37.44 |
| Montreal, QC | 288 a ± 52.95 |
| Vancouver, BC | 219 ab ± 52.95 |
| Luneburg, NS/New Glasgow, NS/Hampton, NB (Atlantic) | 217 ab ± 52.95 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P = 0.0015)
[2] ON – Ontario, QC – Quebec, BC – British Columbia, NS – Nova Scotia, NB – New Brunswick
[3] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[4] Value after the ± is the standard error of the mean

Spiral Nematodes

The factors that influenced the populations of spiral nematodes in Ontario were the season when the green was sampled, the age of the green, and the region in which the golf course was present. Populations were higher in the summer than in the spring. The fall population was not significantly different from the spring or summer.

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-11: SPIRAL NEMATODE POPULATIONS BY SEASON IN ONTARIO (2013 AND 2014 COMBINED).

| Season | LS Mean[1] |
|---|---|
| Spring | 123 b[2] ± 24.66[3] |
| Summer | 260 a ± 24.66 |
| Fall | 178 ab ± 24.66 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P = 0.0005)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

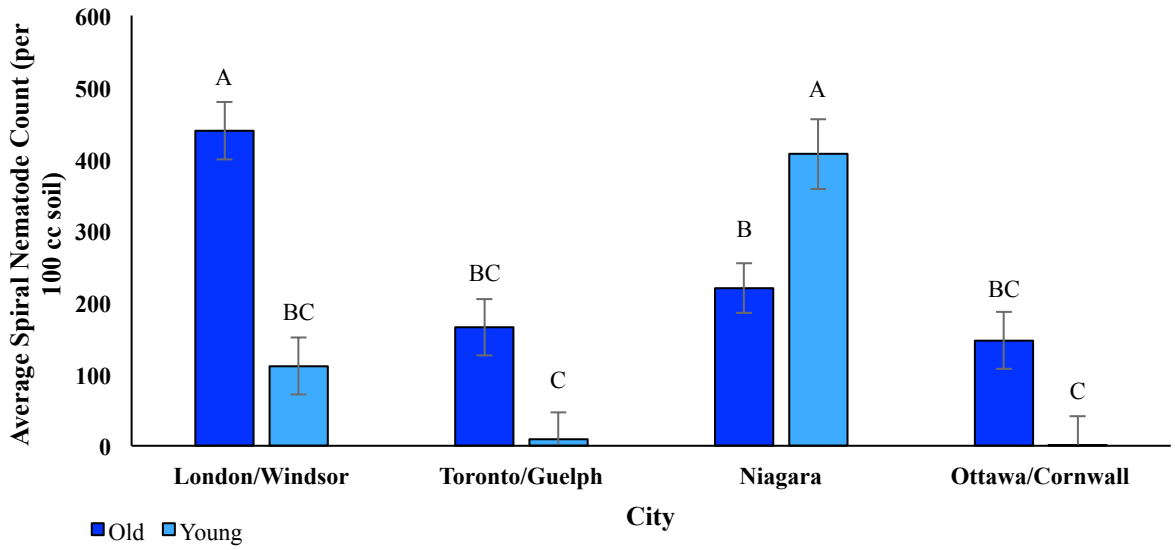


Figure **Error! No text of specified style in document.**-8: Regional variation in spiral nematode population on old (over 20 years of age) and young (under 20 years of age) greens in Ontario (n = 441; P <0.0001). Bars with the same letter are not significantly different at P > 0.05, Tukey's adjustment. The error bars represent the standard error of the mean.

There was also a signification region by green age interaction for spiral nematodes but the trend was different from that of ring nematodes. The interaction found between age of the green and region in ring nematodes was a difference of magnitude, whereas the interaction seen in spiral nematodes was one of direction. In Niagara the young greens had more spiral nematodes than the old greens did. Whereas in the London/Windsor region, the old greens had more spiral nematode that the young greens. The population of spiral nematodes in the young greens in Niagara was comparable to the populations in the old greens in London/Windsor (~ 400 per 100 cc soil) (

Figure **Error! No text of specified style in document.**-8).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-12: SPIRAL NEMATODE POPULATIONS BY SEASON IN CANADA (2014).

| Season | LS Mean[1] |
|---|---|

| | |
|---|---|
| Spring | 399 b[2,3] ± 115.80[4] |
| Summer | 772 a ± 115.80 |
| Fall | 735 a ± 115.80 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P = 0.0443)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05
[3] Data were analyzed using Student's t-test adjustment
[4] Value after the ± is the standard error of the mean

When looking at spiral nematode data across Canada, season and region were important factors influencing the spiral nematode population. Although the effect of season was significant in the ANOVA, the spiral nematode populations from season to season were not significant when the Tukey-Kramer adjustment. Hence the less conservative Student's t-test adjustment was applied to determine the means separation among means (Table Error! No text of specified style in document.-12). The populations of spiral nematodes were lower in the spring than in summer or fall (Table Error! No text of specified style in document.-12).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-13: SPIRAL NEMATODE POPULATION IN CANADA (2014) BY REGION.

| **City/Region** | **LS Mean[1]** |
|---|---|
| London/Windsor, ON[2] | 321 c[3] ± 148.00[4] |
| Toronto/Guelph, ON | 107 c ± 148.00 |
| Niagara, ON | 250 c ± 148.00 |
| Ottawa/Cornwall, ON | 87 c ± 148.00 |
| Montreal, QC | 636 bc ± 209.30 |
| Vancouver, BC | 1563 a ± 209.30 |
| Luneburg, NS/New Glasgow, NS/Hampton, NB (Atlantic) | 1485 ab ± 209.30 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P <0.0001)
[2] ON – Ontario, QC – Quebec, BC – British Columbia, NS – Nova Scotia, NB – New Brunswick
[3] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[4] Value after the ± is the standard error of the mean

Regionally, spiral nematode populations were higher in the Vancouver region than they were in all other regions except for the Atlantic provinces (Table Error! No text of specified style in document.-13). In addition, the average spiral nematode population in Vancouver exceeded the threshold of 1500 spiral nematodes per 100 cc soil set by the University of Massachusetts (Wick 2012).

Stunt Nematodes

The sampling year, age of the green and regional variation were significant factors affecting the stunt nematode population in Ontario. There were no significant interactions found among the factors for the stunt nematodes. Greens that were over the age of 20 years had more stunt nematodes than those less than 20 years of age (Table Error! No text of specified style in document.-14). The population of stunt nematodes was higher 2014 than in 2013 (Table Error! No text of specified style in document.-15).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-14: STUNT NEMATODE POPULATIONS BY AGE CATEGORY IN ONTARIO (2013 AND 2014 COMBINED).

| Age Category | LS Mean[1] |
|---|---|
| Old (< 20 years) | 349 a[2] ± 30.20[3] |
| Young (> 20 years) | 119 b ± 30.20 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P <0.0001)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-15: STUNT NEMATODE POPULATIONS BY YEAR IN ONTARIO (2013 AND 2014 COMBINED).

| Year | LS Mean[1] |
|---|---|
| 2013 | 189 b[2] ± 31.24[3] |
| 2014 | 279 a ± 31.24 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P = 0.0433)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-16: STUNT NEMATODE POPULATIONS BY REGION IN ONTARIO (2013 AND 2014 COMBINED).

| City | LS Mean[1] |
|---|---|
| London/Windsor | 292 ab[2] ± 44.27[3] |
| Toronto/Guelph | 395 a ± 42.83 |
| Niagara | 133 bc ± 46.96 |
| Ottawa/Cornwall | 116 c ± 44.27 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P <0.0001)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

Within Ontario, the Toronto/Guelph region had a higher population of stunt nematodes than the Ottawa/Cornwall or Niagara regions did but it was not significantly different than the London/Windsor region (Table Error! No text of specified style in document.-16). Nationally, region was also an important factor affecting the population of stunt nematodes (Table Error! No text of specified style in document.-17). Toronto/Guelph region had a higher average population than the Ottawa/Cornwall, Montreal, Vancouver, and the Atlantic region. None of the regions were over the threshold of 800 stunt nematodes per 100 cc soil as determined by the University of Massachusetts (Wick 2012).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-17: STUNT NEMATODE POPULATION IN CANADA (2014) BY REGION.

| City/Region | LS Mean[1] |
|---|---|
| London/Windsor, ON[2] | 319 ab[3] ± 68.67[4] |
| Toronto/Guelph, ON | 481 a ± 68.67 |
| Niagara, ON | 206 ab ± 68.67 |
| Ottawa/Cornwall, ON | 121 b ± 68.67 |
| Montreal, QC | 87 b ± 97.12 |
| Vancouver, BC | 112 b ± 97.12 |
| Luneburg, NS/New Glasgow, NS/Hampton, NB (Atlantic) | 37 b ± 97.12 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P =0.0004)
[2] ON – Ontario, QC – Quebec, BC – British Columbia, NS – Nova Scotia, NB – New Brunswick
[3] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[4] Value after the ± is the standard error of the mean

Root-Knot Nematodes

There were differences in the populations of root-knot nematode between the sampling years within Ontario. There was a three-way interaction with region, age of the green, and year in which the samples were collected for root-knot nematode populations. (Figure Error! No text of specified style in document.-9). The average root-knot nematode populations in old and young greens were not significantly different in either year. There were higher root-knot populations found in 2014 in the old greens in Toronto/Guelph and the young greens in Niagara than the populations observed in 2013.

Throughout Canada, region and season were significant factors affecting root-knot nematode populations but the population did not follow the same seasonal trend in all regions (Figure Error! No text of specified style in document.-10). The means were not significantly different from season to season in any region except Vancouver. Vancouver had higher populations of root-knot nematodes in the fall than the spring. No region in Canada exceeded the threshold of 500 root-knot nematodes per 100 cc soil as determined by the University of Massachusetts (Wick 2012).

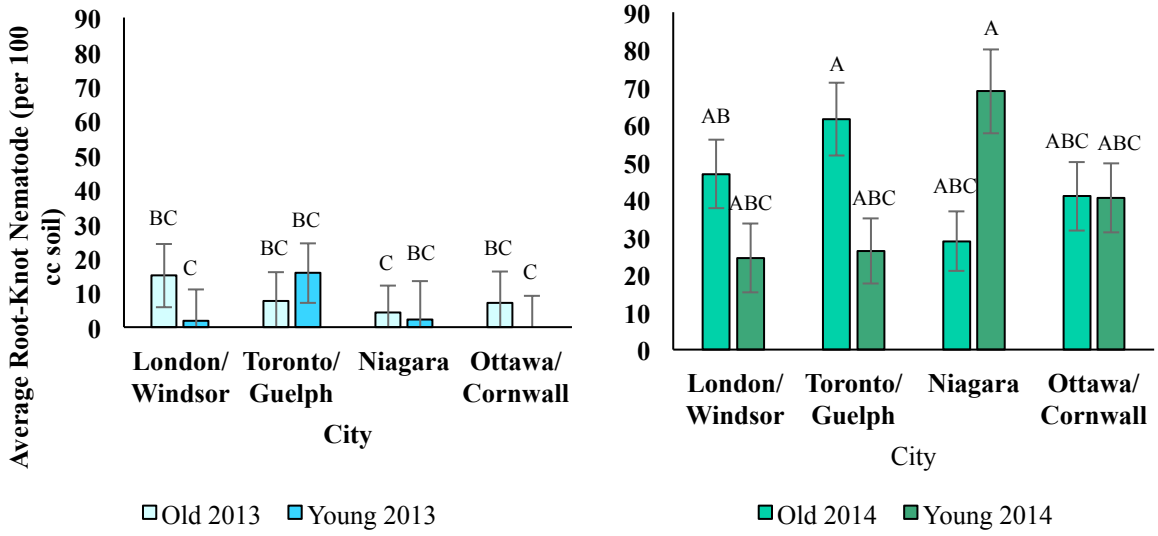


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FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-9: REGIONAL VARIATION IN

ROOT-KNOT NEMATODE POPULATIONS IN OLD (OVER 20 YEARS OF AGE) AND YOUNG (UNDER 20 YEARS OF AGE) GREENS IN ONTARIO IN 2013 AND 2014 (N = 441; P = 0.0131). BARS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT P > 0.05, TUKEY'S ADJUSTMENT. THE ERROR BARS REPRESENT THE STANDARD ERROR OF THE MEAN.

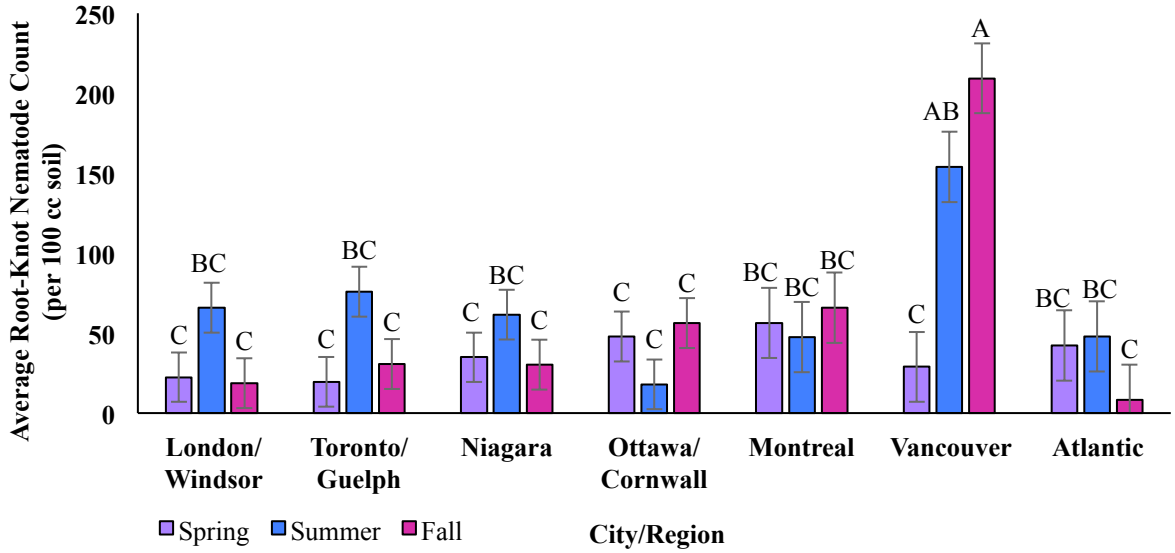


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-10: REGIONAL VARIATION IN ROOT-KNOT NEMATODE POPULATIONS THROUGHOUT CANADA IN THE SPRING, SUMMER, AND FALL OF 2014 (N = 297; P <0.0001). BARS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT P > 0.05, TUKEY'S ADJUSTMENT. THE ERROR BARS REPRESENT THE STANDARD ERROR OF THE MEAN.

Cyst Nematodes

Region and age of the green were significant factors affecting the cyst nematode population within Ontario. Both region and season interacted with the age of the green when estimating cyst nematode populations. The interaction between region and age of the green was due to the low cyst nematode population in old greens in the Niagara region, which was not significantly different from the population in young greens (Figure Error! No text of specified style in document.-11). In the Toronto/Guelph and Ottawa/Cornwall regions, old greens had significantly higher populations of cyst nematodes than the young greens. Similarly the interaction between season and age of the green that affected root-knot nematodes also affected cyst nematode populations (Figure Error! No text of specified style in document.-12). Populations of cyst nematodes in young greens were too low for there to be a significant difference between the seasons. Seasonal variation affecting cyst nematode populations was observed in the old greens but this difference was not significant.

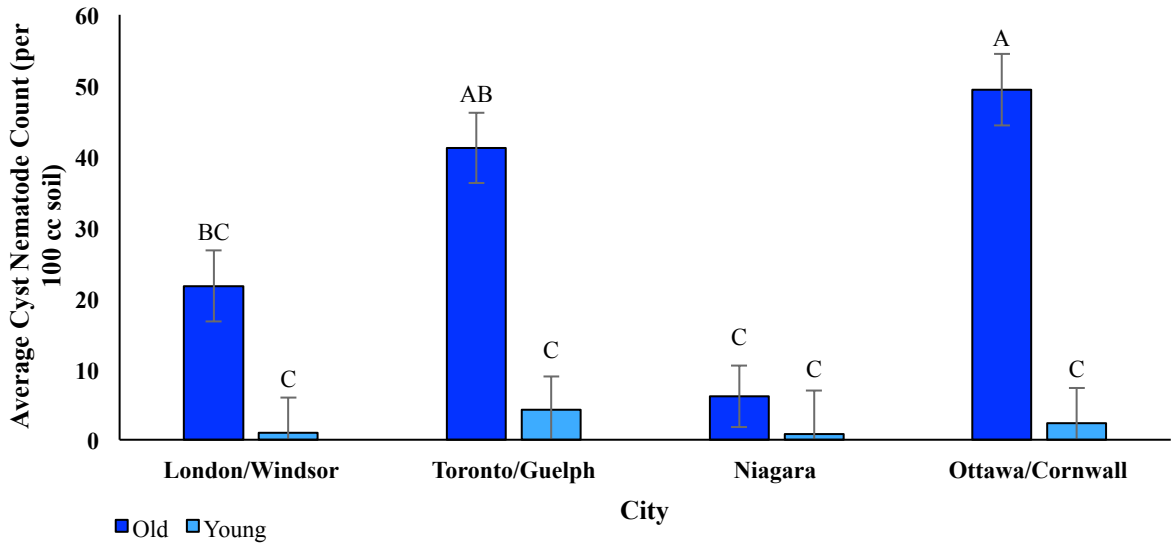


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-11: REGIONAL VARIATION IN CYST NEMATODE POPULATION ON OLD (OVER 20 YEARS OF AGE) AND YOUNG (UNDER 20 YEARS OF AGE) GREENS IN ONTARIO (N = 441; P = 0.0003). BARS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT P > 0.05, TUKEY'S ADJUSTMENT. THE ERROR BARS REPRESENT THE STANDARD ERROR OF THE MEAN.

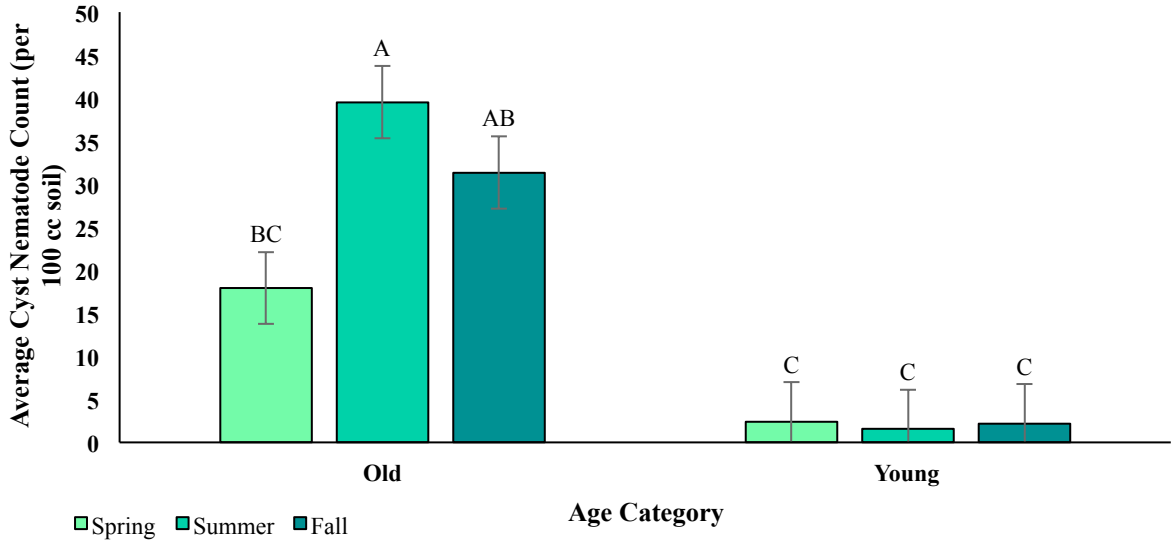


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-12: SEASONAL VARIATION IN CYST NEMATODE POPULATION ON OLD (OVER 20 YEARS OF AGE) AND YOUNG (UNDER 20 YEARS OF AGE) GREENS IN ONTARIO (N = 441; P = 0.0372). BARS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT P > 0.05, TUKEY'S ADJUSTMENT. THE ERROR BARS REPRESENT THE STANDARD ERROR OF THE MEAN.

Throughout Canada, region was an important factor affecting cyst nematode populations (Table Error! No text of specified style in document.-18). The Atlantic region had higher cyst nematode population levels than Montreal and the Niagara region but all other regions were not significantly different from each other. In all regions cyst nematode populations were below the threshold of 500 cyst nematodes per 100 cc of soil as set by the University of Massachusetts (Wick 2012).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-18: CYST NEMATODE POPULATION IN CANADA (2014) BY REGION.

| City/Region | LS Mean[1] |
|---|---|
| London/Windsor, ON[2] | 10 ab[3] ± 5.63[4] |
| Toronto/Guelph, ON | 19 ab ± 5.63 |
| Niagara, ON | 5 b ± 5.63 |
| Ottawa/Cornwall, ON | 27 ab ± 5.63 |
| Montreal, QC | 2 b ± 7.96 |
| Vancouver, BC | 17 ab ± 7.96 |
| Luneburg, NS/New Glasgow, NS/Hampton, NB (Atlantic) | 36 a ± 7.96 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 297; P = 0.0064)
[2] ON – Ontario, QC – Quebec, BC – British Columbia, NS – Nova Scotia, NB – New Brunswick
[3] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, AT P < 0.05, TUKEY-KRAMER ADJUSTMENT
[4] Value after the ± is the standard error of the mean

Free Living Nematodes

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-19: FREE-LIVING NEMATODES BY REGION, SEASON, YEAR SAMPLED, AND AGE OF GREEN IN ONTARIO.

| Region | Age Category | Season | Year | LS Mean[1] |
|---|---|---|---|---|
| **London/ Windsor** | Old | Spring | 2013 | 479 b-h[2] ± 63.60[3] |
| | | | 2014 | 381 c-h ± 63.60 |
| | | Summer | 2013 | 1088 a ± 63.60 |
| | | | 2014 | 628 bcd ± 63.60 |
| | | Fall | 2013 | 577 b-f ± 63.60 |
| | | | 2014 | 322 c-h ± 63.60 |
| | Young | Spring | 2013 | 127 h ± 63.60 |
| | | | 2014 | 158 h ± 63.60 |
| | | Summer | 2013 | 260 e-h ± 63.60 |
| | | | 2014 | 272 d-h ± 63.60 |
| | | Fall | 2013 | 155 h ± 63.60 |
| | | | 2014 | 150 h ± 63.60 |
| **Toronto/ Guelph** | Old | Spring | 2013 | 308 c-h ± 57.53 |
| | | | 2014 | 366 c-h ± 67.46 |
| | | Summer | 2013 | 553 b-h ± 57.53 |
| | | | 2014 | 621 b-e ± 67.46 |
| | | Fall | 2013 | 378 c-h ± 57.53 |
| | | | 2014 | 190 h ± 67.46 |
| | Young | Spring | 2013 | 207 h ± 60.34 |
| | | | 2014 | 301 c-h ± 60.34 |
| | | Summer | 2013 | 273 e-h ± 60.34 |
| | | | 2014 | 777 ab ± 60.34 |
| | | Fall | 2013 | 234 f-h ± 60.34 |
| | | | 2014 | 174 h ± 60.34 |
| **Niagara** | Old | Spring | 2013 | 188 h ± 55.08 |
| | | | 2014 | 196 h ± 55.08 |
| | | Summer | 2013 | 442 c-h ± 55.08 |
| | | | 2014 | 539 b-g ± 55.08 |
| | | Fall | 2013 | 294 d-h ± 55.08 |
| | | | 2014 | 257 f-g ± 55.08 |

| | | | | |
|---|---|---|---|---|
| | Young | Spring | 2013 | 278 c-h ± 77.90 |
| | | | 2014 | 203 f-h ± 77.90 |
| | | Summer | 2013 | 619 b-f ± 77.90 |
| | | | 2014 | 686 a-c ± 77.90 |
| | | Fall | 2013 | 181 f-h ± 77.90 |
| | | | 2014 | 207 f-h ± 77.90 |
| **Ottawa/ Cornwall** | Old | Spring | 2013 | 188 h ± 63.60 |
| | | | 2014 | 354 c-h ± 63.60 |
| | | Summer | 2013 | 251 e-h ± 63.60 |
| | | | 2014 | 343 c-h ± 63.60 |
| | | Fall | 2013 | 178 h ± 63.60 |
| | | | 2014 | 214 gh ± 63.60 |
| | Young | Spring | 2013 | 140 h ± 63.60 |
| | | | 2014 | 306 c-h ± 63.60 |
| | | Summer | 2013 | 346 c-h ± 63.60 |
| | | | 2014 | 335 c-h ± 63.60 |
| | | Fall | 2013 | 227 f-h ± 63.60 |
| | | | 2014 | 336 c-h ± 63.60 |

[1] LS MEAN (LEAST SQUARED MEAN) OF NEMATODES PER 100 CC OF SOIL (N = 441; P = 0.0018)
[2] MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, A P < 0.05, TUKEY-KRAMER ADJUSTMENT
[3] Value after the ± is the standard error of the mean

## Influence of Soil Properties and Management Practices on Plant-Parasitic Nematode Populations

Soil samples collected for the survey outlined in Chapter 4 were analyzed for physical and chemical properties. Samples were collected from every green at three time points over the growing season (spring, summer, and fall). Once the nematodes in the soil had been counted, the three composite samples were combined to reduce the impact of sample collection time on the parameters measured. The samples were mixed thoroughly as previously described prior to sub-sampling for analysis. Data on soil properties and management practices were only determined once for each green. These data were then applied to the nematode population data from all collection dates for each green.

### Soil Physical Properties

Soil samples were analyzed for the physical properties including percent organic matter, bulk density, soil texture, soil temperature, and air temperature. Soil organic matter content was outsourced to A&L Laboratories (London, Ontario, Canada) using method 13.2 of the Soil Analysis Handbook of Reference Methods (Jones Jr 1999). Soil temperature data was collected on site each time a green was sampled using a digital thermometer (Hanna checktemp HI 98501). Air temperature data was downloaded from Environment Canada's historical weather data website (Canada 2015e).

Bulk density was measured once on each green by inserting a 12.7 cm tube, with a beveled edge on one end, 10.2 cm into the soil. The tube was removed from the ground using a screw driver which was inserted through two holes at the top of the tube. The soil in the tube was pressed from the beveled end out of the tube 1-2 cm, depending on thatch level, and the turf and thatch were cut off and measured. The remaining soil was transferred to a small metal pan and placed in a drying oven at 80 °C for 48 hours. Once the soil had dried it was weighed and then discarded and the pan was reweighed. The weight of the soil and the pan were recorded and bulk density was calculated (Equation Error! No text of specified style in document.-1).

$$Bulk\ Density = \ Mass\ of\ Dry\ Soil\ (g)/\text{V}olume\ of\ Sample\ (\ cm^3)$$

EQUATION ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-1: BULK DENSITY CALCULATION (DONAHUE ET AL. 1977).

The soil texture was determined using the Retsch AS 200 sieve shaker (Haan, Germany). Moist soil samples of 500g were placed in metal pans and air-dried. Once the soil was dried it was placed into the top of the shaker apparatus and the lid was placed on top. Eight sieves and a bottom pan were used to separate the soil particles by size. The sieves used were numbers 5, 10, 18, 35, 60, 100, 200 and 270 with pore sizes of 4 mm, 2 mm, 1 mm, 500 µm, 250 µm, 150 µm, 75 µm, and 53 µm, respectively. Soil particles that were smaller than 53 µm fell into the bottom pan of the shaker apparatus. Soil samples were shaken at amplitude 40 for 30 minutes. When the cycle was complete the sieves containing soil were weighed to two decimal places. The sieves were then cleaned by tapping them over a bin and carefully brushing them with a small hand brush. Once cleaned the sieves were reweighed. Both weights were recorded and the percent gravel, sand, and clay/silt were calculated. The soil particle sizes were grouped into seven different categories (Table Error! No text of specified style in document.-20).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-20: SOIL PARTICLE SIZE CATEGORIES

| Particle Size Category | Particle Size Range | Sieves Used |
|---|---|---|
| Clay/Silt | < 53 µm | Bottom Pan |
| Very Fine Sand | 53 µm to 75 µm | 200 & 270 |
| Fine Sand | 75 µm to 150 µm | 100 |
| Medium Sand | 150 µm to 250 µm | 60 |
| Coarse Sand | 250 µm to 500 µm | 35 |
| Very Coarse Sand | 500 µm to 4 mm | 18 & 10 |
| Gravel | > 4 mm | 5 |

Soil Chemical Properties

The soil chemical analysis was outsourced to A&L Laboratories (London, Ontario, Canada). The soil chemical parameters measured were: potassium, magnesium, calcium, sodium, phosphorus, aluminum, hydrogen, pH, and cation exchange capacity (CEC). The parts per million (ppm) and base saturation were reported for potassium, magnesium, calcium, sodium, phosphorus, and aluminum. The base saturation was also reported for hydrogen. The chemical parameters were reported as ppm and base saturation because ppm refers to the amount of a compound present while base saturation refers to the amount of a compound which is bound to the soil colloid; this has some impact on the compound's availability to plants (Birch 1951).The bicarbonate phosphorus was measured using the Olsen Bicarbonate Phosphorus method. Method 7.3, Mehlich 3 Extraction was used to measure phosphorus (weak Bray), potassium, magnesium, calcium, sodium, and aluminum. Dried soils were extracted with the aforementioned method and analyzed using an inductively couple plasma optical emission spectrophotometer (Jones Jr 1999). Base saturation and CEC were calculated from the ppm values. The pH was measured by mixing soils 1:1 with deionized water and measured with a pH electrode (A&L Laboratories, personal communication)

Ten soil samples were analyzed for heavy metal content. Soil was analyzed for arsenic, cadmium, cobalt, chromium, copper, mercury, molybdenum, nickel, lead, selenium, and zinc. The analysis was outsourced to A&L Laboratories (London, Ontario, Canada). Metal concentration in soils was determined using the modified EPA 3050B and EPA 6010 methods (2012a; 2012b). Dried soils were weighed and digested at 105°C for two hours in a mixture of hydrochloric and nitric acid. The digestate was analyzed with an inductively coupled plasma optical emission spectrophotometer for all metals except mercury. The digestate was analyzed for mercury with cold vapour atomic absorption spectrophotometer. All data were reported as microgram metal per gram of soil and compared to the total PPN population of that sample.

Collection of Golf Course Management Information

Golf course manager participants were asked to fill out a questionnaire on the management practices conducted on their golf course. The 33-question document contained questions pertaining to the height of cut, type of mower used, frequency and amount of topdressing, number of rounds per year, disease issues, and other management-related topics. Of the questions asked, six of these questions were selected for analysis based on number of participants who answered and based on relevance to the study. These questions were:

1. How many rounds per year are played on the course?
2. Are tarps used on the greens?
3. What grass species are present on the green and in what proportions are they?
4. Is drainage installed in the greens?
5. What is the mid-season height of cut?
6. How frequently are the greens rolled per week?

The answers that provided a numerical response were entered into the spreadsheet as they were given. The questions that yielded a yes or no response were entered into the spreadsheet as 1 for a response of yes and 0 for a response of no.

Results

The population counts of the, stunt, spiral, ring, root-knot and cyst nematodes, plus the total PPN and free-living nematode populations were analyzed to identify relationships with the soil properties and management techniques. The minimum, maximum, and average values for each parameter are presented in Table Error! No text of specified style in document.-21,

Table Error! No text of specified style in document.-23, and Table Error! No text of specified style in document.-26. Soil chemical properties were split into three categories: total concentration in soil (ppm), percent base saturation, and heavy metals.

Soil Physical Properties

The soil physical properties that were positively correlated with total PPN populations were: percent organic matter, percent clay/silt, percent coarse sand, percent gravel, and air temperature (Table Error! No text of specified style in document.-22). The soil physical properties that were negatively correlated with total PPN population were: percent very fine sand, percent very coarse sand, and soil temperature.

Ring, cyst, and free-living nematodes had the strongest correlations with soil physical properties. Ring nematode populations were influenced by percent organic matter, percent very fine sand, percent very coarse sand, percent gravel, soil temperature, and air temperature (adjusted $R^2 = 0.20$; p value $<0.0001$) (Table 5-3). Percent organic matter, percent gravel, and soil temperature were positively correlated with ring nematode populations. Percent very fine sand, percent very coarse sand, and air temperature were negatively correlated with ring nematode populations. Cyst nematodes were positively correlated with percent very fine sand, fine sand, and coarse sand (adjusted $R^2 = 0.15$; p value $<0.0001$). Free-living nematode populations were positively correlated with percent medium sand, percent gravel, bulk density, and soil temperature (adjusted $R^2 = 0.21$; p value $<0.0001$).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-21: SOIL PHYSICAL PROPERTIES OF GOLF COURSE GREENS BY REGION OF CANADA.

| Province | Ontario[1] | | | Quebec[1] | | | British Columbia[1] | | | Atlantic[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Statistic** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** |
| **Organic Matter (%)** | 0.40 | 5.40 | 2.12 | 1.30 | 3.20 | 2.13 | 1.70 | 2.10 | 1.84 | 1.00 | 2.80 | 1.94 |
| **Clay/Silt (%)** | 0.09 | 17.14 | 1.50 | 0.24 | 0.61 | 0.41 | 0.19 | 0.47 | 0.33 | 0.33 | 1.00 | 0.60 |
| **Very Fine Sand (%)** | 1.57 | 32.09 | 6.92 | 2.21 | 6.07 | 3.51 | 0.96 | 2.16 | 0.17 | 2.58 | 3.95 | 2.99 |
| **Fine Sand (%)** | 5.18 | 65.69 | 13.97 | 8.40 | 13.11 | 10.50 | 5.05 | 8.48 | 7.04 | 6.89 | 9.16 | 7.78 |
| **Medium Sand (%)** | 12.31 | 56.59 | 36.75 | 36.11 | 45.61 | 40.52 | 38.64 | 44.07 | 41.89 | 28.86 | 34.54 | 32.20 |
| **Coarse Sand (%)** | 1.82 | 51.70 | 28.94 | 29.79 | 40.01 | 35.36 | 36.77 | 39.51 | 38.23 | 39.18 | 44.12 | 41.65 |
| **Very Coarse Sand (%)** | 1.16 | 37.29 | 11.25 | 6.95 | 12.27 | 9.42 | 6.68 | 16.29 | 10.64 | 10.54 | 17.66 | 14.08 |
| **Gravel (%)** | 0.00 | 6.69 | 0.68 | 0.09 | 0.61 | 0.28 | 0.01 | 0.41 | 0.17 | 0.23 | 2.85 | 0.69 |
| **Bulk Density (g/cm$^3$)** | 1.16 | 1.99 | 1.55 | 1.40 | 1.67 | 1.55 | N/A | N/A | N/A | N/A | N/A | N/A |
| **Soil Temperature (°C)[2]** | 7.60 | 29.70 | 17.55 | 9.80 | 23.50 | 17.85 | 9.50 | 23.00 | 16.54 | 9.30 | 23.20 | 16.86 |
| **Air Temperature (°C)[2]** | 7.30 | 24.00 | 15.92 | 12.60 | 23.60 | 18.10 | 13.80 | 20.40 | 16.56 | 7.10 | 19.80 | 12.46 |
| **Sample Size** | | 75 | | | 9 | | | 9 | | | 9 | |

[1] All sites from the province were pooled.
[2] Sample size for soil and air temperatures were 441, 27, 27, and 27 for Ontario, Quebec, British Columbia, and the Atlantic region, respectively.

Spiral, stunt, and root-knot nematodes had few correlations with soil physical properties. Spiral nematodes populations were positively correlated with percent clay/silt and percent coarse sand (adjusted $R^2 = 0.06$; p value = 0.0002) (Table 5-3). Stunt nematode populations were influenced by percent organic matter, percent medium sand, bulk density, soil temperature, and air temperature (adjusted $R^2 = 0.08$; p value = 0.0002). Percent organic matter, percent medium sand, bulk density, and air temperature were positively correlated with stunt nematode populations. Soil temperature at the time of sampling was negatively correlated with stunt nematode populations. Root-knot nematodes were negatively correlated with percent very fine sand and positively correlated with bulk density (adjusted $R^2 = 0.05$; p value = 0.0008).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-22: SLOPE COEFFICIENTS OF STEPWISE REGRESSION OF SOIL PHYSICAL PROPERTIES AND THE PREDOMINANT PLANT-PARASITIC NEMATODE (PPN) GENERA, TOTAL PPN, AND FREE-LIVING NEMATODES (N = 243). BLANK CELLS INDICATE THE PARAMETER WAS NOT SIGNIFICANT.

| Soil Physical Property | Ring | Spiral | Stunt | Cyst | Root-Knot | Total PPN | Free-living |
|---|---|---|---|---|---|---|---|
| **Organic Matter (%)** | 115.73 | | 125.02 | | | 259.10 | |
| **Clay/Silt (%)** | | 36.21 | | | | 62.20 | |
| **Very Fine Sand (%)** | -11.82 | | | 1.33 | -1.93 | -36.86 | |
| **Fine Sand (%)** | | | | 1.25 | | | |
| **Medium Sand (%)** | | | 8.26 | | | | 2.37 |
| **Coarse Sand (%)** | | 8.58 | | 2.79 | | 15.34 | |
| **Very Coarse Sand (%)** | -22.41 | | | | | -69.12 | |
| **Gravel (%)** | 86.26 | | | | | 227.11 | 27.10 |
| **Bulk Density (g/cm$^3$)** | | | 660.45 | | 52.47 | | 315.51 |
| **Soil Temperature (°C)** | 8.97 | | -26.90 | | | -28.92 | 28.24 |
| **Air Temperature (°C)** | -13.64 | | 40.62 | | | 43.27 | |
| **Adjusted $R^2$** | 0.20 | 0.06 | 0.08 | 0.15 | 0.05 | 0.14 | 0.21 |
| **Model p Value** | <0.0001 | 0.0002 | 0.0002 | <0.0001 | 0.0008 | <0.0001 | <0.0001 |

Soil Chemical Properties

Soil chemical parameters are important in nematode reproduction and feeding as they directly influence plant health (Walker et al. 2002). The total amount of plant nutrients present in the soil does not indicate how much of the compound is available to the plant while base saturation does (Donahue et al. 1977). The soil chemical properties, were reported as the concentration of the chemical in units of parts per million (ppm) (Table Error! No text of specified style in document.-24) and nutrient saturation of the soil colloids by the soil chemicals in terms of percent base saturation (Table Error! No text of specified style in document.-25). Heavy metals were reported in units of micrograms of metal per gram of soil. The total soil chemical properties that were negatively correlated with the total PPN populations were magnesium, aluminium, and pH; potassium was positively correlated with the total PPN population (adjusted $R^2 = 0.18$; p value <0.0001).

Spiral, stunt, and free-living nematodes had the strongest correlations with total soil chemical concentration parameters (Table Error! No text of specified style in document.-24). Spiral nematodes were negatively correlated with phosphorus (bicarbonate test), magnesium, and pH and positively correlated with potassium (adjusted $R^2 = 0.18$; p value <0.0001). Stunt nematodes were positively correlated with phosphorus (bicarbonate test) and sodium and negatively correlated with potassium and aluminium (adjusted $R^2 = 0.14$; p value <0.0001). Free-living nematodes were

positively correlated with potassium and negatively correlated with phosphorus (Bray), sodium, and pH (adjusted $R^2$ = 0.11; p value <0.0001).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-23: SOIL CHEMICAL PROPERTIES EXPRESSED AS PARTS PER MILLION (PPM) AND PERCENT BASE SATURATION BY REGIONS OF CANADA.

| **Province** | **Ontario** | | | **Quebec** | | | **British Columbia** | | | **Atlantic** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Statistic** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** | **Min** | **Max** | **Ave** |
| **Phosphorus Bicarb (ppm)** | 2.00 | 59.00 | 25.23 | 28.00 | 49.00 | 36.89 | 16.00 | 29.00 | 22.89 | 28.00 | 53.00 | 41.78 |
| **Phosphorus Bray (ppm)** | 1.00 | 134.00 | 53.38 | 50.00 | 120.00 | 73.89 | 25.00 | 49.00 | 38.11 | 51.00 | 134.00 | 92.11 |
| **Potassium (ppm)** | 21.00 | 123.00 | 58.38 | 27.00 | 69.00 | 42.22 | 37.00 | 91.00 | 61.11 | 27.00 | 88.00 | 52.67 |
| **Magnesium (ppm)** | 60.00 | 300.00 | 148.69 | 70.00 | 160.00 | 88.89 | 65.00 | 110.00 | 83.33 | 50.00 | 95.00 | 74.89 |
| **Calcium (ppm)** | 600.00 | 7200.00 | 3520.75 | 360.00 | 1010.00 | 624.44 | 360.00 | 480.00 | 417.78 | 270.00 | 760.00 | 448.89 |
| **Sodium (ppm)** | 11.00 | 41.00 | 19.98 | 19.00 | 35.00 | 23.56 | 9.00 | 23.00 | 13.67 | 8.00 | 20.00 | 13.33 |
| **Aluminum (ppm)** | 5.00 | 832.00 | 157.89 | 190.00 | 395.00 | 286.33 | 191.00 | 335.00 | 269.56 | 247.00 | 549.00 | 380.89 |
| **% Phosphorus Saturation** | 1.00 | 63.00 | 15.64 | 6.00 | 39.00 | 23.11 | 5.00 | 29.00 | 16.56 | 24.00 | 37.00 | 30.56 |
| **% Potassium Saturation** | 0.20 | 3.40 | 1.20 | 1.60 | 3.40 | 2.29 | 2.40 | 5.30 | 3.73 | 1.10 | 4.30 | 2.88 |
| **% Magnesium Saturation** | 2.50 | 22.50 | 9.25 | 11.30 | 21.00 | 15.50 | 14.20 | 21.20 | 16.56 | 4.00 | 19.00 | 13.61 |
| **% Calcium Saturation** | 50.40 | 96.40 | 79.31 | 45.80 | 85.50 | 63.88 | 46.00 | 52.60 | 49.80 | 13.50 | 64.00 | 48.24 |
| **% Sodium Saturation** | 0.10 | 2.50 | 0.70 | 1.50 | 2.90 | 2.16 | 1.00 | 2.20 | 1.39 | 0.70 | 2.80 | 1.29 |
| **% Aluminum Saturation** | 0.00 | 0.80 | 0.07 | 0.10 | 0.30 | 0.16 | 0.20 | 0.60 | 0.30 | 0.30 | 1.90 | 0.54 |
| **% Hydrogen Saturation** | 3.10 | 23.20 | 11.52 | 8.90 | 30.30 | 20.84 | 26.10 | 31.30 | 28.51 | 20.00 | 80.80 | 36.08 |
| **pH** | 5.60 | 7.70 | 6.93 | 6.50 | 7.40 | 6.94 | 6.10 | 6.60 | 6.44 | 5.40 | 6.70 | 6.31 |
| **CEC [1] (meq/100 g soil)** | 5.10 | 43.30 | 20.57 | 3.50 | 6.30 | 4.81 | 3.80 | 4.60 | 4.19 | 3.20 | 10.40 | 5.04 |
| **Sample Size** | | 75 | | | 9 | | | 9 | | | 9 | |

[1]CATION EXCHANGE CAPACITY (CEC)

The population numbers of ring, cyst, and root-knot nematodes had weak correlations with the total soil chemical concentration in the soil as seen in Table Error! No text of specified style in document.-24. Ring nematodes were negatively correlated with phosphorus (Bray) and positively correlated with phosphorus (bicarbonate), magnesium, and aluminum (adjusted $R^2$ = 0.06; p value

= 0.0002). Cyst nematodes were negatively correlated with magnesium, and pH (adjusted $R^2$ = 0.06; p value <0.0001). Root-knot nematodes were positively correlated with magnesium and negatively correlated with phosphorus (Bray), calcium, and pH (adjusted $R^2$ = 0.09; p value <0.0001).

The total PPN population levels were positively correlated with phosphorus base saturation and potassium base saturation and negatively correlated with magnesium base saturation, calcium base saturation, aluminum base saturation, hydrogen base saturation, and pH (adjusted $R^2$ = 0.38; p value <0.0001,Table Error! No text of specified style in document.-25). Spiral nematodes had the strongest correlation with the soil chemical parameters when expressed as percent base saturation. Spiral nematodes were positively correlated with phosphorus base saturation and potassium base saturation and negatively correlated with magnesium base saturation, calcium base saturation, aluminum base saturation, hydrogen base saturation, and pH (adjusted $R^2$ = 0.45; p value <0.0001). Free-living nematodes had the second highest correlation with the soil chemical base saturation parameters, but this was much lower than the total PPN. Free-living nematodes were positively correlated with potassium base saturation, and CEC and were negatively correlated with phosphorus base saturation, calcium base saturation, and aluminum base saturation (adjusted $R^2$ = 0.11; p value <0.0001).

Ring, stunt, root-knot, and cyst nematodes had few correlations with the soil chemical parameters when expressed as percent base saturation (Table Error! No text of specified style in document.-25). Ring nematodes were positively correlated with potassium base saturation and negatively correlated with sodium base saturation and hydrogen base saturation (adjusted $R^2$ = 0.04; p value = 0.0043). Stunt nematodes were positively correlated with calcium base saturation and sodium base saturation and negatively correlated with aluminum base saturation and pH (adjusted $R^2$ = 0.06; p value = 0.0007). Root-knot nematodes were negatively correlated with phosphorus base saturation and calcium base saturation (adjusted $R^2$ = 0.05; p value = 0.0007). Finally, cyst nematodes were positively correlated with hydrogen base saturation and negatively correlated with magnesium base saturation and CEC (adjusted $R^2$ = 0.08; p value <0.0001). There were no correlations between any of the heavy metals and total PPN populations (Appendix A).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-24: SLOPE COEFFICIENTS OF STEPWISE REGRESSION OF SOIL CHEMICAL PROPERTIES IN UNITS OF PARTS PER MILLION (PPM) AND THE PREDOMINANT PLANT-PARASITIC NEMATODE (PPN) GENERA, TOTAL PPN, AND FREE-LIVING NEMATODES (N = 297). BLANK CELLS INDICATE THE PARAMETER WAS NOT SIGNIFICANT.

| Soil Chemical Property | Ring | Spiral | Stunt | Cyst | Root-Knot | Total PPN | Free-living |
|---|---|---|---|---|---|---|---|
| **Phosphorus Bicarb[1] (ppm)** | 14.71 | -5.37 | 13.59 | | | | |
| **Phosphorus Bray[1] (ppm)** | -5.18 | | | | -0.56 | | -1.95 |
| **Potassium (ppm)** | | 14.20 | -2.17 | | | 14.52 | 1.63 |
| **Magnesium (ppm)** | 1.02 | -7.88 | | -0.12 | 0.15 | -9.36 | |
| **Calcium (ppm)** | | | | | -0.01 | | |
| **Sodium (ppm)** | | | 18.23 | | | | -10.67 |
| **Aluminum (ppm)** | 0.32 | | -0.47 | | | -1.24 | |
| **pH** | | -688.49 | | -18.66 | -32.19 | -817.59 | -178.07 |
| **CEC [2] (meq/100 g soil)** | | | | | | | |
| **Adjusted $R^2$** | 0.06 | 0.18 | 0.14 | 0.06 | 0.09 | 0.18 | 0.11 |
| **Model p Value** | 0.0002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

[1] Two different tests were used to determine the phosphorus content depending on the pH of the soil. Phosphorus bicarbonate (Bicarb) test measures readily available phosphorus for plants in soils with a pH

over 7 and phosphorus weak Bray (Bray) test measures the amount of phosphorus available to plants.
[2] Cation exchange capacity (CEC)

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-25: SLOPE COEFFICIENTS OF STEPWISE REGRESSION OF SOIL CHEMICAL PROPERTIES IN UNITS OF PERCENT BASE SATURATION AND THE PREDOMINANT PLANT-PARASITIC NEMATODE (PPN) GENERA, TOTAL PPN, AND FREE-LIVING NEMATODES (N = 237). BLANK CELLS INDICATE THE PARAMETER WAS NOT SIGNIFICANT.

| Soil Chemical Property | Ring | Spiral | Stunt | Cyst | Root-Knot | Total PPN | Free-living |
|---|---|---|---|---|---|---|---|
| **% Phosphorus Saturation** | | 13.27 | | | -0.75 | 15.40 | -3.37 |
| **% Potassium Saturation** | 79.05 | 369.11 | | | | 453.28 | 109.27 |
| **% Magnesium Saturation** | | -235.88 | | -2.35 | | -264.24 | |
| **% Calcium Saturation** | | -111.15 | 12.01 | | -1.34 | -123.51 | -5.35 |
| **% Sodium Saturation** | -75.13 | | 170.12 | | | | -99.13 |
| **% Aluminum Saturation** | | -1801.85 | -799.81 | | | -2652.28 | |
| **% Hydrogen Saturation** | -4.12 | -48.10 | | 0.59 | | -69.37 | |
| **pH** | | -502.96 | -617.36 | | | -1092.74 | |
| **CEC [1] (meq/100 g soil)** | | | | -1.25 | | | 5.18 |
| **Adjusted $R^2$** | 0.04 | 0.45 | 0.06 | 0.08 | 0.05 | 0.38 | 0.11 |
| **Model p Value** | 0.0043 | <0.0001 | 0.0007 | <0.0001 | 0.0007 | <0.0001 | <0.0001 |

[1]Cation Exchange Capacity (CEC)

Golf Green Management

Golf course management practices, as reported by the superintendents, had a stronger correlation with the nematode populations than the soil chemical and physical properties. The management practices summary table (Table Error! No text of specified style in document.-26) includes only values that had a numerical response. The management techniques that were positively correlated with the total PPN population were percent annual bluegrass (*Poa annua)* and presence of drainage (adjusted $R^2 = 0.34$; p value <0.0001).

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-26: MANAGEMENT PRACTICES SUMMARY STATISTICS FOR GOLF COURSES IN CANADA (N=153).

| Management Practice[1] | Minimum | Maximum | Average |
|---|---|---|---|
| **Rounds of Golf (per year)** | 15000 | 3160000 | 202222 |
| **Annual Bluegrass (%)** | 0 | 90 | 42.7 |
| **Creeping Bentgrass (%)** | 10 | 100 | 57.3 |
| **HOC[2] (mm)** | 2.921 | 3.556 | 3.179 |
| **Rolling Frequency (per week)** | 0 | 7 | 3 |

[1] Only the management practices with numerical responses were included in this table.
[2] Height of cut (HOC)

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-27: SLOPE COEFFICIENTS OF STEPWISE REGRESSION OF MANAGEMENT PRACTICES AND THE PREDOMINANT PLANT-PARASITIC NEMATODE (PPN) GENERA, TOTAL PPN, AND FREE-LIVING NEMATODES (N = 153). BLANK CELLS INDICATE THE PARAMETER WAS NOT SIGNIFICANT.

| Management Practice | Ring | Spiral | Stunt | Cyst | Root-Knot | Total PPN | Free-living |
|---|---|---|---|---|---|---|---|
| **Rounds of Golf (per year)** | 6.97E-5 | | | -7.45E-6 | | | |
| **Tarps (Y/N)[1]** | 104.02 | | | | | | |
| **Creeping Bentgrass (%)** | | -4.66 | | | | | |
| **Annual Bluegrass (%)** | | | 13.58 | 0.57 | 0.32 | 21.56 | |
| **Drainage (Y/N)[1]** | -49.79 | | 444.03 | 9.32 | | 299.33 | |
| **Height of Cut (mm)** | -145.84 | | | | | | -207.67 |
| **Rolling Frequency (per week)** | 46.83 | 86.18 | -91.54 | -3.03 | 4.77 | | 18.49 |
| **Adjusted $R^2$** | 0.31 | 0.14 | 0.28 | 0.37 | 0.03 | 0.34 | 0.05 |
| **Model p Value** | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0312 | <0.0001 | 0.0116 |

[1] Yes (Y)/No (N)

Populations of ring, spiral, stunt, and cyst nematodes had the strongest correlation with the management practices (Table Error! No text of specified style in document.-27). Ring nematodes were positively correlated with the rounds of golf per year, the use of tarps, and rolling frequency and were negatively correlated with the presence of drainage and the height of cut (adjusted $R^2$ = 0.31; p value <0.0001). Spiral nematodes were negatively correlated with percent creeping bentgrass (*Agrostis stolonifera* L.) and positively correlated with rolling frequency (adjusted $R^2$ = 0.14; p value <0.0001). Stunt nematodes were positively correlated with percent annual bluegrass and drainage and were negatively correlated with rolling frequency (adjusted $R^2$ = 0.28; p value <0.0001). Cyst nematodes were positively correlated with annual bluegrass and drainage and were negatively correlated with rounds per year and rolling frequency (adjusted $R^2$ = 0.37; p value <0.0001).

Root-knot and free-living nematodes had few correlations with management practices. Root-knot nematodes were positively correlated with percent annual bluegrass and rolling

frequency (adjusted $R^2$ = 0.03; p value = 0.0312). Free-living nematodes were positively correlated with rolling frequency and negatively correlated with height of cut (adjusted $R^2$ = 0.05; p value = 0.0116).

**Low-Risk Nematicide Trial**

The five research greens at the Guelph Turfgrass Institute, in Guelph, Ontario were sampled and their base nematode populations were determined. The USGA East and West greens were chosen for this experiment as the nematode population was more diverse than the other greens and the root zones were of similar construction, (Table Error! No text of specified style in document.-28). The study greens were divided into 1 $m^2$ plots with a 0.5 m buffer from the adjacent plots. The treatments were set up in a randomized complete block design with four blocks on each of the two greens.

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-28: PRELIMINARY NEMATODE POPULATIONS PER 100 CC SOIL FROM THE TEST GREENS AT THE GUELPH TURFGRASS INSTITUTE (N=5).

| Green | Ring | Spiral | Stunt | Root Knot[1] | | | Cyst[1] | | | Free-living |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | F | J | M | F | J | M | |
| USGA West | 8 | 0 | 32 | 16 | 64 | 56 | 64 | 120 | 0 | 444 |
| USGA East | 96 | 0 | 328 | 0 | 24 | 0 | 116 | 520 | 0 | 320 |
| California | 0 | 0 | 40 | 0 | 56 | 88 | 32 | 48 | 0 | 1000 |
| Native Soil | 24 | 4 | 648 | 0 | 8 | 0 | 44 | 164 | 0 | 1080 |
| Pathology | 0 | 0 | 180 | 0 | 96 | 32 | 60 | 144 | 0 | 616 |

[1] Females (F), juveniles (J), and males (M) for root-knot and cyst nematodes were counted separately.

Pre-treatment samples were taken before the products were applied to establish the baseline nematode population levels in each 1 $m^2$ plot. The treatments applied to the plots were: no treatment (negative control), oxamyl (Vydate™, positive control), chitosan (Nature's Cure™), isothiocyanate and related compounds from *Brassica junica* (Mustgro™ and Dazitol™), and abamectin $B_1$ (Avid™). Each product was applied at the Canadian label rate (Table Error! No text of specified style in document.-29). Vydate™, Nature's Cure™, Avid™, and Dazitol™ were in liquid form and applied using a custom built bicycle sprayer (Teejet 8001VS flat fan nozzles - 5 mL/second/nozzle at 20 psi). The granular product, Mustgro™, was weighed and applied by hand to the surface of the green. The products were applied from 10 am to 12 pm on August 21st, 2014; the weather at time of application was 22°C and sunny. All treatments, including the control, were watered in with overhead irrigation to a depth of 10 cm immediately after application.

TABLE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-29: NEMATICIDE ACTIVE INGREDIENTS, ACTIVE INGREDIENT CONTENT, AND APPLIED RATE.

| Nematicide Common Name | Active Ingredient | Concentration of Active Ingredient in Product | Rate Applied (kg/$m^2$) |
|---|---|---|---|
| Vydate | Oxamyl | 240 g/L | 0.00625 L/$m^2$ |

| | | | |
|---|---|---|---|
| Nature's Cure | Chitosan and yucca plant extract | 11% | 0.00023 L/m$^2$ |
| Mustgro | Isothyocyanate and related compounds from *Brassica juncea* seed meal | 100% | 0.20 kg/m$^2$ |
| Dazitol | Allyl isothyocyanate | 3.70% | 0.0058 L/m$^2$ |
| | Capsaicin and related capsaicinoids | 0.42% | |
| Avid | Abamectin $B_1$ | 19 g/L | 0.001 L/m$^2$ |

The plots were sampled one, two, and four weeks following treatment application, Five soil cores were taken from each plot during each of the four time points and the variation from the pre-treatment counts was analyzed for each time point. Soil samples were collected and stored for no more than two weeks, and nematodes were extracted and identified as stated in chapter 4.

Results

Total PPN, ring, spiral, stunt, cyst, root-knot, and free-living nematode populations in each week post-treatment were compared to the week zero counts. The nematode populations in each of the treatment plots did not differ significantly from the starting population nematode present. Only PPN and free-living nematodes are shown in the graphs (Figure Error! No text of specified style in document.-13 and Figure Error! No text of specified style in document.-14) because none of the nematicides tested significantly influenced the nematode populations.

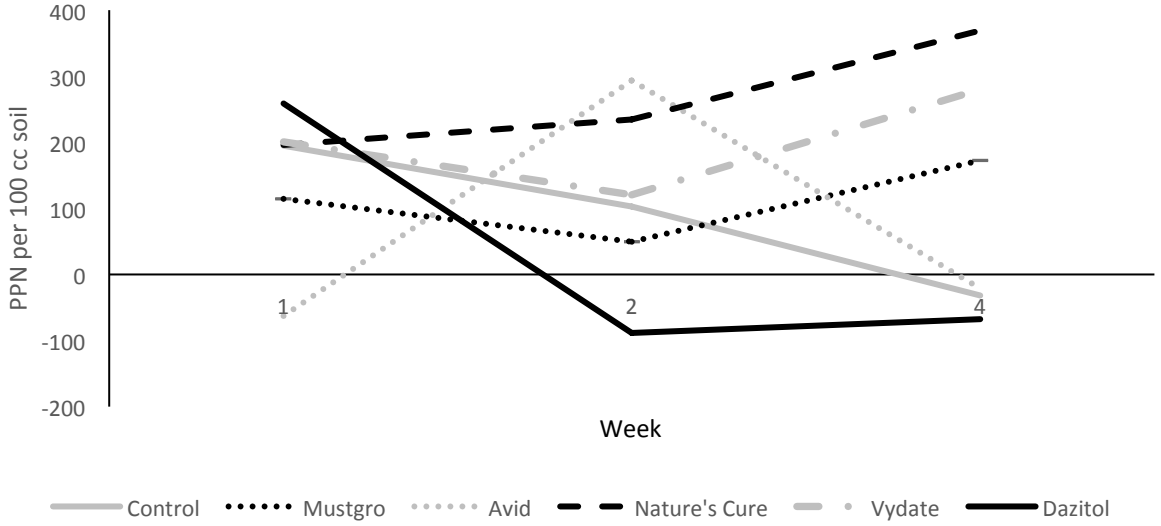


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-13: AVERAGE PPN COUNTS PER 100 CC SOIL AFTER ONE, TWO, AND FOUR WEEKS FROM TREATMENT. ZERO REPRESENTS THE MEAN PRE-TREATMENT COUNT OF PPN FOR EACH TREATMENT (N=197, ALL SAMPLE DATES).

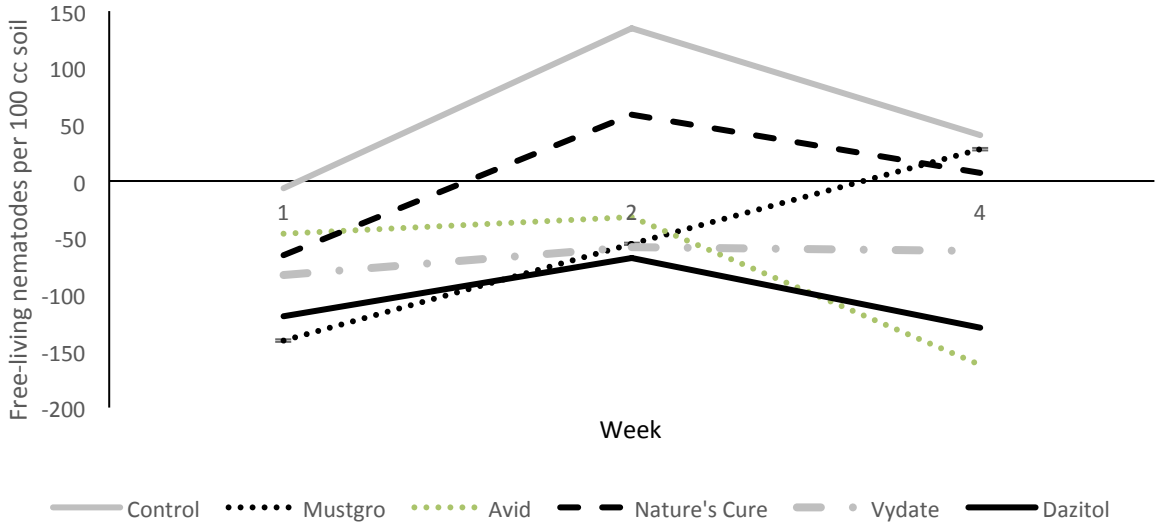


FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT.-14: AVERAGE FREE-LIVING NEMATODE COUNTS PER 100 CC SOIL AFTER ONE, TWO, AND FOUR WEEKS FROM TREATMENT. ZERO REPRESENTS THE MEAN PRE-TREATMENT COUNT OF FREE-LIVING NEMATODES FOR EACH TREATMENT (N=197, ALL SAMPLE DATES).

| **Goals for completion [Interim Report only ]** | Outline the goals and milestones left to complete the project. Will the original objectives be delivered as outlined in the project proposal? |
|---|---|
| | |

| **Conclusions [Final Report]** | Explain the key outcomes of the project, noting the success or obstacles of achieving the original objectives. Interpret the results and explain how the research and results will benefit the turfgrass industry. Recommendations for further studies may be included. |
|---|---|

The goals of this project were to determine the population dynamics of PPN in golf course greens throughout Canada, determine which soil factors influenced their populations, determine the best nematode extraction technique for golf course genera, and to test several nematicides for use on golf course green turfgrass. We designed a survey that would focus on Ontario, determining the influence of region, season, sampling year, and age of the green. In 2013 samples were collected from sites in London, Windsor, Toronto, Guelph, Niagara, Ottawa, and Cornwall, Ontario. The sites were visited in May, July, and September. In 2014 the same sites from Ontario were sampled again, in the same way. We added sites to the survey from British Columbia, Alberta, Quebec, Nova Scotia, and New Brunswick. The sites in Ontario and Quebec were sampled by our team while for the remaining sites we had the golf course superintendents mail their samples to the University of Guelph. The sites outside of Ontario were only sampled during 2014 and were not selected based on green age so the only inferences that could be made from those data were based on region and season.

The data from the survey yielded some interesting results. Plant-parasitic nematodes are an issue in certain areas of Canada but, as expected, they do not seem to be as much of a problem as they are in warmer climates. Regardless, in our study there were approximately 25% sites across Canada had populations of spiral, stunt, ring, and cyst nematodes that were over the threshold levels commonly used in Canada (Wick 2012). These sites had nematodes that were typically over the threshold for only one genus; however, close to threshold population levels of multiple PPN were not uncommon in sites that were over the threshold for one genus. In order to determine how detrimental PPN are on golf course greens in Canada, it is important to develop thresholds for each of the regions of the country. Developing thresholds that accurately represent the risk of damage from PPN populations in Canada will help turfgrass managers to understand when they have a problem and at what point they should intervene.

Sites in British Columbia and the Atlantic region had the highest counts of PPN. The air and soil temperatures remain in the hospitable range for nematode reproduction, feeding, and movement for a longer portion of the year in the coastal regions of Canada, as compared to the inland areas. Spiral nematodes were more prevalent in the coastal areas than the inland areas and stunt nematodes were more prevalent in Ontario than anywhere else in Canada. We saw a diverse population in most sites but the most abundant genera were ring, spiral, and stunt nematodes. To a smaller extent we also found cyst and root-knot nematodes and rarely found pin, lance, and lesion nematodes. Climate appeared to be a major factor in the variation in the nematode populations. The seasonal variation we saw in the nematode populations was not consistent throughout the nematode genera studied and for certain genera, such as root-knot nematode, there were interactions between seasonal variation and the region in which the samples were collected.

Data pertaining to the age of the green were only collected for sites within Ontario. An equal number of greens were selected which were over 20 years of age and less than 20 years of age. For most nematode genera, with the exception of root-knot nematodes, age of the green was an important factor in the nematode population. Older greens had larger and more diverse populations of PPN present. There are no nematicides registered in Canada for use on golf courses. This can explain why there were larger populations in older greens. Nematodes are naturally present in soil. Golf greens are an ideal habitat, and the populations can increase in an unchecked manner over the years. Fungal and bacterial antagonists may be killed by the application of fungicides and bacteriocides, Without nematicides, nematode numbers can increase until they reach the carrying capacity of the soil (Rodriguez-Kabana and Curl 1980).

Some of the nematode genera, such as free-living and root-knot nematodes, were difficult to model. The population fluctuations appeared to be influenced by factors that were outside the scope of this survey. The free-living nematodes in Ontario had a four-way interaction among year, age of the green, season, and region. There was no discernible pattern and probably other factors caused the variation in population. Root-knot nematodes were also difficult to model as the influence of the age of the green, season, and year appeared to be strongly influenced by the region in which the samples were collected from in both the Ontario data set and the Canadian data set. The factors had a different effect on the population depending on where the samples came from. However, populations of root knot nematodes were generally below the threshold at all sites.

The nematode population in Ontario increased from 2013 to 2014. This was probably caused winter weather that preceding each growing season. The winter of 2012/2013 was mild and the temperature often fluctuated above and below freezing. In contrast, the winter of 2013/2014 started earlier, lasted longer, and temperatures were well below freezing for the duration of the season. A winter season with high temperature fluctuations and with more thaw and re-freeze events could lead to greater mortality and lower populations of nematodes. This study demonstrated that the population of PPN in golf course greens can fluctuate drastically from year to year.

Soil chemical and physical properties of each participating green were measured and recorded

and the values were compared to the nematode populations for each of the golf courses sampled across Canada. All of the soil samples from a golf course over a year were combined to produce a composite sample that was sent for analysis. This was done in 2013 for the sites in Ontario and during 2014 for the sites outside of Ontario. The soil analysis results along with information gathered about the management practices used were subjected to stepwise regression to determine what influence they had on the nematode populations that were determined by the survey.

Physical soil properties have a significant impact on the water holding capacity of soils and the soil matrix structure. The physical properties of the soil were often correlated with nematode populations in golf greens in Canada. However, the correlation coefficients were low, only accounting for at most 21% of the variation in the nematode population. The same result was found with the chemical properties of the soil. Although there were relationships between the chemical properties and the nematode populations, the correlations were not very strong and the biological significance of these correlations remains unclear. Many of the factors were inter-related, making it difficult to attribute a nematode population to the factors that were measured. Additionally the composition and management of golf course greens is strictly controlled, leaving little variation between sampling locations. It is also inadvisable to change many of these management practices and soil properties to reduce nematode populations as they are managed with important goals such as plant health and drainage in mind. Designing an experiment that controls certain aspects of the environment will allow for clear linkages to be made between soil properties, management practices, and nematode populations.

The questionnaire that was developed to gather information about management techniques could have been executed better. In the future the questions should be posed with multiple choice options to increase participation and to reduce variability in responses. Although this would have taken longer to develop, the answers would have been unambiguous and subjected to less interpretation by the participants. The delivery of the questionnaire was also flawed as many participants were reluctant to release their information in an unsecured word file. Using a protected pdf or survey platform may have alleviated some of the concerns and allowed for a higher rate of participation in the questionnaire. The management techniques had stronger correlations with the nematode populations than the soil physical or chemical properties. The questions we posed focused on factors that may affect the water holding capacity of the soil and the amount of compaction seen in the soil profile as these factors are strongly linked to nematode populations.

In previous studies of PPN in golf course greens in Canada, the nematode extraction technique used was likely not suited to the types of nematodes most prevalent in golf course greens soils (Barker et al. 1969). It was important to determine the best extraction technique for the genera that were found in golf course greens. This was a vital aspect of the project as it will help to unify the community of nematologists on which extraction technique is ideal for golf course genera. We found that the sugar centrifugal flotation method was better at extracting PPN from golf course soils. The Baermann pan method extracted fewer PPN but performed the same as the sugar centrifugal flotation method for free-living nematodes. This was expected because of the combination of mobile and immobile nematodes found in golf course greens in Canada. Literature on the topic suggests that ring nematodes cannot be adequately quantified when the Baermann pan extraction technique is used to remove them from the soil (Mai and Mullin 1996). This was consistent with our study.

A nematicide trial was conducted to identify low-risk alternatives to some of the broad spectrum nematicides used in the past. The carbamate nematicide, Vydate™ with the active ingredient oxamyl, was the positive control and a selection of low-risk products were selected for the trial: Nature's Cure™, Dazitol™, Mustgro™, Avid™. None of the products tested, even the positive control, caused a reduction in the nematode populations. This may be due to the inherent variability of nematode populations within a green. To avoid this some changes to commonly used research design should be made for future studies. Specifically, the number of replications should

increase to allow for the variation. Additionally, as little information is known about the efficacy of many of these products, additional treatments that include multiple applications should be included in the trial. The experiment was intended to be a preliminary analysis of the nematicides to identify candidates for further testing. With more replicates and an area with higher populations an effect of the treatments may have been seen.

Microorganisms and plant materials for management of PPN have been widely studied and utilized in many types of cropping systems. Modifying these practices for use in turfgrass is an area of research that needs attention since there are currently few management options available. There are a plethora of options that turfgrass managers can utilize to manage PPN in turf, but the unrealistic demands of the end users of the putting surfaces has limited the managers' ability to explore these options.

PPN are an issue for turfgrass managers regardless of their location and climate. Raising awareness of this pest may help to reduce the amount of unnecessary fertilizers and pesticides applied to golf course greens, as managers may interpret nematode damage as nutrient deficiency or pathogen infestation. In Canada, PPN populations were moderate compared to those found in the United States, especially in the southern regions of the United States but spiral, stunt, ring, cyst, and root-knot nematodes were frequently found in samples. Many golf courses in Canada had populations over published threshold levels. Over the duration of the study the PPN populations were over threshold values 63 times out of 531 samples. The seasonal variations were not consistent from year to year but the total population of PPN appears to be increasing as time passes. Populations of root-knot, cyst, and ring nematodes did not pose a significant threat to golf green health in Canada. The populations of stunt and spiral nematodes were higher than those of the other predominant genera. Spiral and stunt nematodes may pose an issue in some areas of Canada. Spiral nematode appears to be the most virulent genus of PPN present in Canada since populations were found as high as 11,000 per 100 cc soil. Soil physical properties accounted for some of the variation in the nematode populations as they are linked to the structure and water holding capacity of the soil. Soil chemical properties were often correlated with nematode populations but it is difficult to discern which part of the food web they affected. Chemical properties that increased plant growth may have also increased certain nematode populations by increasing the growth of healthy roots that the nematodes could feed on. Management techniques linked to water retention and soil compaction were often correlated with nematode populations. The factors identified in Chapter 5 showed some correlation with nematode populations but many of them may be difficult or impractical to alter. The sugar centrifugal flotation nematode extraction from soil method was found to be superior to the Baermann pan nematode extraction method for all PPN. Both methods were deemed appropriate for the extraction of free-living nematodes.

Monitoring nematode populations on a regular basis should be a test incorporated in the regular management of golf courses in Canada rather than a final option after attempts to adjust fertility and apply fungicides fails to return the turf to a healthy state. Understanding PPN populations is important for management which is why regular testing is essential. If golf course superintendents can catch a PPN issue before it gets out of hand cultural practices can mitigate the damage and slow the rate of population increase. Using the sugar centrifugal flotation method to extract nematodes from soil will provide an accurate assessment of the PPN population present in golf course soils and allow superintendents to determine which genera of PPN are most prevalent in their soils, including sedentary nematodes. The soil's properties and the management practices conducted do influence the nematode populations but more focus should be placed on cultural practices like deep and infrequent watering, reducing shade on the green, and allowing the grass to grow a little longer while managing speed of the green with rolling. The information gathered by this study will assist turfgrass growers as it provides them with the invaluable tool of knowledge of an unfamiliar pest.

| **Graduate Student** | Provide a brief update of the status of any graduate student involved on project. |
|---|---|
| The graduate student involved in this project will be defending her thesis on January 21, 2016. | |

| **Project Expenses** | Using the project budget in the proposal, report the approximate expenditure of each line item. Submission of proof of expenditures will normally not be required |
|---|---|
| The budget ($25,000 per year) were spent on the graduate student's stipend at $19,000 per year and the remaining funds were used for travel to sites, laboratory testing of samples, travel to a scientific conference to present findings (Taylor Wallace received 1st place for the industry scholarship based on her poster presentation of this work), and to hire research assistants to aid in sample collection and analysis. | |

| **Project Communication** | List all industry and academic presentations and submitted publications |
|---|---|
| **Ontario Turfgrass Symposium,** Plant-Parasitic Nematodes in Managed Turfgrass Systems in Ontario, oral presentation, Guelph, Ontario, February 2015.<br><br>**Crop Science Society of America**, Plant-Parasitic Nematodes in Managed Turfgrass Systems throughout Canada, poster presentation, C-5 Division, Long Beach, California, November 2014.<br><br>**Ontario Turfgrass Symposium,** Plant-Parasitic Nematodes in Managed Turfgrass Systems in Ontario, oral presentation, Guelph, Ontario, February 2014. | |

NOTE: Portions of this report will be posted on the OTRF website