# PLANT-PARASITIC NEMATODES ON SUGARBEET IN NORTH DAKOTA AND MINNESOTA

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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# ABSTRACT

Field surveys were conducted in the Red River Valley (RRV) of North Dakota and Minnesota during 2016 and 2017 to determine the incidence, abundance, and distribution of plant-parasitic nematodes (PPNs) on sugarbeet. Seventy-two and 65 % of the fields surveyed were positive for PPNs in 2016 and 2017, respectively. The major genera of PPNs identified from sugarbeet production fields were *Heterodera*, *Helicotylenchus, Tylenchorhynchus, Paratylenchus, Pratylenchus, Paratrichodorus, Hoplolaimus,* and *Xiphinema*. Eight of PPNs were identified at the species level using species-specific PCR assays, and sequencing of the ribosomal rDNA gene.

Stubby-root nematode, *Paratrichodorus allius*, is one of the important nematode pests for sugarbeet production worldwide. An experiment was conducted to determine the host status of sugarbeet and their rotational crops for *P. allius* under greenhouse conditions. The results from two experiments indicated sugarbeet and most rotational crops support the reproduction of *P. allius*.

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# DEDICATION

This work is dedicated to my parents Mr. Min Bahadur Khatri and Mrs. Sulochana KC. I also like to dedicate this work to my sister, Mrs. Manisha KC, brother in law, Mr. Dipendra Basnet and my nephew, Mr. Devarsh Basnet.

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# LIST OF ABBREVIATIONS

BLAST.....Basic local alignment search tool

CLE.....CLAVATA3/ESR-RELATED

CRD .....Completely Randomized Design

DNA.....Deoxyribonucleic acid

DNA.....Deoxyribonucleic acid

FAO.....Food and Agriculture Organization

GPS .....Global Positioning System

ITS..... Internal transcribed spacer

LOI.....Loss on Ignition

LSD.....Least Significance Difference

MN .....Minnesota

MSU.....Michigan State University

MT.....Montana

NC.....North Carolina

ND.....North Dakota

NDSU.....North Dakota State University

NJ .....New Jersey

NPK.....Nitrogen Phosphorous Potassium

NTC.....Non-template control

NY.....New York

OR.....Oregon

PCR.....Polymerase Chain Reaction

PH .....Poor-Host

PPNs.....Plant-parasitic Nematodes

PV .....Prominence Value

RF.....Reproductive Factor

RNA .....Ribonucleic acid

RPV.....Relative Prominence Value

RRV .....Red River Valley

rRNA.....Ribosomal ribonucleic acid

SBCN .....Sugarbeet Cyst Nematode

SCN.....Soybean Cyst Nematode

SH .....Suitable Host

UMn .....University of Minnesota

US .....United States

US$ .....United States Dollar

USA.....United States of America

USDA-ERS…….United States Department of Agriculture-Economic Research Service

UV.....Ultra Violet

WI .....Wisconsin

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# CHAPTER 1. INTRODUCTION

Plant-parasitic nematodes (PPNs) impact growth of different plants and crops worldwide causing significant yield and economic loss. Many researchers worldwide (Abad et al., 2008; Koenning et al., 1999; Nicol et al., 2011; Sasser and Freckman, 1987; Singh et al., 2013), reported an annual crop loss of 8.8-14.6% and an economic loss of US$100-157 billion worldwide from PPNs. Chitwood (2003) reported an estimated annual crop loss of 10 billion US$ in the United States (US), from infection of PPNs. PPNs possess different shapes and sizes and are usually cylindrical and are tapered towards the head and tail. They range from 250 μm to 12 mm in length and from 15-35 μm in width. They have different survival strategies, among which mobility within the deeper soil environment and invasion of host and survival within plant tissues, is one of them. They disseminate from one field to another by various means which helps in the movement of soil particles such as farm tools, shoes, birds, animals, dust, rainwater, flooding, wind, insects, and human interventions. It can also disseminate from nematode infested plants or plant parts from one field to the other. Noel (1992) have explained in detail about the dissemination of soybean cyst nematode (SCN) from Midwest US to other parts of the country. Its migration is on its own and somehow limited, but it generally takes place by the help of environmental factors and/or human activities. It can interact with host plants and infects roots and other plant tissues for the feeding and has a broad range of hosts.

Sugarbeet is one of the important crops in the US which is affected by the plant-parasitic nematodes. *Helicotylenchus* spp. (Spiral nematode), *Heterodera* spp. (Cyst Nematode), *Meloidogyne* spp. (Root-knot nematode), *Paratylenchus* spp. (Pin nematode), *Pratylenchus* spp. (Root-lesion nematode), *Paratrichodorus* spp. (Stubby-root nematode), and *Tylenchorynchus* spp. (Stunt nematode) are some of the plant-parasitic nematodes found in sugarbeet fields. In the

US, North Dakota (ND) and Minnesota (MN) are major producers of sugarbeet. The Red River Valley (RRV), a geographical region along the ND and MN border, is the major producer of sugarbeet and have significant production historically. However, with higher production, more problems of diseases and pests have been reported. Research has been conducted to investigate various root diseases, but very few studies consider the impact of PPNs on crop production and only limited nematode surveys have been conducted in this region. *Paratrichodorus allius* (stubby-root nematode), one of the important pests for sugarbeet production, has been reported in parts of Europe, California, and Eastern Idaho (Hafez, 1998) and were detected in a sugarbeet field in MN (Yan et al., 2015; 2016b). However, no experiments exist on determining the host status of sugarbeet and other crops to this plant-parasitic nematode in this region. Therefore, the objectives of this study were:

1. Determine the incidence, abundance, and distribution of cyst and vermiform plant-parasitic nematodes in sugarbeet production fields in ND and MN.
2. Perform plant-parasitic nematode species identification and quantification.
3. Determine the host status of seven sugarbeet cultivars and twenty-one cultivars of most common sugarbeet rotational crops including wheat, corn, dry bean, barley, sunflower, and soybean to *Paratrichodorus allius.*

# CHAPTER 2. LITERATURE REVIEW

## Sugarbeet (*Beta vulgaris* L.)

### Background of sugarbeet

Sugarbeet (*Beta vulgaris* L.) is the economically most important crop of the large order Caryophyllales. It is cultivated as a source of sugar and has a high level of sucrose in its juice, making it the second major source of sugar after sugarcane (*Saccharum officinarum* L.) worldwide. It is mainly grown in Europe, North America, and Asia. It supplies approximately 35% of sugar worldwide (Harveson et. al., 2009). It is a biennial crop with a sugar-rich taproot in the first year and a flowering seed stalk in the second (Zhang et al., 2016). Sugarbeet passes through different phases of vegetative development: shoot growth, storage root growth, sugar storage, reproductive development stage of flower shoot elongation and flowering, and seed development (Bouillene et al.,1940; Van de Sande Bakhuyzen, 1949). Sugarbeet grown for sugar is an annual crop– from seed to roots that are harvested. Sugarbeet is extensively used to produce sugar and its by-products such as tops, pulp, and molasses used as animal feed. Sugarbeet is grown in rotation with other crops such as soybean, corn, and cereal grains. Sugarbeet thrives in temperate climatic conditions and are grown annually for sugar.

### History and production status of sugarbeet in the world, United States, North Dakota, and Minnesota

Sugarbeet is believed to be introduced from Arabia to China some 1500 years ago. Greek and Roman culture used sugarbeet as a food source for both humans and animals during the ancestral time (Cooke and Scott, 1993). Andreas Marggraf was first credited for extraction of sugar from white beetroot during 1744 in Europe (Prussia), and by the 19$^{th}$ century there was increased production of sugarbeet throughout the Europe (Harveson et al., 2009). In 2014, the

top five countries producing sugarbeet were listed as France, Russia, Germany, United States, and Turkey in the order of highest production of sugarbeet in million tons (FAO, 2015). Sugarbeet was produced in the US once the first sugar factory was established in California in 1870 by E. H. Dyer and since then, there has been a rapid development of the beet industries in the US (Winner, 1993). The US plays a major role in world sugar production by producing 10.6 % of the world sugarbeet which is equivalent to 28.7 million tons (FAO, 2015). It is grown in Michigan, Minnesota, North Dakota, Colorado, Montana, Nebraska, Wyoming, California, Idaho, and Oregon (USDA-ERS, 2016). In the US, sugarbeet provides about 55 % of the total sugar produced domestically since the mid-1990s (Benoit et al., 2015). MN and ND are the two largest producers of sugarbeet in the US. Although corn, soybean, and wheat are produced on more areas in RRV, sugarbeet economic contribution is significant (USDA, 2010). The seven sugarbeet factories owned by three grower-owned cooperatives: American Crystal Sugar Company, Minn-Dak Farmers' Cooperative, and Southern Minnesota Beet Sugar Cooperative in the region of ND and MN account for 51% of the national total sugarbeet production (USDA-ERS, 2016).

**Diseases affecting sugarbeet production**

Sugarbeet like many other crop species is affected by pest and several plant pathogens. Hanson (2009) reported sugarbeet can be affected by viruses, fungi, bacteria, oomycetes, parasitic plants, arthropods, and nematodes. Common diseases of sugarbeet in ND and MN includes *Fusarium*, *Rhizomania*, *Cercospora* leaf spot, and *Rhizoctonia* root and crown rot. Nematodes also are considered one of the important pests of sugarbeet worldwide.

**Nematode pests of sugarbeet**

Different plant-parasitic nematodes have been identified in sugarbeet in the world. In Iran, 37 known species of plant-parasitic nematodes have been reported in the sugarbeet (Karegar A., 2006). Some of the reported plant-parasitic nematodes from sugarbeet include *Helicotylenchus* spp. (Spiral nematode), *Heterodera* spp. (Cyst Nematode), Meloidogyne spp. (Root-knot nematode), *Paratylenchus* spp. (Pin nematode), *Pratylenchus* spp. (Root-lesion nematode), *Paratrichodorus* spp. (Stubby-root nematode), and *Tylenchorynchus* spp. (Stunt nematode). In Idaho and eastern Oregon, the most common sugarbeet nematodes were reported to be sugarbeet cyst nematode (SBCN) (*Heterodera schachtii*), root-knot nematode (*Meloidogyne* spp.), and stubby-root nematodes (*Paratrichodorus* spp.) (Hafez, 1998). SBCN (*Heterodera schachtii*) considered as one of the major pests of sugarbeet worldwide, is found in forty different countries and seventeen states in the US (Hafez, 1998). Other important nematode species such as, stubby-root nematode (*Paratrichodorus* spp.), have been reported in parts of Europe, California and Eastern Idaho (Hafez, 1998). *Heterodera schachtii*, and *Paratrichodorus allius* are among the important species of nematodes affecting sugarbeet production, and also more than two dozen species of the nematodes can damage the sugarbeet worldwide and the yield losses have been estimated to be between 10-80 percent (Hafez, 1998). Detail studies on the life cycle and symptoms, economic impact, and management strategies are very important for proper identification and effective control of the nematode species for obtaining a better yield of the sugarbeet.

***Heterodera schachtii***

*Heterodera schachtii* was first reported in the US in Utah in 1895 (Stewart et al., 2014). It also was detected in western ND (Nelson et al., 2012) but so far, SBCN has not been reported in eastern ND and MN (KC et. al., Unpublished). Sugarbeet planted in late June or grown in warmer soils can incur a 25-50% loss from SBCN (Rudolph et al., 2013). SBCN shows signs of wilting, yellowing and stunting of older plants due to their infection. SBCN-infected sugarbeet also displayed a hairy-root phenotype (Agrois, 2005). Infected plants show wilting symptoms even with adequate soil moisture condition on warmer days. Hafez (1998) reported that SBCN had multiple hosts including field crops and vegetables and the crop species were red table beet (*Beta vulgaris subsp. vulgaris* L), broccoli (*Brassica oleracea var. italica*), Brussels sprouts (*Brassica oleracea var. gemmifera*), mustard (*Brassica* spp.), radish (*Raphanus raphanistrum subsp. sativus*), kohlrabi (*Brassica oleracea Gongylodes Group*), and rapeseeds (*Brassica napus*). SBCN pass through six life stages: egg, first, second, third, and fourth stage larvae, and adult stage. The second stage juvenile is the infective stage and can invade the roots for feeding. Males are thread-like in shape whereas females are lemon-shaped and swollen. Upon maturation, females die, and their body becomes a cyst. The life cycle of SBCN completes in four to six weeks depending upon optimal soil moisture and temperature and their cyst can hold up to 500 eggs and those eggs within the cyst can survive without a suitable host (SH) for over 12 years (Hafez, 1998). Like many other nematodes, SBCN has relatively shorter migration. However, SBCN moves between fields by the aid of humans, irrigation water, soil, livestock, and farm machinery. Upon heavy infestation, most of the small seedlings could not resist the infection and dies but those which survive remain small with excessive hairy roots. The yield reduction of

sugarbeet is higher with the increase in the infection severity and entire sugarbeet seedling stands can be lost under severe infestation.

***Paratrichodorus allius***

*Paratrichodorus allius* are migratory ectoparasites, which feed on roots of plants for their survival and can transmit tobravirus (Jensen, 1983). It was first reported from an onion field in Oregon and was later reported to be present in other Pacific North West states including Oregon, Washington, and California (Norton, 1984). In our region, the stubby-root nematode *P. allius* was first detected from sugarbeet field in MN (Yan et al., 2015; 2016a; 2016b) and a potato field in ND (Yan et al., 2016). *P. allius* infection causes symptoms in plants including poor growth, yellowing, stunting and reduced taproot with abnormal branched lateral roots (Khan et al., 2016). Other damage symptoms include 'fanging' of the tap root (Gratwick, 1992; Jones and Dunning, 1972). Docking disorder due to *P. allius* in the sugarbeet taproot also has been reported by Jones and Dunning (1972). This symptom is often found in sandy soil with low organic matter. Stubby-root nematode feeds on the epidermal root cells and after their feeding, the roots are branched and distorted (Hafez, 1998). Damage caused by stubby-root nematode is higher in wet seasons, but plants are rarely killed by this group of nematodes (Hafez, 1998). They have six life stages including eggs, four larval stages, and adults. Adults are wormlike and are found in soil. The population of stubby-root nematode increases with the availability of suitable host (SH) crops whereas it declines upon the absence of the SH. The lifecycle of this group of nematodes completes in three to seven weeks depending upon the optimum soil moisture and temperature condition. They undergo dormancy under severe cold weather and can migrate up to 40 inches soil depth. Stubby-root nematodes have a wide range of hosts including cereal crops and potatoes (Hafez, 1998). They are transmitted from one field to the other by the aid of irrigation water,

wind, farm animals, human beings, and types of farm machinery. Appropriate control measures need to be implemented for managing this group of nematodes as a large population of stubby-root nematode develops quickly within a year.

**Interaction of plant-parasitic nematodes associated with sugarbeet and fungal pathogens**

Singh et al. (2013) elaborated about the disease complex, formed by interaction among nematodes, fungi, bacteria, and viruses. One of the important pests of sugarbeet, SBCN, can enhance the infection process by other sugarbeet pathogens, such as *Rhizoctonia*, viruses, and *Cercospora* to increase the yield loss (Agrios, 2005). SBCN does not directly interact with the fungus, such as *Rhizoctonia* and *Verticillium* but promotes the infection process and pathogenicity after root penetration (Agrios, 2005). Many endo- or ectoparasitic nematodes cause wounds on host roots and tissues. Wound can later serve as an entrance for other fungal pathogens. Ecto-parasitic nematodes such as *Paratrichodorus* spp. and *Tylenchorynchus* spp. make a small wound on the epidermis of plant root and endo-parasitic nematode such as *Heterodera* spp. can cause more damage to the host root (Back et al., 2002).

Studies have shown that sudden death syndrome (SDS) in soybean, when associated with SCN, have increased crop loss (Xing and Westphal, 2009). SDS occurs in most soybean-producing states and yield loss depends upon plant age at the time of infection, plant resistance, and environmental conditions (Agrios, 2005). The puncturing of roots by SCN can enhance the entrance for the soil-borne pathogens. Sugarbeet infected by SCN have the possibility to further support the growth of *Fusarium* and *Rhizoctonia* as SCN can survive in the soil for a long time without the presence of host crops (Harveson et al., 2009). The presence of SBCN along with fungi *R. solonai* on sugarbeet can enhance the fungal infection process and harm the sugarbeet crops (Hillnhütter et al., 2012). Polychronopoulos et al. (1969) reported the increased infection

and disease severity of *R. solonai* after beets were further infected by SBCN. Thus, the association of nematodes and fungal pathogens can be a great concern for sugarbeet production worldwide.

**Host range of *P. allius* for sugarbeet and its rotational crops**

*P. allius* has been associated with various crops and is believed to cause significant yield loss (Mojtahedi and Santo 1999). *P. allius* is found in many crop species in different parts of the world, including Chile, South Africa, Italy, Portugal, Israel, and Tanzania (Decreamer, 1995). *P. allius* has been reported in the different parts of the US and has a wide range of hosts (Norton, 1984). Goodey et al., (1965) suggested that onion serves as the host of *P. allius* in their research paper. Their suspicions were even confirmed by the studies of Jensen et al., (1983) in *P. allius*. *P. allius* were detected from a sugarbeet field in MN (Yan et al., 2015; 2016a; 2016b) and a potato field in ND (Yan et al., 2016). *P. allius* has been identified from one of the pea field of Ward county, ND during PPNs survey (Upadhyay et al., 2018). Crops including potatoes (Charlton et al., 2010; Gieck et al., 2007; Ingham et al., 2007; Mojtahedi and Santo, 1999), corn and wheat (Mojtahedi et al., 2002a), beans and sunflower (Ayala et al., 1970), barley (Mojtahedi and Santo, 1999), and soybean and sugarbeet (Yan et al., 2015; 2016a; 2016b) have been found to be associated with *P. allius*.

**Management of plant-parasitic nematodes in sugarbeet fields**

Plant-parasitic nematode management mainly relies on estimation and detection of the nematode population. They can be managed once detected, through integrated strategies including chemical, biological, cultural, and host plant resistance (Hague and Gowen, 1987; Halbrendt and LaMondia, 2004; Heald, 1987; Kerry, 1987; Starr and Roberts, 2004). Plant-parasitic nematodes disseminate from one field to another by various means such as infested farm equipment, farm tools, shoes, birds, dust, water, wind, insects, and human interventions. Cultural pest control techniques which include manipulation of planting and cultivation practices, preventive practices like sanitation and use of nematode-free plant materials, and an appropriate quarantine method can be implemented to prevent further nematode dissemination (Bird, 1981).

Human health and environmental concern restrict or limit use of nematicides in different parts of the world (Martin, 2003; Schierow, 2000). Thus, an integrated approach for plant-parasitic nematode management is recommended (Brown, 1987; McKenry, 1987). Integration of physical, biological, and limited chemical management strategies can help reduce the damage potential of different plant-parasitic nematodes (Robinson, 2004; Stirling, 1991). Crop rotations, planting cover crops, management of planting and harvesting date, use of trap crop, and weed host management are important integration methods for nematode management (Bird 1981; Brown 1978). Biological control agents (Siddiqui and Mahmood, 1999), organic soil amendments (Akhtar and Malik, 2000), and host resistance (Williamson and Hussey, 1996) are different measures used for management of plant-parasitic nematodes. In addition, resistance genes have been identified to attain host resistance against plant-parasitic nematodes. Genes *rhg1, rhg2, rhg3, and rhg4* were obtained from resistance line Peking, which is resistant to SCN

(Matthews et al., 2013). Mi-mediated resistance has been identified which prevents the formation of giant cell in host plant required by nematode for infection when invaded by *Meloidogyne incognita* (Williamson and Hussey, 1996). For SBCN, genetic resistance has been identified with the gene, $Hs1^{pro-1}$ (Cai et al., 1997). $Hs1^{pro-1}$ were cloned using genomic-specific satellite markers and chromosomal break-point analysis. The resistance of SBCN was observed by an expression of complementary DNA in a susceptible sugarbeet. This gene is believed to encode 282-amino acid protein with similar characteristics as shown by disease resistance genes which have been cloned from higher plants (Cai et al., 1997).

Management strategies should be focused on *Heterodera* spp. and *Paratrichodorus* spp. since they are of major concern in our region. Resistant/tolerant sugarbeet cultivars such as BTS 73MN which is available in our region can be used as an alternate strategy for managing SBCN since nematicides are uneconomical at large scales. Rotation with non-host crops, including wheat, barley, corn, bean, potato, and alfalfa, and use of trap crops, including oil seed radish and white mustard ae also considered effective control measure for SBCN. A resistance gene $Hs1^{pro-1}$, has been identified against SBCN. Other management strategies include sugarbeet planting when soil temperature is below $50^{o}F$, maintaining weed-free fallow land for certain period, and maintenance of farm sanitation. The best option available for the management of stubby-root nematode is to maintain proper sanitization. We can avoid use of farm tools from areas with the problem of stubby-root nematode to prevent its dissemination to unaffected field. Use of alternative non-hosts crop can help lessen the nematode population from an infected area. With a more negative impact of nematicides in environment, human health and input cost, the use of nematicides is limited. Thus, management strategies relying on an integrated approach will be the best option as it is a basis for sustainable management of PPNs.

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# CHAPTER 3. INCIDENCE, ABUNDANCE, AND DISTRIBUTION OF PLANT-PARASITIC NEMATODES IN SUGARBEET FIELDS OF NORTH DAKOTA AND MINNESOTA


## Abstract

Sugarbeet throughout the world may be parasitized by one or several PPN species. Surveys were conducted in the RRV area of ND and MN to determine the incidence, abundance, and distribution of PPNs on sugarbeet. A total of 217 soil samples were collected in 2016 and 2017 from fields with sugarbeet or a history of sugarbeet production and 48 samples were collected from tare soils in sugarbeet piling stations in ND and MN in 2016, and two samples from sugarbeet production fields in eastern Montana (MT) and western ND border area in 2017. Soil samples were collected randomly using a zig-zag pattern across each field. The incidence and abundance of major genera of PPNs identified from sugarbeet production fields in 2016 and 2017 were: *Heterodera* ( incidence = 15%, abundance = 1,351/ 200 gm of soil); *Helicotylenchus* (38%, 157 / 200 gm of soil)*; Tylenchorhynchus* (37%, 121 / 200 gm of soil)*; Paratylenchus* (28%, 108 / 200 gm of soil)*; Pratylenchus* (6%, 38 / 200 gm of soil); *Paratrichodorus* (7%, 37 / 200 gm of soil); and *Xiphinema* (3%, 32 / 200 gm of soil). *Hoplolaimus* (0.4%, 20 / 200 gm of soil) were not detected in 2016, while they were detected at low densities in 2017. Four genera of plant-parasitic nematodes such as *Helicotylenchus, Paratylenchus, Xiphinema,* and *Heterodera* were identified at very low densities from tare soils in sugarbeet piling stations in 2016. Species-specific PCR assays and direct sequencing of the ribosomal rDNA gene were used to confirm the species identities. Species identification revealed that the cyst nematodes from one of the counties of eastern MT were *Heterodera schachtii* and the cyst nematodes analyzed from 31 samples from 12 counties in ND and MN were *Heterodera glycines.* We have not identified any

*H. schachtii* so far from twelve counties in eastern ND and MN. Other nematode species identified include *Paratrichodorus allius, Pratylenchus neglectus, Tylenchorhynchus* sp., *Paratylenchus nanus, Helicotylenchus microlobus,* and *Helicotylenchus pseudorobustus*. Accurate identification of these nematodes and their distribution across the region will help determine effective pest management strategies for improved sugarbeet production.

**Key Words –** Sugarbeet, plant-parasitic nematodes, nematode incidence, nematode abundance, nematode distribution, species identification.

## Introduction

Sugarbeet (*Beta vulgaris* L.) is the economically most important crop of the large order Caryophyllales, supplying approximately 35% of sugar worldwide (Harveson et. al., 2009). Sugarbeet was introduced to China from Arabia about 1500 years ago. It is a biennial crop with a sugar-rich taproot in the first year and a flowering seed stalk in the second (Zhang *et al.*, 2016). Rapid development in the beet industries in the US took place following establishment of the first sugar factory in California by E. H. Dyer in 1870 (Winner, 1993). Sugarbeet today is grown in 10 states including Michigan, Minnesota, North Dakota, Colorado, Montana, Nebraska, Wyoming, California, Idaho, and Oregon and are raw materials for commercial sugar (USDA-ERS, 2016). In the US, sugarbeet provides approximately 55 % of the total sugar produced domestically since the mid-1990s (Benoit et al., 2015). Sugarbeet are grown in rotation with other crops including soybean, corn, and many cereal grains in temperate climatic conditions. Sugarbeet plants grown in the western region of the US have shown higher yield as compared to the eastern region. It is because western region agriculture practices irrigated farming whereas the eastern regions agricultural practices generally have dryland farming. The RRV of western MN and eastern ND is the most dynamic and largest producers of sugarbeet in the US. American

Crystal Sugar Company, Minn-Dak Farmers' Cooperative, and Southern Minnesota Beet Sugar Cooperative contributes 51% of the national total sugarbeet production (USDA-ERS, 2016).

PPNs possess a risk to agriculture crops worldwide. They mainly feed on the roots of plants and reduce crops ability to absorb nutrient and water. It causes annual crop loss of 8.8-14.6% and 100-157 billion US$ worldwide (Abad et al., 2008; Koenning et al., 1999; Nicol et al., 2011; Sasser and Freckman, 1987; Singh et al., 2013). Many PPNs have been associated with sugarbeet production fields. Thirty-seven known species of PPNs have been reported in sugarbeet fields in Iran (Karegar A. 2006). Approximately 500 million US$ are spent in nematode control worldwide (Keren-Zur et al., 2000). Some of the reported plant-parasitic nematodes from sugarbeet fields includes *Helicotylenchus* spp. (Spiral nematode), *Heterodera* spp. (Cyst Nematode), *Meloidogyne spp.* (Root-knot nematode), *Paratylenchus* spp. (Pin nematode), *Pratylenchus* spp. (Root-lesion nematode), *Paratrichodorus* spp. (Stubby-root nematode), and *Tylenchorynchus* spp. (Stunt nematode). Hafez, (1998) reported sugarbeet cyst nematode (*Heterodera schachtii*), root-knot nematode (*Meloidogyne* spp.), and stubby-root nematodes (*Paratrichodorus* spp.) as the most common sugarbeet nematodes in Idaho and eastern Oregon. SBCN (*Heterodera schachtii*.) which is considered as one of the major pests of sugarbeet worldwide is found in forty countries and seventeen states in the US (Saad L. Hafez 1998). Other important nematode genera, stubby-root nematodes (*Paratrichodorus* spp.), have been reported in parts of Europe, California, and Eastern Idaho (Hafez, 1998). In the RRV, the stubby-root nematode *Paratrichodorus allius* was first detected from a sugarbeet field (Yan et al., 2015; 2016a; 2016b) and a potato field (Yan et al., 2016) in ND.

Although many PPNs are associated with sugarbeet production, few of them have been further studied for its damage and yield loss. The experiment conducted by Michigan Sugar

Company demonstrated heavily infested SBCN fields could cause yield loss of more than 15 tons per acre (Stewart et al., 2014). The annual economic loss caused by SBCN to the Michigan Sugar Cooperative has been reported to be 5-10 million dollars by reducing yield and sucrose content (Stewart et al., 2014). Root-knot nematode is another important nematode genus affecting sugarbeet. High infestation level of root-knot nematode and its interaction with other pathogen groups are considered a major factor hindering sugarbeet production in Egypt (Abd El-Massih et al., 1986; El-Nagdi et al., 2004; Ibrahim 1982; Korayem, 2006; Maareg et al., 1998; Oteifa and El-Gindi, 1982). Stubby-root nematode is also considered as one of the economically important groups of nematodes affecting sugarbeet. They are reported to cause yield loss of more than 50 percent in the cool and wet growing season (Khan et al., 2016).

Western ND and eastern MN is one of the major production regions of sugarbeet and has more sugar processing factories and facilities available compared to other sugarbeet growing regions in the US. Cold weather in this region aid for proper storage and quality products. Thus, it has a direct economic impact on this area through higher sugarbeet production and many farmers today are interested in growing this crop. More threats of diseases and pests might prevail with increasing cultivation in this area. There are experiments on various soilborne and foliar diseases affecting sugarbeet production, but research is lacking regarding the impact of PPNs on crop production. Even though eastern ND and western MN are one of the major production regions of sugarbeet, interaction among various PPNs with sugarbeet is still unknown. Limited surveys have been conducted in this region, but no any comprehensive nematode survey has been conducted so far. Thus, a survey was conducted in the RRV and the sugarbeet growing region in western ND and eastern MT. A comprehensive field survey of sugarbeet production fields in ND and MN was initiated with the objectives to determine the

incidence, abundance, and distribution of cyst and vermiform plant-parasitic nematodes in sugarbeet production fields in ND/MN and identify them at the species level. The desired outcome is beneficial for developing effective pest management strategies for improved sugarbeet production in the future which is achievable through accurate identification, quantification and documentation of distribution of these nematodes across the region.

## Materials and Methods

### Soil sample collection and nematode extraction

Soil samples were collected in 2016 and 2017. Soil samples were collected primarily from RRV and secondarily from location near the ND and MT border region. Samples collected in western ND and eastern MT were done with cooperation from Williston Research Extension Center, Williston, ND 58801, USA (Fig. 3.1). Samples were collected across the counties of ND and MN, where sugarbeet are grown in rotation with major crops including corn, soybean, sunflower, wheat, barley, and dry bean. For counties with higher sugarbeet production area, soil samples were collected from more than 10 different fields across each county, whereas fewer soil samples of around three to seven samples were collected from counties with low production area. Most of the soil samples were collected during the growing season. A total of 217 field soil samples were collected during 2016 and 2017 from our surveyed locations (Table 3.1). In 2016, 48 tare soil samples were collected from the sugarbeet piling stations and from locations which has a history of receiving tare soils (Table 3.1). Combination of unwanted sugarbeet plant parts during harvesting and soil adhered to harvested sugarbeet is often considered as a tare soil. They are relocated when roots are mechanically piled at a piling station (Vermeulen et al., 2002).

Table 3.1. The total number of soil samples collected, and states and counties surveyed in 2016 and 2017

| Year [a] | Number of Samples | States/Counties | Total Counties [b] |
|---|---|---|---|
| **2016** | 108 | ND (Richland, Walsh, Cass, Pembina, Traill, Grand Forks) / MN (Clay, Norman) | 8 |
| | 48 (Sugarbeet piling station) | MN (Swift, Stearns, Marshall, Polk, Clay, Norman, Wilkin), ND (Cass, Richland, Walsh, Pembina, Grand Forks, Traill) | 13 |
| **2017** | 109 | ND (Richland, Walsh, Pembina, Grand Forks, Cass, Traill, Benson) / MN (Clay, Norman, Carver, Aitkin) / MT (Richland) | 13 |

[a] Years in which samples were collected.
[b] Total counties covered during 2016 and 2017.
ND, MN, and MT indicate the states of North Dakota, Minnesota, and Montana.

GPS navigator system (Garmin Drive 51 USA LM GPS Navigator System, OR, USA) was used to identify Global Positioning System (GPS) coordinates across each field. Sampling was conducted in a zig-zag pattern across each field. Five-meter distance was maintained between sampling cores while sampling. Top dry soil of about 1-2 cm was excluded while sampling because nematode populations are usually low under dry condition. The cores were maintained with a normal sampling standard including maintenance of 30 cm depth and 2.5 cm diameter. The collected soil samples were handled and stored properly at 4°C before performing nematode extraction.

Processing of surveyed soil samples was performed in the Nematology Laboratory, NDSU. Soil samples were thoroughly mixed, and a subsample of 200 gm was taken from each properly mixed composite soil samples before each nematode extraction. Nematodes were extracted using sieving and decanting, and sugar floatation method as described by Jenkins

(1964) and were collected in 50 ml suspension tubes for further nematode identification and quantification. First, we weigh around 200gm or 100cc of soil in a large beaker. If there are any clumps in the soil, we break them into fine pieces. Second, we fill the beaker with water and continuously stir the soil and water solution. After stirring, we wait for 10 seconds for heaviest soil particles to settle. The wait time depends on soil texture. For sandy soil, the wait time is about 5 seconds and for heavy clay soil, it is around 15 seconds. For cyst extraction, 1/3 top portion of the solution in the beaker is poured through #25 (710 µm) sieve which is nested over #60 (250 µm) mesh, and #635 (20 µm) mesh sieves. The cyst particles obtained are then collected into 2-3 reusable plastic centrifuge tubes (50 ml). For crushing the cyst to obtain eggs from the collected cyst, #200 numbered sieve (75 µm) is then nested over #635 (25 µm) mesh . The cyst is kept in trays and poured into the circular mesh and later crushed by the help of crusher/driller to obtain the eggs in the 25-µm mesh. The numbers of juveniles and eggs from the cysts collected in vials are then counted.

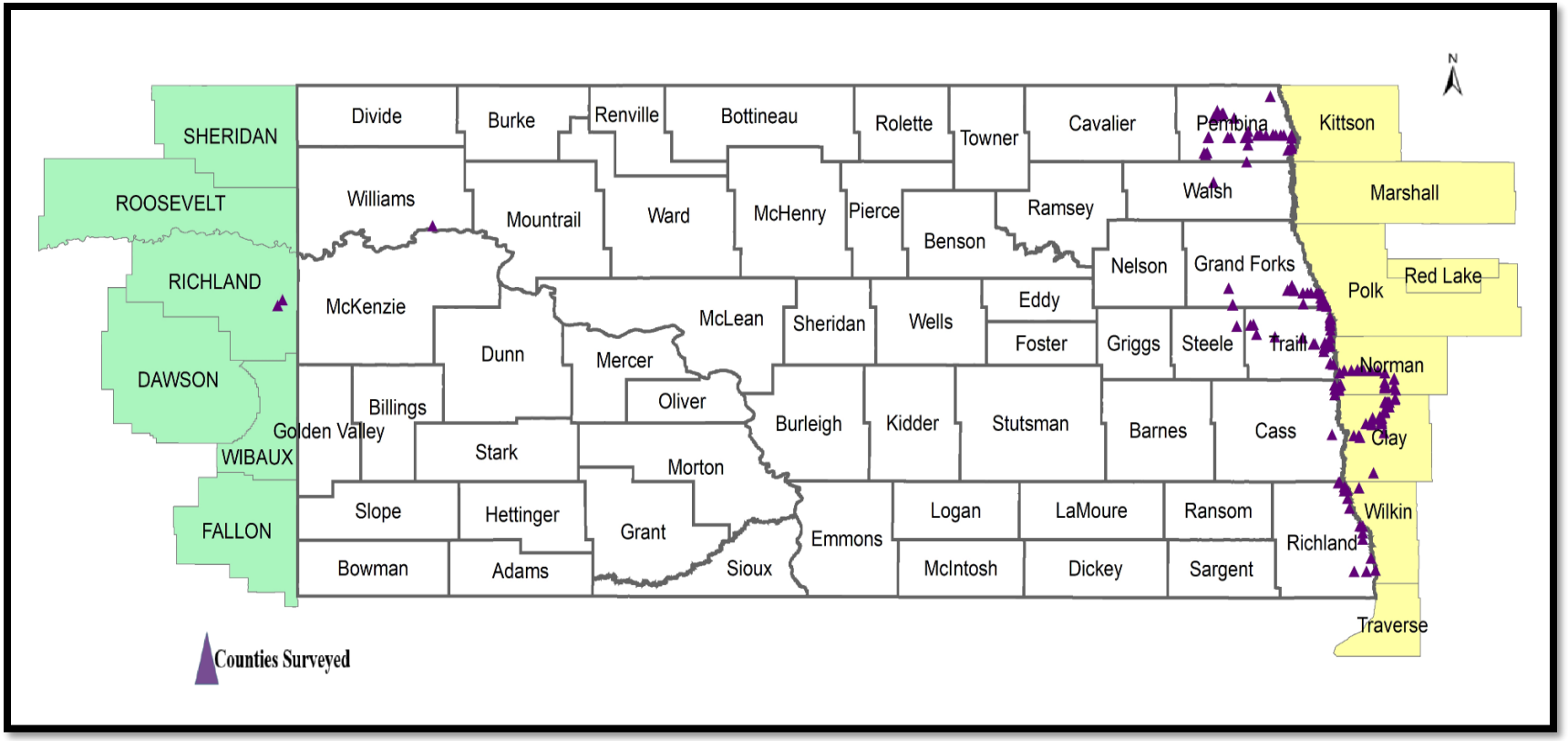


Fig. 3.1. Triangular index representing sampled locations across thirteen counties for plant-parasitic nematodes in sugarbeet field; 2016 to 2017. Map of North Dakota (white color), partial Minnesota (yellow color) and partial Montana (light green color).

**Plant-parasitic nematode identification and quantification**

Morphological and molecular characteristics were used to identify the most commonly occurring and abundant nematodes. An inverted transmitted light microscope at 100x magnification (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA) was used for PPNs identification morphologically at the genus level. Nematode population (the number of nematodes per 200 gm soil) was determined by counting the number of nematodes of each genus.

For molecular identification of nematode species, single nematode samples (n=4 per field) were used for DNA extraction as described by Huang et al. (2017). Nematodes were chopped and 0.5 ml sterile Eppendorf tube with 10 µl of buffer solution [2 µl of 10x PCR buffer, 2 µl of Proteinase K (600 µg/ml), and 6 µl of double-distilled water] was pipetted with nematode suspension (10 µl). They were then incubated at -20ºC for 30 mins followed by 65ºC for 1 hour and then 95ºC for 10 mins. DNA was then processed directly for PCR assays or sequencing and then stored at -80ºC for further use. The primers for *Pratylenchus neglectus* (Pn-ITS-F2/Pn-ITS-R2) (Yan *et al.*, 2013), *Heterodera glycines* (GlyF1/rDNA2) (Subbotin et al., 2001a), *Heterodera schachtii* (SHF6/AB28) (Amiri et al., 2002) and *Paratrichodorus allius* (PaF11/PaR12) (Huang *et al.*, 2017) were used for species-specific PCR assays (Table 3.2). *Heterodera glycines* were distinguished from *H. schachtii* using melt curve analysis of the CLAVATA3/ESR-RELATED (CLE) gene (Fig. 3.5). For sequencing, forward primer ACAAGTACCGTGAGGGAAAGTTG and reverse primer TCGGAAGGAACCAGCTACTA (Tenente et al., 2004) were used for amplifying D2-D3 expansion region of the 28s ribosomal RNA (rRNA) and similarly, forward primer CGCGAATRGCTCATTACAACAGC and reverse primer GGGCGGTATCTGATCGCC (Vrain et al., 1992) were used for amplifying 18S rRNA. 18 µl of the PCR mixture in PCR tubes

with 0.8 µl of each primer (10 µM), 0.4 µl dNTP, 1.2 µl $MgCl_2$, 4.0 µl 5× PCR buffer, and 0.15 U of Taq DNA Polymerase (Promega Corp., Madison, WI) were mixed with template DNA (2 µl). Initial denaturation (94ºC for 3 min), followed by 40 cycles of denaturation at 94ºC for 45 secs, annealing at 55ºC for 1 min, and extension at 72ºC for 1 min, and a final extension for 10 min at 72ºC that was set up as a PCR amplification protocol during this work. Subsequently, 2 µl of the PCR product was mixed with 3 µl of 2× loading dye. Finally, 5µl of the total mixture was loaded in 2% agarose gel for gel electrophoresis at 100 V for 25 min. Gel visualization was conducted under UV light, and AlphaImager Gel Documentation System (Proteinsimple Inc., Santa Clara, CA) was used for image processing. E.Z.N.A. Cycle Pure Kit (Omega BIO-TEK, Norcross, Georgia) was used for separating amplified DNA. Purified DNA was sent for DNA sequencing by GenScript (GenScript, Piscataway, NJ). Sequences were aligned using ClustalX, and they were finally deposited in the GenBank and compared with known sequences using BLAST tool in NCBI (www.ncbi.nlm.nih.gov) (Table 3.3).

**Data analysis**

The frequency or incidence (%) of each nematode genus were calculated by dividing the number of positive samples with nematode population by the total number of samples collected during the entire period and multiplying it by 100. Abundance is defined as the relative representation of a species in an ecosystem (Verberk, 2011). Abundance/population density was calculated as average nematode population density per 200 gm of sampled soil. The highest population density was expressed as the value which is highest among the range of population densities of a nematode genus in 200 gm of soil (Chen et al., 2012). The prominence value was calculated as abundance value multiplied by square root of incidence. The relative prominence value was calculated as the prominence value of a nematode genus divided by the sum of

prominence values for all nematode genera and multiplying by 100 (Chen et al., 2012). Log (1+X) was used to transform raw nematode abundance data to maintain the homogeneity of variance between sampled locations. Heatmap was generated by R package (R Development Core Team, 2017) to visualize the abundance of PPNs genera in sugarbeet production fields of thirteen sampled counties during the entire survey period (Fig. 3.6). Dendrogram of nematode genera sampled in different counties was presented in an upper level of the heat map. Normalized nematode abundance using log (1+X) transformation was presented by a color key scale with dark blue color representing the highest nematode abundance and light color being the lowest nematode abundance per 200 gm of soil in the sampled counties. Finally, ArcGIS software was used to analyze geospatial data, symbolize sampled counties and create maps of ND, MN and MT (Fig. 3.1).

## Results

### Plant-parasitic nematode genera and species in sugarbeet fields in North Dakota and Minnesota.

Eight genera of PPNs were detected including *Heterodera* (cyst nematode), *Paratrichodorus (*stubby-root nematode), *Helicotylenchus* (spiral nematode), *Tylenchorhynchus* (stunt nematode), *Paratylenchus* (pin nematode), *Pratylenchus* (root-lesion nematode), *Xiphinema* (dagger nematode), and *Hoplolaimus* (lance nematode). The species of the cyst, spiral, stunt, and pin nematodes were identified as *Heterodera schachtii, Heterodera glycines, Helicotylenchus pseudorobustus, Helicotylenchus microlobus, Tylenchorhynchus* sp., and *Paratylenchus nanus* by the aid of DNA sequencing (Table 3.3) (Fig. 3.2 – 3.4). Species of root-lesion nematode were identified as *Pratylenchus neglectus,* based upon amplification of the ITS region of rDNA with species-specific primers Pn-ITS-F2/Pn-ITS-R2 (Yan *et al.*, 2013) (Table

3.2). Cyst nematodes were identified as *Heterodera glycines* with species specific primers GlyF1/rDNA2 (Subbotin et al., 2001a) and *Heterodera schachtii* with species-specific primers SHF6/AB28 (Amiri et al., 2002) (Fig. 3.5 - 3.6). Stubby-root nematode was identified as *Paratrichodorus allius* with species-specific primers PaF11/PaR12 (Huang *et al.*, 2017) (Table 3.3). *Heterodera glycines* were also distinguished from *H. schachtii* using melt curve analysis of the CLE gene with *Heterodera glycines* CLE melting at 81.5°C and *H. schachtii* CLE melting at 83/83.5°C (Fig. 3.7). The cyst nematodes from RRV were identified to be *Heterodera glycines* whereas the cyst nematodes from eastern MT were identified as *H. schachtii*. From our surveyed locations in eastern ND and western MN, we have not identified *H. schachtii*. Among species identified, *P. allius* and *H. schachtii* can be of great concern for sugarbeet production, as these nematodes have been reported to cause significant yield loss in sugarbeet growing regions.

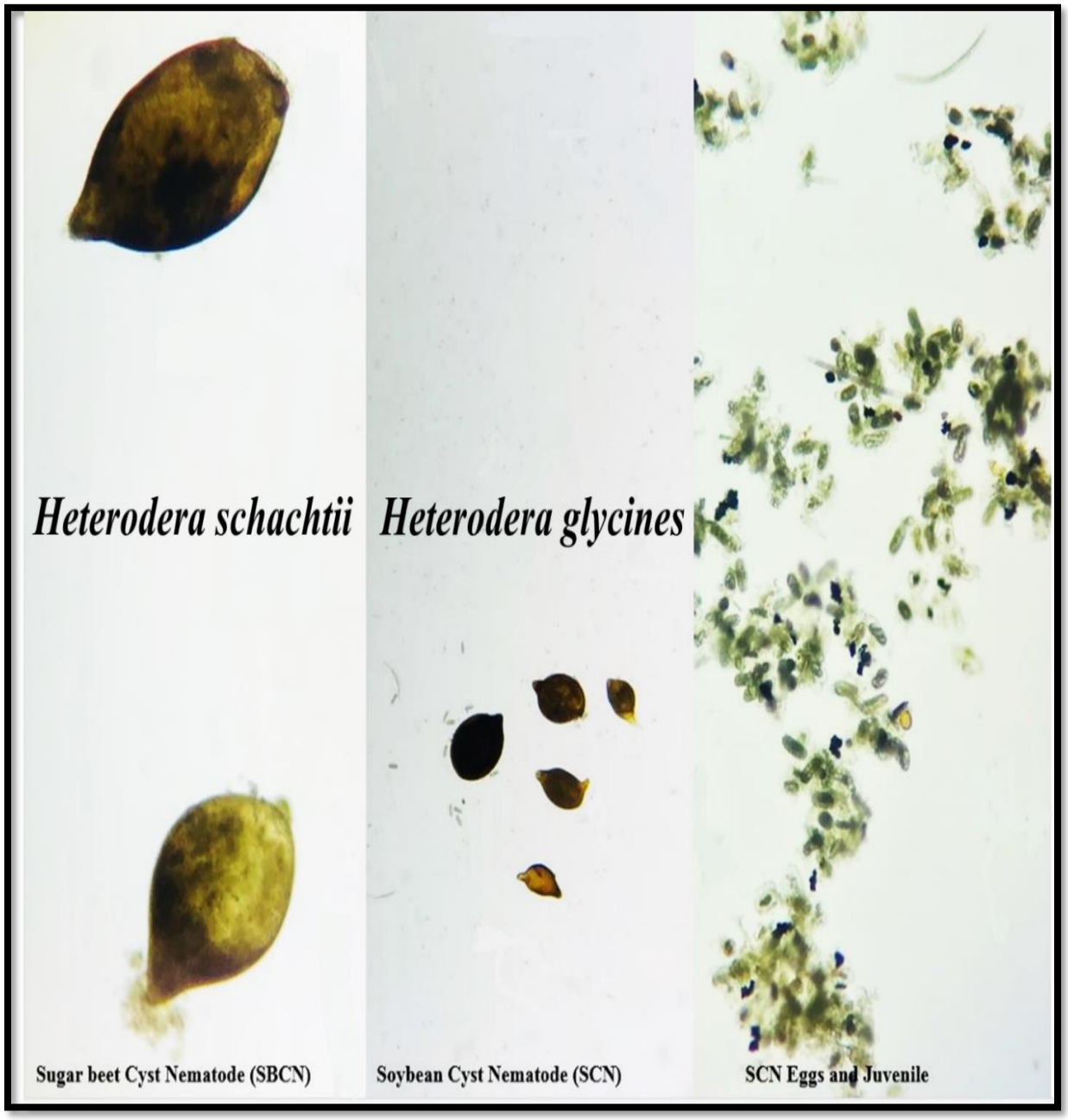


Fig. 3.2. Microscopic image of *Heterodera schachtii*, sugarbeet cyst nematode (SBCN) and *Heterodera glycines*, soybean cyst nematode (SCN).


Fig. 3.3. Microscopic image of *Pratylenchus neglectus* (root-lesion nematode), *Paratrichodorus allius* (stubby-root nematode), *Helicotylenchus* sp. (spiral nematode), and *Tylenchorhynchus* sp. (stunt nematode).

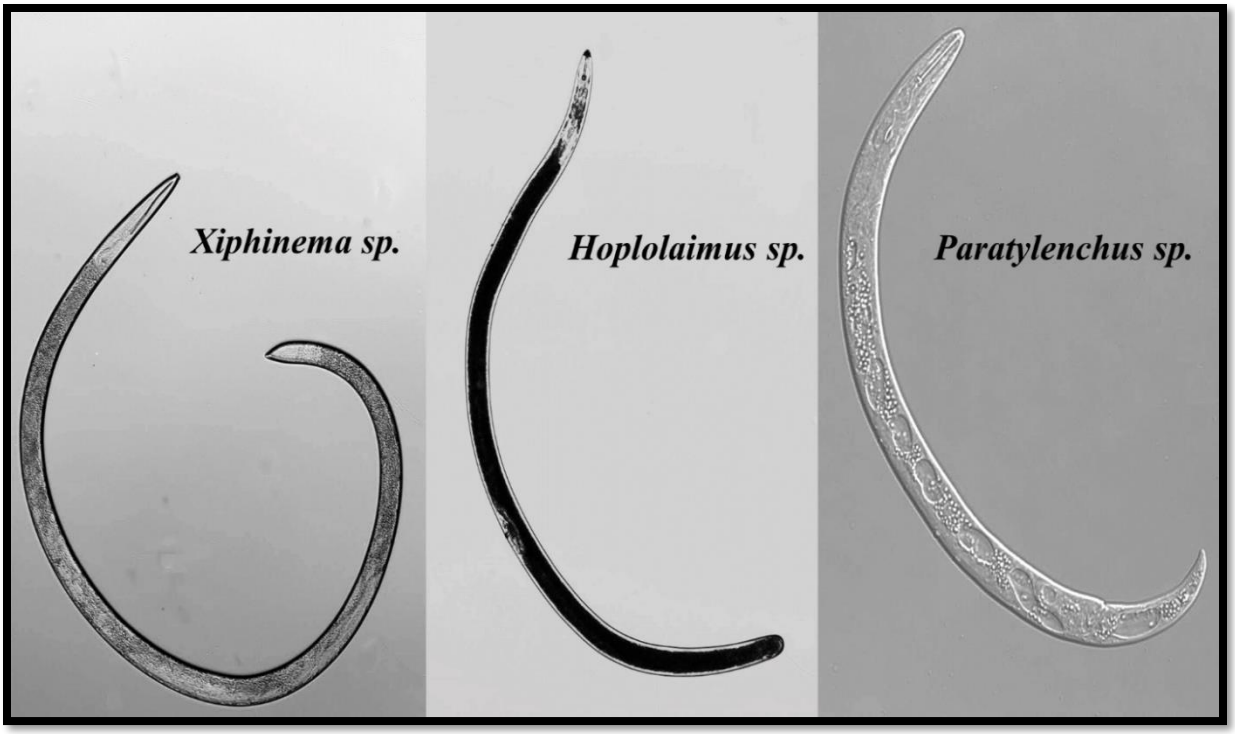


Fig. 3.4. Microscopic image of *Xiphinema* sp. (dagger nematode), *Hoplolaimus* sp. (lance nematode), and *Paratylenchus* sp. (pin nematode).

Table 3.2. Molecular identification of plant-parasitic nematodes from species-specific PCR assays and direct sequencing method

| Nematode Species | Identification Method | Primers Used | References for species specific PCR assays | Deposited accession no.[a] | Compared accession no.[a] | Expect (E) value[b] | Identity |
|---|---|---|---|---|---|---|---|
| ***Heterodera schachtii*** | Direct Sequencing/species specific PCR assays | SHF6/AB28 | Amiri et al., 2002 | MH790255.1 | JQ040527.1 | 0.0 | 99% |
| ***Heterodera glycines*** | Direct Sequencing/Species-specific PCR assays | GlyF1/rDNA2 | Subbotin et al., 2001a | MK262900.1 | KY795944.1 | 0.0 | 99% |
| ***Paratrichodorus allius*** | Species-specific PCR assays | PaF11/PaR12 | Huang *et al.*, 2017 | - | - | - | - |
| ***Pratylenchus neglectus*** | Species-specific PCR assays | Pn-ITS-F2/Pn-ITS-R2 | Yan *et al.*, 2013 | - | - | - | - |
| ***Helicotylenchus pseudorobustus*** | Direct Sequencing | | | MK358143.1 | KU722387.1 | 0.0 | 99% |
| ***Helicotylenchus microlobus*** | Direct Sequencing | | | MH790254.1 | KM506861.1 | 0.0 | 99% |
| ***Tylenchorhynchus* sp.** | Direct Sequencing | | | MH818454.1 | KY200667.1 | 0.0 | 98% |
| ***Paratylenchus nanus*** | Direct Sequencing | | | MH790252.1 | KF242201.1 | 0.0 | 99% |

[a] Deposited accession number and compared accession number refers to unique identifier of the query and comparison sequence respectively;

[b] E value is the expected value and the percent similarity between the deposited and comparison sequences after sequence BLAST in NCBI database is represented as identity %.

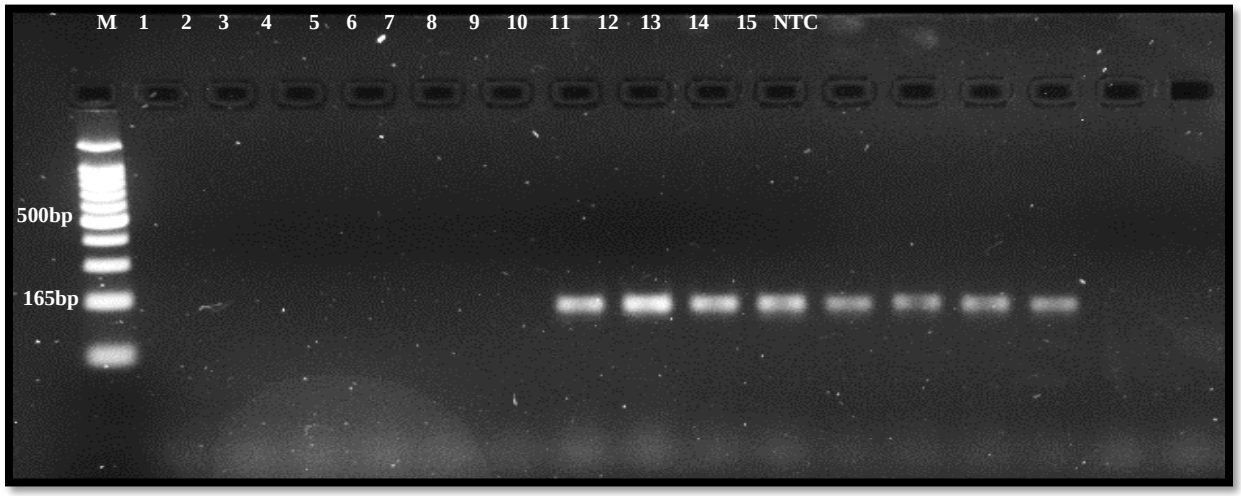


Fig. 3.5. PCR amplification of cyst nematode samples using soybean cyst nematode specific primers GlyF1 and rDNA2 primers (Subbotin et al., 2001a). 1-3 = MT samples, 4-6 = MT samples, 7 = *H. glycines*, 8 -14 = RRV area samples, 15 = *H. schachtii*, NTC = non-template control using double-distilled $H_2O$, M = 100bp ladder.

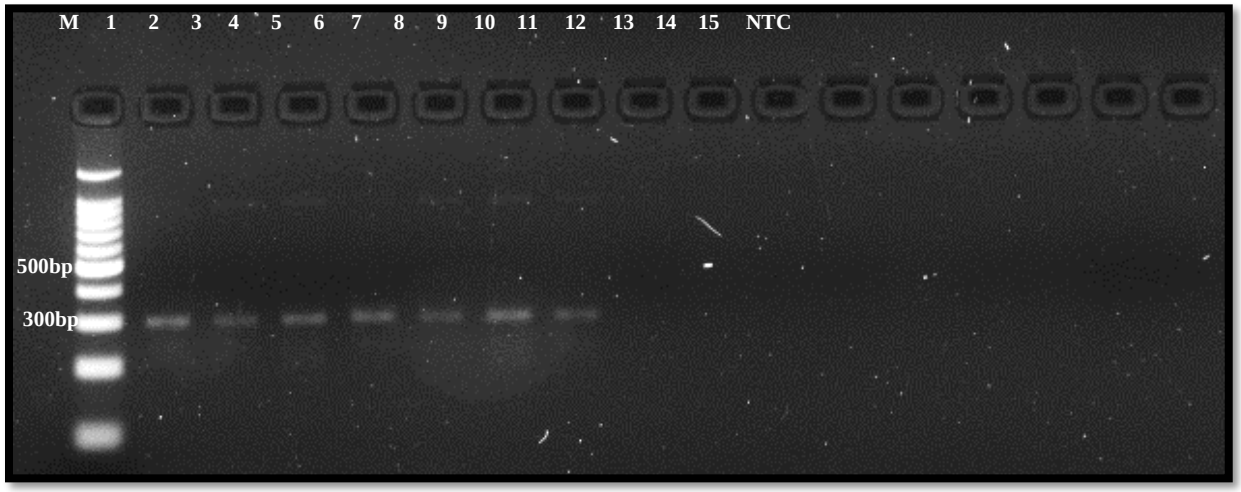


Fig. 3.6. PCR amplification of cyst nematode samples using SBCN specific primers SHF6 and AB28 primers (Amiri et al., 2002). 1= *H. schachtii*, 2-4 = MT samples, 5-7 = MT samples, 8 = *H. glycines*, 9-15 = RRV area samples, NTC = non-template control using double-distilled $H_2O$, M = 100bp ladder.

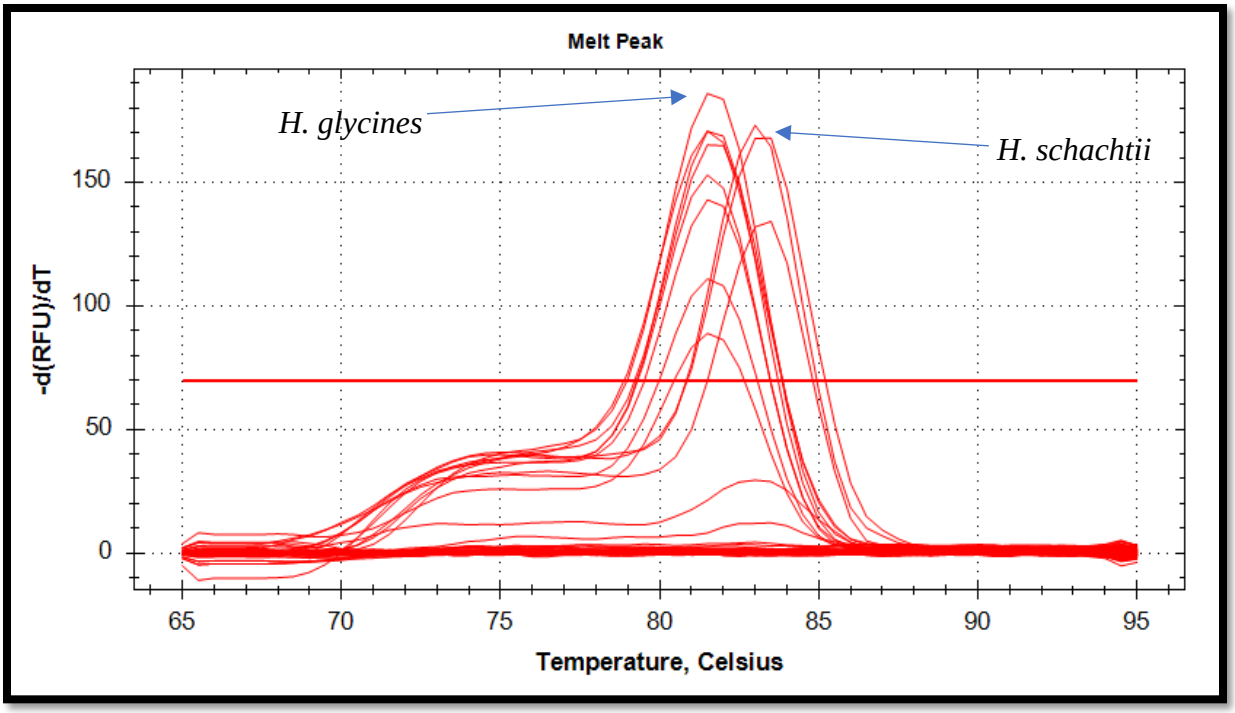


Fig. 3.7. Distinguishing *H. glycines* from *H. schachtii* using CLE primer pair (CLE2F/CLE2R). *H. glycines* CLE melts at 81.5°C whereas *H. schachtii* CLE melts at 83/83.5°C (represented by blue arrows).

**Incidence, and abundance of cyst and vermiform plant-parasitic nematodes and their distribution among counties**

Seventy-two and 65 % of the fields surveyed were positive for PPNs in 2016 and 2017. The incidence and average population density of major genera of PPNs identified from sugarbeet production fields were shown as follows: *Heterodera* (incidence = 11-18%; average population density = 1,609 / 200 gm of soil), *Helicotylenchus* (24-53%; 167 / 200 gm of soil), *Tylenchorhynchus* (35-40%; 159 / 200 gm of soil), *Paratylenchus* (24-32%; 114 / 200 gm of soil), *Pratylenchus* (4-10%; 40 / 200 gm of soil), *Paratrichodorus* (5-10%; 38 / 200 gm of soil) and *Xiphinema* (1-5%; 34 / 200 gm of soil) for the year 2016 and 2017 (Table 3.3). *Hoplolaimus* (0-1%; 20 / 200 gm of soil) were not detected in 2016 but were detected at low densities in 2017 (Table 3.3). Four genera of PPNs such as *Helicotylenchus*, *Paratylenchus*, *Xiphinema*, and *Heterodera* were identified at very low densities from tare soils in sugarbeet piling stations in 2016 (Table 3.3).

The highest prominence value (PV) and relative prominence value (RV) was recorded for *Heterodera* (PV = 5,074-5,336; RV = 64-72) and *Helicotylenchus* (652-1,216; 9-15) followed by *Tylenchorynchus* (491-980; 7-12) from soil samples in 2016 and 2017 (Table 3.3). For sugarbeet piling station, highest PV and RV were recorded for *Helicotylenchus* (773; 80). For both of the years combined, the major genera identified were *Heterodera* ( incidence = 15%; average population density = 1,351 / 200 gm of soil), *Helicotylenchus* (38%; 157 / 200 gm of soil), *Tylenchorhynchus* (37%; 121 / 200 gm of soil), *Paratylenchus* (28%; 108 / 200 gm of soil), *Pratylenchus* (6%; 38 / 200 gm of soil), *Paratrichodorus* (7%; 37 / 200 gm of soil), *Hoplolaimus* (0.4%; 20 / 200 gm of soil), and *Xiphinema* (3%; 32 / 200 gm of soil) (Table 3.3). For tare soil samples, sampling was done in Cass, Richland, Walsh, Pembina, Grand Forks, and Traill

counties of ND and Swift, Stearns, Marshall, Polk, Clay, Norman, and Wilkin counties of MN. The major genera of PPNs identified from sugarbeet piling stations were *Heterodera* (incidence = 2%; average population density = 20 / 200 gm of soil), *Helicotylenchus* (77%; 88 / 200 gm of soil), *Paratylenchus* (6%; 53/ 200 gm of soil), and *Xiphinema* (2%; 20 / 200 gm of soil). Overall, four nematode genera were found from tare soil samples at very low densities (Table 3.3).

*Paratylenchus* nematodes were detected in Traill, Benson, Carver, Williams, Aitkin, Richland (MT), Norman, Pembina, Grand Forks, Richland, and Clay counties (Fig 3.8). *Tylenchorhynchus* were detected in Traill, Benson, Williams, Aitkin, Richland (MT), Cass Norman, Pembina, Grand Forks, Richland, and Clay counties (Fig 3.8). *Helicotylenchus* nematodes were detected in Walsh, Traill, Aitkin, Richland (MT), Norman, Pembina, Grand Forks, Richland, and Clay counties (Fig 3.8). *Heterodera* were detected in Benson, Richland (MT), Cass, Norman, Pembina, Grand Forks, Richland, and Clay counties (Fig 3.8). *Pratylenchus* were detected in Cass, Benson, Norman, Pembina, Grand Forks, Richland, and Clay counties (Fig 3.8). *Xiphinema* were detected in Clay, Richland, Grand Forks, and Walsh counties (Fig 3.8). *Paratrichodorus* were detected in Richland, Pembina, and Norman counties whereas *Hoplolaimus* were detected in Pembina county (Fig 3.8). The seven nematode genera were identified in Richland and Pembina counties, and counties of Carver, Walsh, and Traill were found to have one, two and three nematode genera respectively (Fig 3.8). Spiral and stunt nematodes had the highest incidence with 38 and 37% in sampled counties, respectively (Table 3.3.). Pin, cyst, stubby-root, root-lesion, dagger, and lance nematode were found in 28, 15, 6, 3, 0.4, and 0.4% fields, respectively (Table 3.3). Overall, PPNs genera, *Heterodera, Helicotylenchus, Tylenchorhynchus,* and *Paratylenchus* were the more dominant and abundant genera during two-year sampling periods across 13 sampled counties. However, *Paratylenchus*

were not identified from Walsh and Cass counties. Likewise, *Tylenchorhynchus* were not detected in Carver and Walsh counties, *Helicotylenchus* remained undetected in Benson, Carver, and Aitkin counties, and *Heterodera* were not identified from Aitkin, Williams, Carver, Traill, and Walsh counties (Fig 3.6). Nematode genera *Paratylenchus*, and *Tylenchorhynchus* had similar distribution pattern across counties identified with those genera of nematodes. Their distribution was comparable to the distribution of *Heterodera*, and *Helicotylenchus*, across counties identified with those group of nematodes. However, distribution pattern of such dominant nematode genera across those sampled counties were dissimilar to the distribution pattern of *Pratylenchus*, *Xiphinema*, *Paratrichodorus*, and *Hoplolaimus* (Fig 3.6).

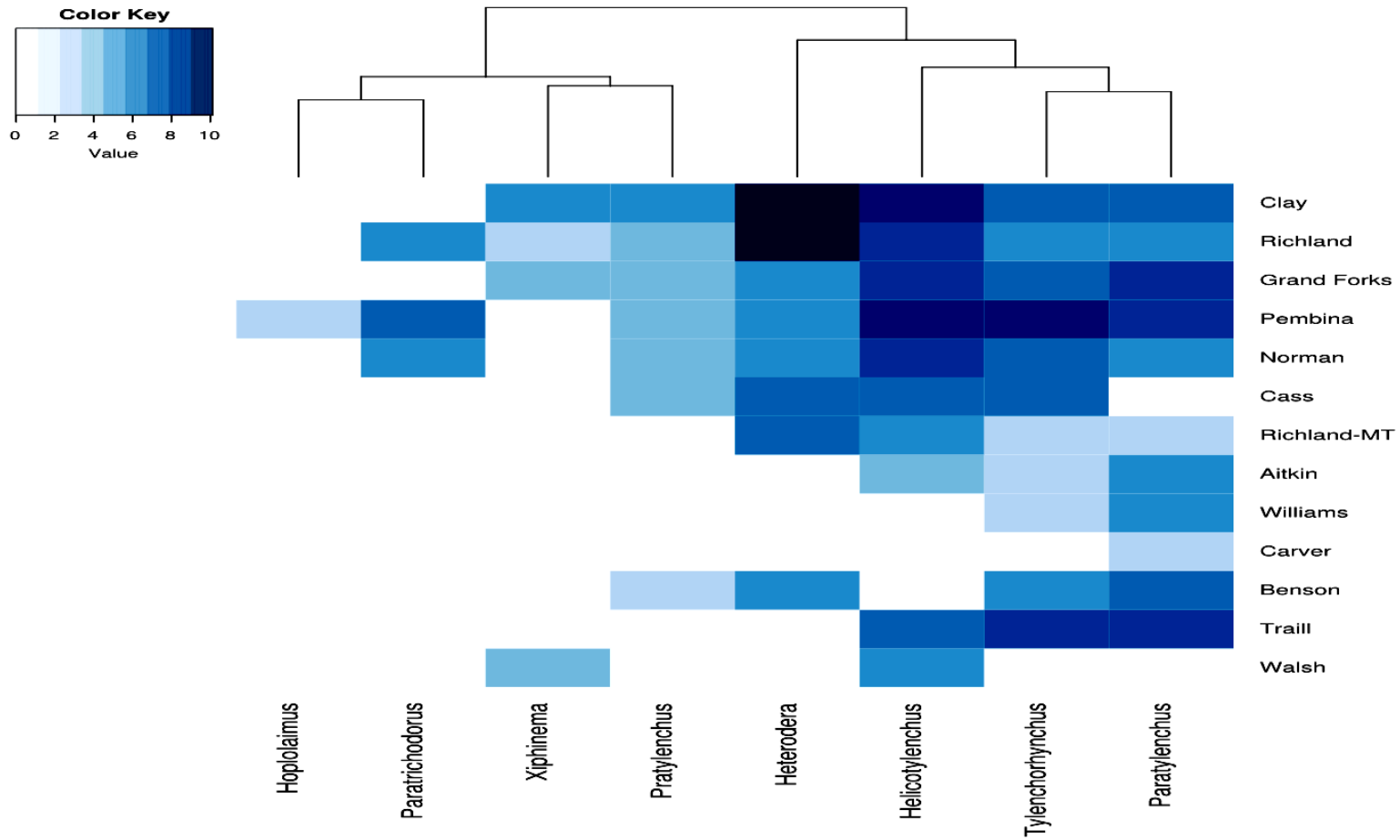


Fig. 3.8. Heatmap of plant-parasitic nematode genera in sugarbeet fields of thirteen sampled counties during survey period. Dendrogram of nematode genera sampled in different counties are represented in upper level. Normalized nematode abundance is represented by color key scale with dark blue color representing highest nematode abundance and light color being the lowest nematode abundance per 200 g of soil in the sampled counties.

Table 3.3. Incidence (Frequency) and Abundance (Average Population Densities) of plant-parasitic nematodes genera during sampling years, 2016 and 2017 (Field soil samples and tare soil sample from sugarbeet piling station in 2016)

| Nematode Genera | Total no. of positive nematode samples | Incidence (%) [a] | Abundance/ Average population density per 200 gm of soil [a] | Highest population density per 200 gm of soil [a] | Prominence values (PV) [b] | Relative Prominence values (RV) [c] |
|---|---|---|---|---|---|---|
| | | | **2016 (N = 108)** | | | |
| ***Heterodera*** | 12 | 11 | 1,609 | 8,600 | 5,336 | 64 |
| ***Paratrichodorus*** | 5 | 5 | 38 | 60 | 85 | 1 |
| ***Helicotylenchus*** | 57 | 53 | 167 | 1,530 | 1,216 | 15 |
| ***Tylenchorhynchus*** | 43 | 40 | 155 | 600 | 980 | 12 |
| ***Paratylenchus*** | 25 | 24 | 115 | 320 | 563 | 7 |
| ***Pratylenchus*** | 10 | 10 | 37 | 66 | 117 | 1 |
| ***Xiphinema*** | 5 | 5 | 34 | 60 | 76 | 1 |
| ***Hoplolaimus*** | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | **2017 (N = 109)** | | | |
| ***Heterodera*** | 20 | 18 | 1,196 | 8,560 | 5,074 | 72 |
| ***Paratrichodorus*** | 11 | 10 | 36 | 100 | 114 | 2 |
| ***Helicotylenchus*** | 26 | 24 | 133 | 720 | 652 | 9 |
| ***Tylenchorhynchus*** | 38 | 35 | 83 | 620 | 491 | 7 |
| ***Paratylenchus*** | 35 | 32 | 104 | 500 | 588 | 8 |
| ***Pratylenchus*** | 4 | 4 | 40 | 60 | 80 | 1 |
| ***Xiphinema*** | 1 | 1 | 20 | 20 | 20 | 0 |
| ***Hoplolaimus*** | 1 | 1 | 20 | 20 | 20 | 0 |

[a] Incidence = (number of positive samples with nematode population during survey period) / (total number of samples collected during that period) × 100; Abundance = average nematode population density per 200 gm of sampled soil; Highest density = the value which is higher among the range of population densities of a nematode genus (Chen et al., 2012). [b] Prominence values = absolute density x square root (incidence); absolute density = mean number of nematodes of a genus per 200 gm soil in positive samples (Chen et al., 2012). [c] Relative prominence values = (prominence value of a nematode genus)/ (sum of prominence values for all nematode genera) x 100 (Chen et al., 2012).

Table 3.3. Incidence (Frequency) and Abundance (Average Population Densities) of plant-parasitic nematodes genera during sampling years, 2016 and 2017 (Field soil samples and tare soil sample from sugarbeet piling station in 2016) (Continued).

| Nematode Genera | Total no. of positive nematode samples | Incidence (%) [a] | Abundance/ Average population density per 200 gm of soil [a] | Highest population density per 200 gm of soil [a] | Prominence values (PV) [b] | Relative Prominence values (RV) [c] |
|---|---|---|---|---|---|---|
| | | | **2016 and 2017 (N=217)** | | | |
| ***Heterodera*** | 32 | 15 | 1,351 | 8,600 | 5,232 | 67 |
| ***Paratrichodorus*** | 16 | 7 | 37 | 100 | 98 | 1 |
| ***Helicotylenchus*** | 83 | 38 | 157 | 1,530 | 968 | 12 |
| ***Tylenchorhynchus*** | 81 | 37 | 121 | 620 | 736 | 9 |
| ***Paratylenchus*** | 60 | 28 | 108 | 600 | 571 | 7 |
| ***Pratylenchus*** | 14 | 6 | 38 | 66 | 93 | 1 |
| ***Xiphinema*** | 6 | 3 | 32 | 60 | 55 | 1 |
| ***Hoplolaimus*** | 1 | 0.4 | 20 | 20 | 13 | 0 |
| | | | **Sugarbeet piling station, 2016 (N=48)** | | | |
| ***Heterodera*** | 1 | 2 | 20 | 20 | 28 | 3 |
| ***Helicotylenchus*** | 37 | 77 | 88 | 320 | 773 | 80 |
| ***Paratylenchus*** | 3 | 6 | 53 | 80 | 133 | 14 |
| ***Xiphinema*** | 1 | 2 | 20 | 20 | 28 | 3 |

[a] Incidence = (number of positive samples with nematode population during survey period) / (total number of samples collected during that period) × 100; Abundance = average nematode population density per 200 gm of sampled soil; Highest density = the value which is higher among the range of population densities of a nematode genus (Chen et al., 2012). [b] Prominence values = absolute density x square root (incidence); absolute density = mean number of nematodes of a genus per 200 gm soil in positive samples (Chen et al., 2012). [c] Relative prominence values = (prominence value of a nematode genus)/ (sum of prominence values for all nematode genera) x 100 (Chen et al., 2012).

## Discussion

This survey was conducted to determine the incidence, and abundance of cyst and vermiform PPNs and their distribution among different counties in sugarbeet growing fields in eastern ND and MN in 2016 and 2017. Eight morphological genera of PPNs were identified from 217 field soil samples and four morphological genera were identified from 48 tare soil samples from sugarbeet piling station. Nematode genera identified includes *Heterodera*, *Helicotylenchus*, *Paratylenchus*, *Tylenchorhynchus*, *Paratrichodorus*, *Pratylenchus*, *Xiphinema*, and *Hoplolaimus*. Eight species identified were *Heterodera glycines*, *H. schachtii*, *Paratrichodorus allius*, *Pratylenchus neglectus*, *Tylenchorhynchus* sp., *Paratylenchus nanus*, *Helicotylenchus microlobus*, and *Helicotylenchus pseudorobustus*. Among those identified species, stunt nematode, *Tylenchorynchus* sp. is a new and unnamed species. Most of these nematode genera have been identified in the sugarbeet field in Iran (Karegar A., 2006). Many of these nematode genera have also been reported as important nematode genera in ND (Yan et al., 2016a; 2016b; 2016c). Similarly, many of these nematode genera were identified as an important group in crop fields in MN (Crow and MacDonald, 1978; MacDonald, 1979; Taylor et al., 1958; Taylor and Schleder, 1959).

Sugar beet cyst nematode is one of the important pests of sugarbeet worldwide. In 2012, SBCN was first reported officially in the Yellowstone Valley in western ND (Nelson et al., 2012). Comparisons of restriction fragment patterns of mitochondrial DNA from *H. schachtii* and *H. glycines* have shown that out of 90 scorable fragments, 10% of them were shared by both the species and had the nucleotide sequence divergence of $p = 0.145$ (Radice et. al., 1988). It suggests that these two species diverged from a common ancestors. Fertile progenies were also

obtained from the hybridization between *H. schachtii* males and *H. glycines* females (Potter and Fox, 1965). Thus, it was biggest challenge for us to differentiate between soybean cyst nematode (SCN) and sugarbeet cyst nematode (SBCN) as they are morphologically similar and are closely related with each other (Miller, 1976; Potter and Fox, 1965; Radice et. al., 1988). The occurrence frequency of *Heterodera* obtained in this study was 15% for both the sampling years and their abundance was 1,351 per 200 gm of soil and the highest population density recorded was 8,600 per 200 gm of soil. The cyst nematodes analyzed from 31 samples by means of species-specific PCR assays and/or amplification and sequencing of the ribosomal rDNA were *Heterodera glycines*. *H. schachtii,* a nematode known to cause damage to sugarbeet, was identified from three samples from western ND and eastern MT. Thus, *H. schachtii* does not exist in the surveyed locations of RRV of eastern ND and western MN but it exists in western ND and eastern MT. Even though *H. schachtii* was not found in the RRV, the presence of *H. glycines* in sugarbeet fields can be a major concern for soybean grown in rotation with sugarbeet. *H. glycines* can infect different crop species based on its virulence, environmental factors, and the cultivars used (Acharya et al., 2016) and were reported to penetrate non-hosts crop (Riggs, 1987; Schmitt and Riggs, 1991). Hence, there is a possibility that it can penetrate sugarbeet and create an entrance pathway for root diseases. Previous research (Adeniji et al., 1975; Tabor et al., 2003; Xing and Westphal, 2009) reported *Heterodera*-fungal interaction does exist, and it can increase disease severity or incidence from a fungal pathogen. More disruption in host root was reported to be caused by *Heterodera* spp. (Back et al., 2002). SBCN increases yield loss caused by *Cercospora, Rhizoctonia,* and viruses and interaction of cyst nematodes with either *Rhizoctonia*

and *Verticillium* and does not vector the fungus, but promotes the activity of pathogen after they penetrate the root system (Agrios, 2005)

Stubby-root nematodes feed on roots and can transmit Tobacco Rattle Virus causing corky ringspot disease of potato (Mojtahedi and Santo, 1999). Stubby-root nematode *Paratrichodorus allius* was identified from sampled fields with an incidence of 7%, an average population density of 37 per 200 gm of soil and highest nematode population was recorded as 100 per 200 gm of soil. Stubby-root nematodes have a wide host range such as cereal crops, potatoes, and sugarbeet (Hafez, 1998). More studies are required in our region to investigate the effect of stubby-root nematode and its impact on various sugarbeet and rotational crop cultivars, commonly grown in ND and MN because this nematode was identified in our region and has been reported to be one of the threats for sugarbeet production in Europe, California, and Idaho (Hafez, 1998). Although, our survey work will create awareness among farmers, more detailed experiment is required to assist them choosing effective pest management strategies in the future against the important nematode species identified.

We were able to characterize one of the economically important nematode species, *Pratylenchus neglectus* from our samples across different counties in our survey. Root-lesion nematode was observed with the incidence of 6 % and the highest population density of 66 per 200 gm of soil. Root-lesion nematodes have a wide host range including potato, corn, wheat, and soybean (Mai et al., 1977; Smiley et al., 2005). Such host crops can help increase nematodes population level, and chances are high for them to indirectly affect the sugarbeet by creating an entrance for fungal pathogens and other sugarbeet root diseases when sugarbeet is grown frequently with these crops in rotation. During our survey work, *Helicotylenchus* group, the

spiral nematodes, had the highest incidence of 38 % and the highest population density recorded was 1,530 per 200 gm of soil. They are one of the most prevalent nematode genera found in different counties across ND and MN. Among the identified species of spiral nematode from our sampled locations, *H. pseudorobustus* is believed to exist in Northcentral USA and is considered as a mild pathogen (Norton, 1977; Norton et al., 1978). Sometimes mild pathogens are neglected in many studies, but they can cause serious problems when present in higher population level. However, the economic importance of these nematodes is yet to be studied at a higher scale. Thus, a follow up research is required to investigate its economic impact in sugarbeet and determine economic threshold levels for better management of different groups of PPNs

Complex nematode genus *Tylenchorhynchus,* was also found from our surveyed locations. They are denoted as a complex nematode genus because of their phenotypic plasticity, which leads to its misidentification due to their overlapped morphology in morphometry (Handoo et. al., 2014). It had the second highest incidence of 37% and the highest population density recorded was 620 per 200 gm of soil. The economic impact of genus *Tylenchorhynchus* is yet to be studied but they have been considered as a mild pathogen, but with higher density, one of the species of *Tylenchorynchus* has shown the significant yield loss in soybean microplot (Ross et al., 1967). Since soybean is grown in rotation with sugarbeet in our region, study on *Tylenchorhynchus* can be beneficial. Another important nematode genus, *Paratylenchus* was identified from our surveyed locations as well. It had an incidence of 28% and the highest population density measured was 600 per 200 gm of soil. Although limited research has been established to demonstrate pathogenicity of *Paratylenchus* spp. in sugarbeet, research conducted by Braun et al. (1975) reported that they could damage fruit trees. The identified species *P.*

*nanus,* has shown to cause damage to several field pea cultivars under greenhouse experiment condition in our region (Upadhaya *et al.*, 2018). Thus, further research is needed to investigate damage level of this group of nematodes in sugarbeet.

Two nematode genera, *Xiphinema, and Hoplolaimus*, were identified with minor incidence of 3 and 0.4%, respectively. The highest population densities for them were 60 and 23 per 200 gm of soil, respectively. The impact of *Xiphinema* and *Hoplolaimus* has not been studied for sugarbeet but their impact has been studied in corn in several states. Corn is grown in rotation with sugarbeet in eastern ND and MN. Therefore, there are opportunities for these groups of nematodes to impact sugarbeet. The study conducted by Niblack (2009), reported that 41-75 *Hoplolaimus* per 100 $cm^3$ of soil could act as moderate risk population and the study by Tylka et al. (2011), suggested 30-40 *Xiphinema* per 100 $cm^3$ of soil could cause severe-risk damage threshold, meaning lower population level of those two genera could possess a serious threat for a host crop. *Xiphinema americanum* has been reported to cause a decline in corn yield (Norton et al., 1978) in MN and the neighboring states. Since we found the highest population density of 23 for *Hoplolaimus* and 60 for *Xiphinema* per 200 gm of soil, it is an alert that these group of nematode need to be further identified and studied at the species level to find out their damage threshold level in sugarbeet. They were identified with lower frequency and have not been shown to cause economic significance in our region, but they might affect specific fields if found at a higher level. Although these groups of nematodes were at lower densities, it cannot be concluded that they have no economic importance in our region. More experiments are recommended to draw some solid conclusions on economic importance.

Our study shows that *Heterodera, Helicotylenchus, Tylenchorhynchus,* and *Paratylenchus* were distributed almost similarly across 13 sampled counties. However, *Paratylenchus* in Walsh and Cass counties, *Tylenchorhynchus* in Carver and Walsh counties, *Helicotylenchus* in Benson, Carver, and Aitkin counties, and *Heterodera* in Aitkin, Williams, Carver, Traill, and Walsh counties remained undetected. *Paratylenchus* and *Tylenchorhynchus* had similar distribution pattern across counties identified with those genera of nematodes and was comparable to the distribution of *Heterodera*, and *Helicotylenchus,* across counties identified with those group of nematodes. The distribution pattern of *Pratylenchus*, *Xiphinema*, *Paratrichodorus,* and *Hoplolaimus* were however, different than the distribution pattern of such dominant nematode genera across sampled counties. Various factors including cropping history, soil type, and varying climatic and weather conditions during the sampling period determines the variability of nematode type and numbers across sampled counties.

In this research, various sugarbeet fields in eastern and western ND, western MN, and eastern MT were assessed to determine PPN populations. Several PPNs with different levels of incidence and population density were detected from various sugarbeet fields across different counties surveyed. Population densities of some nematode genera such as *Pratylenchus, Hoplolaimus,* and *Xiphinema* were lower from our finding but it might be high enough for certain crop species to cause a significant yield loss as reported by different experiments conducted across the US (Niblack, 2009; Tylka et al., 2011). Higher abundance of *Heterodera, Helicotylenchus, Tylenchorhynchus,* and *Paratylenchus* from our survey results can be an alert that follow-up research is required for these groups of nematodes in relation to sugarbeet. It was a challenge for us to distinguish the cyst nematodes as they look alike morphologically. *H.*

*schachtii* is one of the major pests of sugarbeet and *H. glycines* is more prevalent in our surveyed locations, identification of those nematodes at species level was necessary. With three cyst samples from western ND and eastern MT identified as *H. schachtii* and around 31 samples from the RRV area being *H. glycines,* we concluded that no SBCN has been detected in the RRV.

However, since SBCN was identified near the eastern MT border, we need to have better prevention strategies as they might disseminate from those field locations to the RRV area in the future. Since SBCN cyst remains viable for more than 10 years, chances are high for them to disseminate from eastern MT to eastern ND as unsterilized farm equipment used in those locations might be transported and used in our region without proper sanitation. It is important to monitor SBCN and its potential infestation in our region. One of the important nematodes for sugarbeet, *Paratrichodorus allius,* has been confirmed from our survey results. Thus, another study was conducted for this group of nematodes. Morphological and molecular characterization of PPNs populations is must for those nematode genera which are found in abundant amount and are considered as an important pest for sugarbeet. Such work in future can help determine important nematode genera and estimate yield loss caused by those group specifically. Furthermore, identification and distribution of different PPNs across the region will be the critical first step which can help determine effective pest management strategies for improved sugarbeet production.

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# CHAPTER 4. HOST STATUS OF SUGARBEET AND COMMON CROPS IN ROTATION WITH SUGARBEET FOR THE STUBBY-ROOT NEMATODE, *PARATRICHODORUS ALLIUS*


## Abstract

*Paratrichodorus allius,* commonly known as stubby-root nematode is an important pest of many crops including sugarbeet. With little information on the host range of this nematode, a second study was conducted to determine the host status of sugarbeet and crops in rotation with sugarbeet for *P. allius* under greenhouse conditions. In this study, host status of seven sugarbeet and twenty-one rotational crop cultivars were tested in the greenhouse with two repetitions. The results indicated that most of the sugarbeet and rotational crop cultivars supported the reproduction of *P. allius* with reproductive factors (RF) higher than one. Among all the tested cultivars, 79% (22/28) acted as suitable hosts (SH) whereas, 21 % (6/28) acted as poor-hosts (PH). Sugarbeet cv. BTS 8337 in both experiments and cv. BTS 80RR52 in the second experiment had highest ($P < 0.05$) reproduction of *P. allius* among tested sugarbeet cultivars. Among tested rotational crops, soybean cv. Sheyenne in the first experiment and corn DK 43-46 in both experiments had highest ($P < 0.05$) reproduction of *P. allius.* Average RF from the combination of two experiments were used in this experiment to rank the host status of sugarbeet and rotational crop cultivars. Twenty-two rotational crop cultivars and sugarbeet cultivars were ranked as suitable hosts (SH) for *P. allius* reproduction (RF = 1.08 to 4.08). However, certain cultivars including sugarbeet cultivars (BTS 82RR28, and BTS 8500), corn 139VT2P, soybean SB 8807N, wheat Glenn, and dry bean Montcalm were classified as poor-hosts (PH) (RF= 0.21 to 0.62) for *P. allius* reproduction. This study will help us develop an effective crop rotation

strategy to prevent damage caused by *P. allius* to sugarbeet and its rotational crop cultivars grown in our region.

**Keywords:** *Paratrichodorus allius*, host range, sugarbeet, rotational crop, host status ranking, crop rotation.

## Introduction

The stubby-root nematode, *Paratrichodorus allius* (Jensen, 1983) are migratory ectoparasites feeding on the epidermal root cells. They can transmit tobravirus and cause corky ringspot disease in potatoes (Mojtahedi and Santo, 1999). *Paratrichodorus allius*, has been reported in several world areas including Chile, South Africa, Italy, Portugal, Israel and Tanzania (Decreamer, 1995). In the US, it was first reported from an onion field in Oregon and was later reported to be present in Pacific Northwest states including Oregon and Washington, and in California (Norton et al., 1984). It has also been reported in Eastern Idaho (Hafez, 1998) Recently, *P. allius* were detected in a sugarbeet field in Minnesota (Yan et al., 2015; 2016b; 2016c) and a potato field in North Dakota (Yan et al., 2016a). The general symptoms caused by *P. allius* in sugarbeet and different host crops may include poor growth, yellowing, stunted plants and reduced taproot with abnormal branched lateral roots (Khan et al., 2016). Fanging (Jones and Dunning, 1972; Gratwick, 1992) and docking disorder in the sugarbeet taproot was also reported (Jones and Dunning, 1972). Docking disorder is often found in sandy soil with low organic matter.

Damage caused by stubby-root nematode is highest in wet seasons, but sugarbeet plants are rarely killed by this group of nematodes (Hafez, 1998). Stubby-root nematode have six life stages including eggs, four juvenile stages, and adult stage. Adults are wormlike and are found in

the soil. The lifecycle is three to seven weeks depending upon the species, optimum soil moisture, and temperature condition. They undergo dormancy under the severe cold weather by migrating deep below the soil, up to 40 inches deep. They are transmitted from one field to another by the aid of irrigation water, wind, farm animals, human beings, and farm machinery. Stubby-root nematodes host range includes various cereal crops and potatoes (Hafez, 1998). It is believed to cause significant yield loss in multiple crops (Mojtahedi and Santo 1999). These nematodes have been associated with a wide range of crops including potatoes (Charlton et al., 2010; Gieck et al., 2007; Ingham et al., 2007b; Mojtahedi and Santo, 1999), corn and wheat (Mojtahedi et al., 2002a), beans and sunflower (Ayala et al., 1970), barley (Mojtahedi and Santo, 1999), and sugarbeet (Yan et al., 2016b; 2016c). It has been detected from one of the pea field of Ward County, ND during PPNs survey (Upadhyay et al., 2018) and in one soybean field (Yan et al., 2015).

The RRV of eastern ND and western MN is one of the major sugarbeet production regions in the US. However, no comprehensive study has been carried out for impact of PPNs and their hosts in the region thus far. Our previous comprehensive field survey identified stubby root nematode species as *Paratrichodorus allius* (KC et al., Unpublished). *Paratrichodorus allius*, is one of the important nematode pests for sugarbeet production worldwide. In 2015, a sugarbeet field with sand syndrome was detected to have stubby-root nematode and was also identified as *P. allius* (Yan et al., 2016a). With little information on the host range of *P. allius*, a second study was conducted during 2016 and 2017. Our previous field survey has helped us identify the important *P. allius* but its effect on the growth and yield of sugarbeet and its rotational crop cultivars are yet to be accessed. Since sugarbeet is grown in rotation with many

crop species in our region, the effect of this nematode is necessary to be studied for various rotational crops to maximize crop yield and minimize nematode numbers. Therefore, the objective of this study was to determine the host status of sugarbeet and the common sugarbeet rotational crops grown in the region including wheat, corn, dry bean, barley, sunflower, and soybean to *P. allius*. Effective pest management strategies for improved sugarbeet production can be achieved with the accurate identification and management of the nematodes across the region.

## Materials and Methods

### Nematode collection, extraction and species confirmation

The stubby-root nematodes used for this study were obtained from a sugarbeet field previously surveyed and infested with *P. allius* in Pembina County, ND. All the field soil samples collected were assessed in the Nematology Laboratory, NDSU. Soil samples collected from the field were thoroughly mixed and a subsample of 200 gm was taken from each thoroughly mixed composite soil samples before each nematode extraction. Sieving and decanting, and sugar floatation method were used for nematode extraction (Jenkins, 1964) and were then collected in 50 ml suspension tubes for further nematode identification and quantification. Stubby-root nematodes were identified and counted under an inverted transmitted light microscope at 100x magnification (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA) and tallied as a total number of individuals per 200 gm of soil. Single nematode samples from sixteen different infested fields were used for DNA extraction and DNA samples were utilized for further species confirmation using a molecular method (Huang et al. 2017). Nematodes were chopped and 0.5 ml sterile Eppendorf tube with 10 µl of buffer solution [2 µl of 10x PCR buffer,

2 µl of Proteinase K (600 µg/ml), and 6 µl of double-distilled water] was pipetted with nematode suspension (10 µl). They were then incubated at -20ºC for 30 mins followed by 65ºC for 1 hour and then 95ºC for 10 mins. The DNA was then processed directly for PCR assays. AlphaImager Gel Documentation System (Proteinsimple Inc., Santa Clara, California) was used for documenting banding patterns of PCR products after separating them in 2% agarose gel at 100 V for around 20 min. The remaining DNA was stored at -20ºC for subsequent experiments. *Paratrichodorus allius* specific primers (PaF11/PaR12) (Huang *et al.*, 2017) were used for species-specific PCR analysis. The amplification pattern was analyzed to investigate the presence of *P. allius.*

**Host range study**

A total of seven crops including sugarbeet and six rotational crops including corn, wheat, soybean, barley, sunflower, and dry beans were used for the experiments. Seven sugarbeet cultivars (BTS 73MN, BTS 80RR52, BTS 82RR28, BTS 8337, BTS 8500, Crystal M375, and Maribo MA305) and two to five cultivars for each of the rotational crops were analyzed for testing reproduction abilities of *P. allius* on those crop cultivars . Five corn cultivars used were (DK 43-46, DK 43-48, DK 44-13, 1392 VT2P, and LR 9487 VT2PRIB). For wheat, five cultivars used were (Glenn, Faller, Elgin, Brennan, and Barlow). Soybean cultivars used for this study were Sheyenne, SB 88007N, LS-1335NRR2, H009X7, and Barnes. For barley (Quest, and ND Genesis), sunflower (Mycogen 8N270, and Croplan 306), and dry bean (Red Hawk, and Montcalm) were used in this study. A list of cultivars used in the experiments can be found in Table 4.1. All the seeds were pre-germinated before planting so that it developed adequate roots for nematodes to feed on after planting at the greenhouse conditions.

Evaluations of the host status of sugarbeet and common crops in rotation with sugarbeet for the stubby-root nematode, *P. allius* were conducted in two different greenhouse trials in 2016 and 2017. The soil samples used in this study were the *P. allius* infested field soil and its composition was tested at the Agvise Laboratory (Northwood, ND, USA) from soil property analysis (Table 4.2).

Table 4.1. Sugarbeet and rotational crop cultivars used in this study

| Cultivars | Maturity Days/Groups | Originator [a] | Growing Regions [b] |
|---|---|---|---|
| | | **SUGARBEET** | |
| **BTS 73MN** | 90-100 | BETASEED | ND, MN |
| **BTS 80RR52** | 90-100 | BETASEED | ND, MN |
| **BTS 82RR28** | 90-100 | BETASEED | ND, MN |
| **BTS 8337** | 90-100 | BETASEED | ND, MN |
| **BTS 8500** | 90-100 | BETASEED | ND, MN |
| **Crystal M375** | 90-100 | Crystal Beet Seed | ND, MN |
| **MariboMA305** | 90-100 | MARIBO | ND, MN |
| | | **CORN** | |
| **DK 43-46** | 93 | DEKALB | ND |
| **DK 43-48** | 93 | DEKALB | ND |
| **DK 44-13** | 94 | DEKALB | ND |
| **1392 VT2P** | 92 | Proseed | ND, MN, SD |
| **LR 9487 VT2PRIB** | 87 | Legend Seeds | ND, SD |
| | | **SOYBEAN** | |
| **Sheyenne** | 0.7 | NDSU | ND, MN |
| **SB 88007N** | 00.7 | Thunder | ND, MN |
| **LS-1335NRR2** | 1.3 | Legacy | ND, MN |
| **H009X7** | 00.9 | Hefty | ND, MN |
| **Barnes** | 0.3 | NDSU | ND, MN |

**[a]** Originator refers to the developer of those sugarbeet and rotational crop cultivars.
**[b]** ND, MI, and SD indicate the states of North Dakota, Michigan, and South Dakota, respectively and NDSU, UMn and MSU represents North Dakota State University, University of Minnesota and Michigan State University, respectively. These data were obtained from different varietal trial extension bulletins from North Dakota State University (Kandel et al., 2017, Kandel et al., 2018, Ransom et al., 2018a; 2018b; 2018c; 2018d and Khan et al., 2019). For soybean, maturity group is based on its suitability to different locations. Cultivars of maturity groups 00 (double zero), 0 (zero) and 1 are suitable for eastern North Dakota and northwestern Minnesota.

Table 4.1. Sugarbeet and rotational crop cultivars used in this study (Continued)

| Cultivars | Maturity Days/Groups | Originator [a] | Growing Regions [b] |
|---|---|---|---|
| | **WHEAT** | | |
| **Glenn** | 90-120 | NDSU | ND, MN |
| **Faller** | 90-100 | NDSU | Central/Eastern ND |
| **Elgin** | 90-120 | NDSU | ND, MN |
| **Brennan** | 90-100 | Agripro/Syngenta | ND |
| **Barlow** | 90-120 | NDSU | ND, MN |
| | **BARLEY** | | |
| **Quest** | Medium | UMn | ND, MN |
| **ND Genesis** | Medium-late | NDSU | ND |
| | **SUNFLOWER** | | |
| **Mycogen 8N270** | Medium | Mycogen | ND, SD |
| **Croplan 306** | Late | Croplan | ND, SD |
| | **DRY BEAN** | | |
| **Red Hawk** | Medium | MSU | ND, MN, MI |
| **Montcalm** | Medium/Late | MSU | ND, MN, MI |

[a] Originator refers to the developer of those sugarbeet and rotational crop cultivars.
[b] ND, MI, and SD indicate the states of North Dakota, Michigan, and South Dakota, respectively and NDSU, UMn and MSU represents North Dakota State University, University of Minnesota and Michigan State University, respectively. These data were obtained from different varietal trial extension bulletins from North Dakota State University (Kandel *et al.*, 2017, Kandel *et al.*, 2018, Ransom *et al.*, 2018a; 2018b; 2018c; 2018d *and* Khan *et al.*, 2019). For soybean, maturity group is based on its suitability to different locations. Cultivars of maturity groups 00 (double zero), 0 (zero) and 1 are suitable for eastern North Dakota and northwestern Minnesota.

Table 4.2. The characteristics of soil type used in this study

| Soil characteristics | Soil Parameters |
|---|---|
| **USDA Textural Class:** | Sandy Loam |
| **Texture (%):** | |
| **Sand** | 61.0 |
| **Silt** | 29.0 |
| **Clay** | 10.0 |
| **Organic matter (LOI)*** | 2.1 |
| **pH** | 5.8 |

* LOI represents loss of ignition and is used to determine organic matter content (%) in the soil

The initial population density of *P. allius* for the first trial was 55 per 200 gm of soil and for the second set of experiment, the initial population density of *P. allius* was 67 per 200 gm of soil. All the trials were maintained under the controlled greenhouse conditions with 16 hours of daylight and temperature of 22$^{o}$C (±2) at the North Dakota Agricultural Experiment Station Greenhouse Complex, North Dakota State University. Clay pots of 16.5 cm diameter and 15.2 cm of height were used and filled with one kg of soil naturally infested with *P. allius* and thoroughly mixed for both the experiments. The pre-germinated seeds of rotational corps and small seedling of sugarbeet were later placed and transplanted in the center of the soil-filled pot at 3-4 cm depth. The completely randomized block (CRD) experimental design with five replications for each cultivar was used for this study. Five gram of controlled release fertilizer "Multicote 4" (14-14-16 NPK) was applied as an initial application on each pot to provide the nutrients for the plant growth. The crops were grown to maturity, therefore sugarbeet was harvested at 100 days whereas rotational crops at 90 days after planting. Soil and root samples were stored at the 4$^{o}$C temperature until processing to prevent nematode population decline and to facilitate the quality nematode extraction and counting procedure.

Soil samples collected from each pot were thoroughly mixed and 200 gm of soil was used for nematode extraction using sieving and decanting and sugar centrifugal-floatation method (Jenkins, 1964). Roots were rinsed with tap water to avoid the loss of nematodes from the soil attached to the roots before each extraction. After extraction, nematodes were collected in 50 ml tubes and counted under an inverted transmitted light microscope at 100x magnification (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA). The total number of stubby root nematodes obtained were counted and recorded as final nematode population out of 200 gm of soil. Nematode reproductive factor (RF), was calculated by dividing the final nematode population

density by an initial nematode population density. RF for each sugarbeet and rotational crop cultivar is the mean reproductive factor of five replicates. Host status of sugarbeet and common crops in rotation with sugarbeet for the stubby-root nematode, *P. allius*, was ranked based on reproductive factors categorized into 3 classes; Suitable Host = SH ($RF \geq 1$), Poor-Host = PH ($0.1 < RF < 1$), and Non-Host = NH ($RF \leq 0.1$) as described by Mojtahedi et al. (2003).

**Data analysis**

The statistical analysis was performed using statistical software SAS 9.4 (PROC GLM of SAS 9.4; SAS Institute Inc., Cary, NC). An F-protected least significant difference (LSD) at $P < 0.05$ was used to separate means across cultivars of each crop and to investigate significant differences in reproductive factors across those tested crop cultivars. According to F-protected least significant difference test ($P < 0.05$), RF values with same letters are not significantly different.

## Results

***P. allius* identification and confirmation**

The nematode species was identified by the conventional species-specific PCR assay (Huang *et al.*, 2017). *Paratrichodorus allius* specific primer sets were used for species-specific PCR assays. Conventional PCR using species-specific primer set PaF11/PaR12 (Huang *et al.*, 2017) amplified DNA extracts of 16 stubby-root nematode samples of which lane 14,15, and 16 represents DNA of stubby-root nematode from Pembina County, ND used by us for the experiment. Amplification of 246 bp was observed for 16 DNA samples and the positive control of *P. allius.* No amplification was detected for non-template control using double-distilled $H_2O$ (Fig. 4.1).

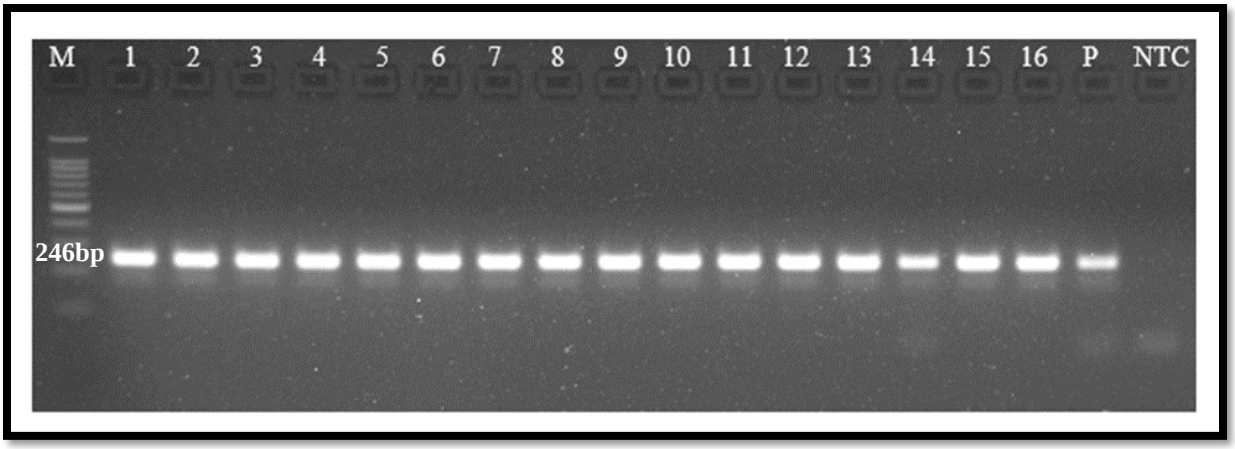


Fig. 4.1. Partial conventional polymerase chain reaction (PCR) results showing amplification using *P. allius* species-specific primers set PaF11/PaR12 (246 bp) (Huang et al., 2017), Lane M = 100-bp DNA ladder (Promega Corp.), lane 1 to 13 represents DNA of stubby-root nematode from potato fields in Sargent County, ND and lane 14,15, and 16 represents DNA of stubby-root nematode from Pembina County, ND used by us for the experiment, lane P = positive control of *P. allius*, and NTC = non-template control using double-distilled $H_2O$.

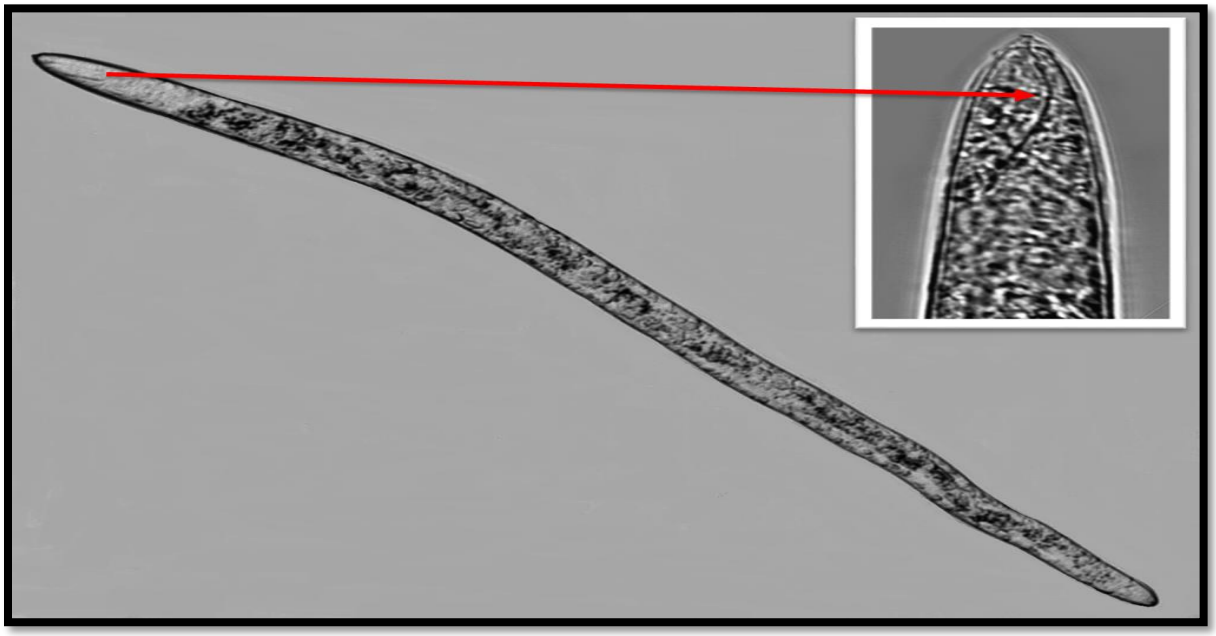

Fig 4.2. Stubby-root nematode *Paratrichodorus allius.* Red Arrow indicates "Onchiostyle", the diagnostic characteristics of this group of nematodes.

**Host range determination**

***First experiment***

Among sugarbeet cultivars tested, *P. allius* reproduced best on cv. BTS 8337, having a significantly higher ($P < 0.05$) reproduction of *P. allius* than other sugarbeet cultivars examined (Fig. 4.3). The RF values of *P. allius* on sugarbeet cultivars (BTS 8337, BTS 80RR52, Maribo MA305, Crystal M375, and BTS 73MN) were ≥ 1 and were ranked as SH (RF = 1.06 to 3.62) (Table 4.3). However, RF values of *P. allius* on sugarbeet cultivars BTS 82RR28 and BTS 8500 were 0.1 < RF < 1 and were ranked as the PH (RF = 0.30 to 0.72) (Table 4.3). Among rotational crop cultivars tested, *P. allius* reproduced best on corn (DK 43-46), and soybean (Sheyenne) having a significantly higher ($P < 0.05$) reproduction of *P. allius* than other rotational cultivars examined (Fig. 4.4). The RF of *P. allius* on wheat cultivars (Elgin, Brennan, and Barlow) were ≥ 1 and were ranked as SH (RF = 1.64 to 3.36) and the RF of *P. allius* on wheat cultivars (Faller and Glenn) were 0.1 < RF < 1 and were ranked as PH (RF = 0.30 to 0.94). The RF of *P. allius* on corn cultivars (DK 43-46, DK 43-48, Dk 44-13, and LR9487VT2PRIB) were ≥ 1 and were ranked as SH (RF = 1.60 to 4.14) and the RF of *P. allius* on corn (cv. 139VT2P) was 0.1 < RF < 1 1 and was ranked as PH (RF = 0.64) (Table 4.3). The RF of *P. allius* on soybean cultivars (Sheyenne, Barnes, HO9X7, and LS1335NRR2) were ≥ 1 and were ranked as SH (RF = 1.02 to 3.92) and the RF of *P. allius* on soybean ( SB 88007N) was 0.1 < RF < 1 and was ranked as PH (RF = 0.74) (Table 4.3). The RF of *P. allius* on dry bean (Red Hawk) was ≥ 1 and was ranked as SH (RF = 1.62) and the RF of *P. allius* on dry bean (Montcalm) was 0.1 < RF < 1 and was ranked as PH (RF = 0.88) (Table 4.3). The RF of *P. allius* on sunflower cultivars Croplan 306 and Mycogen 8N270 were ≥ 1 and were ranked as SH (RF = 1.12 to 1.32) (Table 4.3). The RF of *P. allius* on barley cultivars (Quest and ND Genesis) were ≥ 1 and were ranked as SH (RF = 2.28

to 2.70) (Table 4.3). Sixteen out of twenty-one rotational crop cultivars were SH for *P. allius* (RF = 1.0 to 4.1), except for two wheat cultivars (Faller and Glenn), one dry bean cultivar Montcalm, one soybean cultivar SB 88007N, and one corn cultivar 1392VT2P, which were ranked as PH (RF = 0.3 to 0.9) for *P. allius* (Table 4.3). Non- planted control had significantly lower reproduction (RF = 0.08) of *P. allius* for the first set of experiment (Table 4.3).

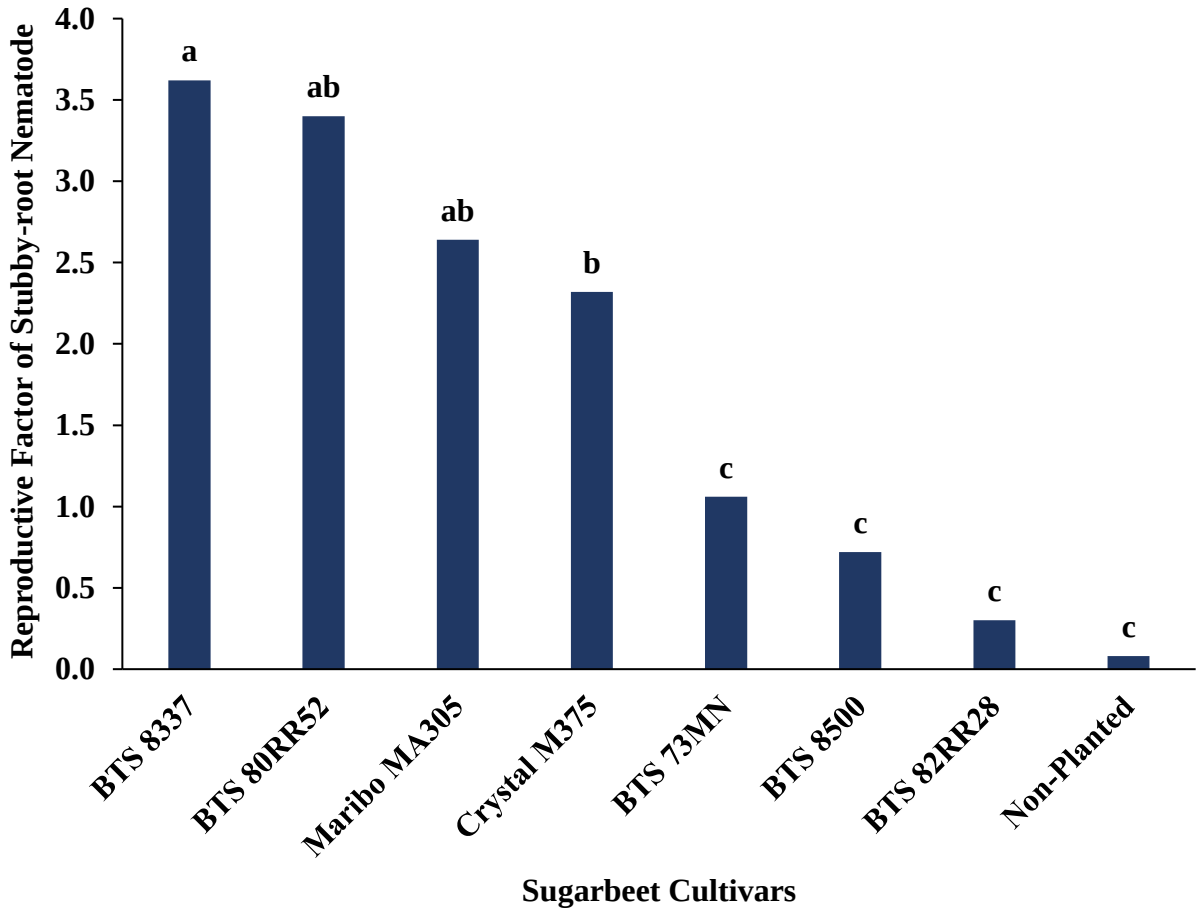


Fig. 4.3. Reproductive factor (RF) values (final nematode population/initial nematode population) of *Paratrichodorus allius* on seven sugarbeet cultivars in greenhouse. Naturally infested field soil with 55 *P. allius* / 200 gm of soil was used at planting. Means of five replications was analyzed to calculate average RF for each cultivar. According to F-protected least significant difference test ($P < 0.05$), RF values with same letters are not significantly different.

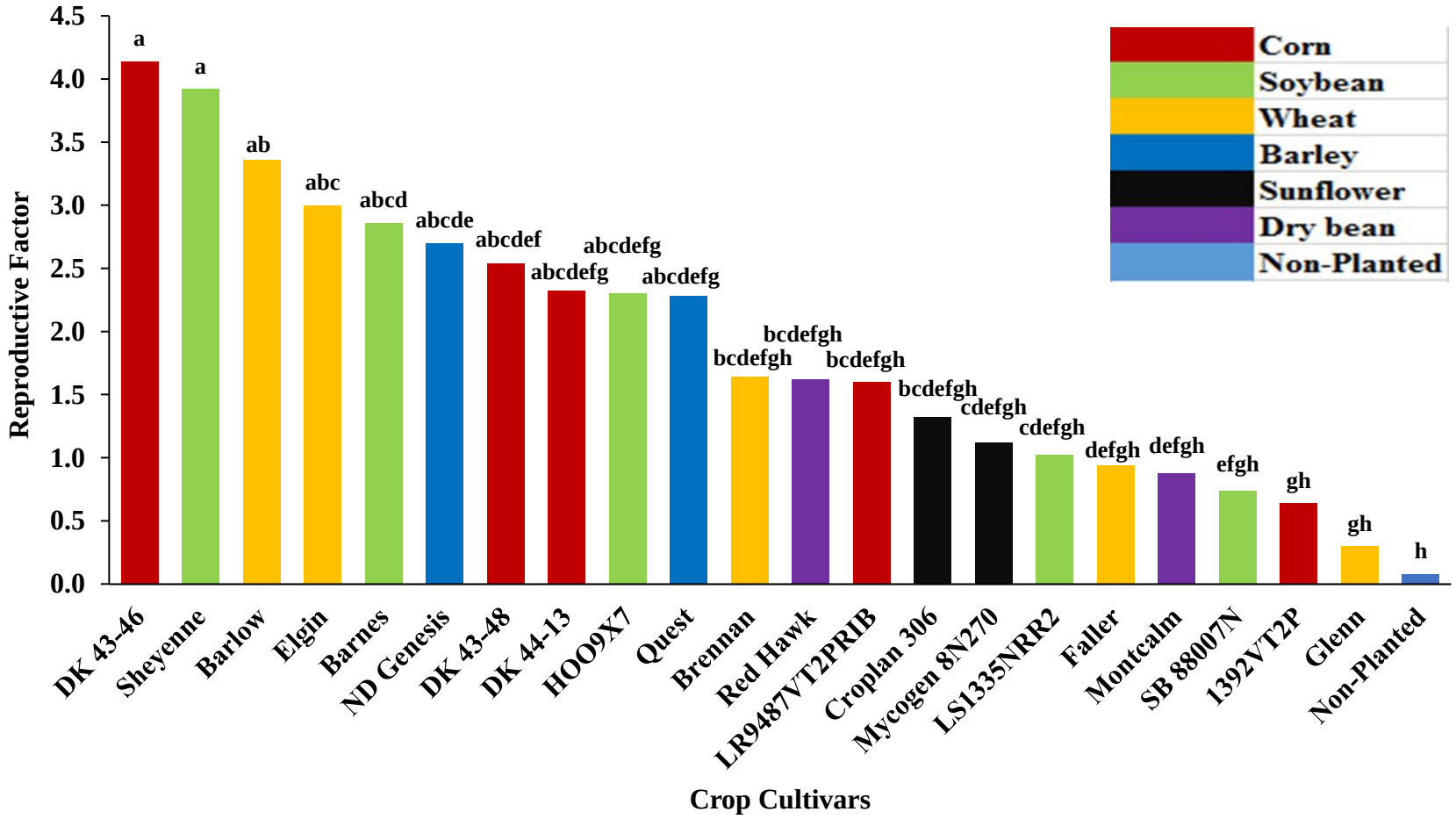


Fig. 4.4. Reproductive factor (RF) values (final nematode population/initial nematode population) of *Paratrichodorus allius* on 21 common crop cultivars grown in rotation with sugarbeet under greenhouse conditions from the first experiment. Naturally infested field soil with 55 *P. allius* / 200 gm of soil was used at the time of planting in greenhouse conditions. For, each cultivar, means of five replications was analyzed to calculate average RF. According to F-protected least significant difference test ($P < 0.05$), RF values with same letters are not significantly different.

***Second experiment***

Among sugarbeet cultivars tested, *P. allius* reproduced best on sugarbeet cultivars (BTS 8337 and BTS 80RR52), having a significantly higher ($P < 0.05$) reproduction of *P. allius* than other sugarbeet cultivars examined (Fig. 4.5). The RF of *P. allius* on sugarbeet cultivars (BTS 8337, BTS 80RR52, Crystal M375, and BTS 73MN) were ≥ 1 and were ranked as SH (RF = 1.46 to 3.32) (Table 4.3). However, RF of *P. allius* on sugarbeet cultivars (BTS 82RR28, BTS 8500, and Maribo MA305) were 0.1 < RF < 1 and were ranked as the PH (RF = 0.12 to 0.96) (Table 4.3). Among rotational cultivars tested, *P. allius* reproduced best on corn ( DK 43-46), having a significantly higher ($P < 0.05$) reproduction of *P. allius* than other rotational cultivars examined (Fig. 4.6). The RF of *P. allius* on wheat cultivars (Faller, Elgin, and Barlow) were ≥ 1 and were ranked as SH (RF = 2.04 to 2.84) and the RF of *P. allius* on wheat cultivars (Brenan and Glenn) were 0.1 < RF < 1 and were ranked as PH (RF = 0.94 to 0.98). The RF of *P. allius* on corn cultivars (DK 43-46, DK 43-48, Dk 44-13, and LR9487VT2PRIB) were ≥ 1 and were ranked as SH (RF = 1.02 to 4.02) and the RF of *P. allius* on corn (139VT2P) was 0.1 < RF < 1 and was ranked as PH (RF = 0.36) (Table 4.3). The RF of *P. allius* on soybean cultivars (Sheyenne, Barnes, HO9X7, and LS1335NRR2) were ≥ 1 and were ranked as SH (RF = 1.14 to 3.72) and the RF of *P. allius* on soybean ( SB 88007N) was 0.1 < RF < 1 and was ranked as PH (RF = 0.42) (Table 4.3). The RF of *P. allius* on dry bean (Red Hawk) was ≥ 1 and was ranked as SH (RF = 1.02) and the RF of *P. allius* on dry bean (Montcalm) was 0.1 < RF < 1 and was ranked as PH (RF = 0.24) (Table 4.3). The RF of *P. allius* on sunflower (Mycogen 8N270) was ≥ 1 and was ranked as SH (RF = 1.58) and the RF of *P. allius* on sunflower (Croplan 306) was 0.1 < RF < 1 and was ranked as PH (RF = 0.94) (Table 4.3). Finally, the RF of *P. allius* on barley cultivars (Quest and ND Genesis) were ≥ 1 and were ranked as SH (RF = 2.02 to 3.00) (Table 4.3).

Fifteen out of twenty-one rotational crop cultivars were SH for *P. allius* (RF = 1.02 to 4.02), except for few wheat cultivars (Brenan and Glenn), dry bean cultivar Montcalm, soybean cultivar SB 88007N, sunflower cultivar Croplan 306 and corn cultivar 1392VT2P, which were ranked as PH (RF = 0.3 to 0.9) for *P. allius* (Table 4.3). Non- planted control had 100% declination in reproduction (RF = 0.08) of *P. allius* for the second set of experiment (Table 4.3).

**Combination of first and second experiments**

The total number of nematodes reproduced from the initial nematode population present in a pot can give us an indication of the host status of a plant to nematodes. From the combination of two experiments, most of the sugarbeet and rotational crop cultivars tested (22/28 = 79 %) supported the reproduction of *P. allius* with RF being greater than one and served as suitable hosts. Other crop cultivars tested (21 %) acted as poor-hosts with RF being less than one. Average RF from the combination of two experiments were used in this experiment to rank the host status of sugarbeet and rotational crop cultivars to *P. allius*. Sugarbeet cultivars (BTS 82RR28 and BTS 8500), corn (139VT2P), soybean (SB 8807N), wheat (Glenn) and dry bean (Montcalm) were PH (RF= 0.21 to 0.62), whereas, all other sugarbeet and rotational crops tested were ranked as SH based on reproduction (RF = 1.08 to 4.08) of *P. allius* (Table 4.3). Among the host crops examined in our experiments, corn was most preferred host by *P. allius* with an RF value up to > 4 (Table 4.3). Overall, corn was an excellent host for *P. allius* with RF values ranging from 1.31 to 4.08 (Table 4.3). Soybean (RF = 1.08 to 3.82) and sugarbeet (RF = 1.26 to 3.47) were also an excellent host for *P. allius* with varying RF values among the cultivars tested (Table 4.3). Under the experimental conditions, wheat cultivars (Faller, Elgin, Brenan, and Barlow), barley cultivars (Quest and ND Genesis), sunflower cultivars (Croplan 306 and Mycogen 8N270), and dry bean (cv. Red hawk) were rated as SH for *P. allius* ( RF $\geq$ 1) (Table

4.3). Overall, our results indicated that the *P. allius* reproduction varied among cultivars of sugarbeet, corn, soybean, wheat, and dry bean based on average RF from the combination of two experiments (Table 4.3), and most of the sugarbeet and rotational crop cultivars tested were suitable hosts.

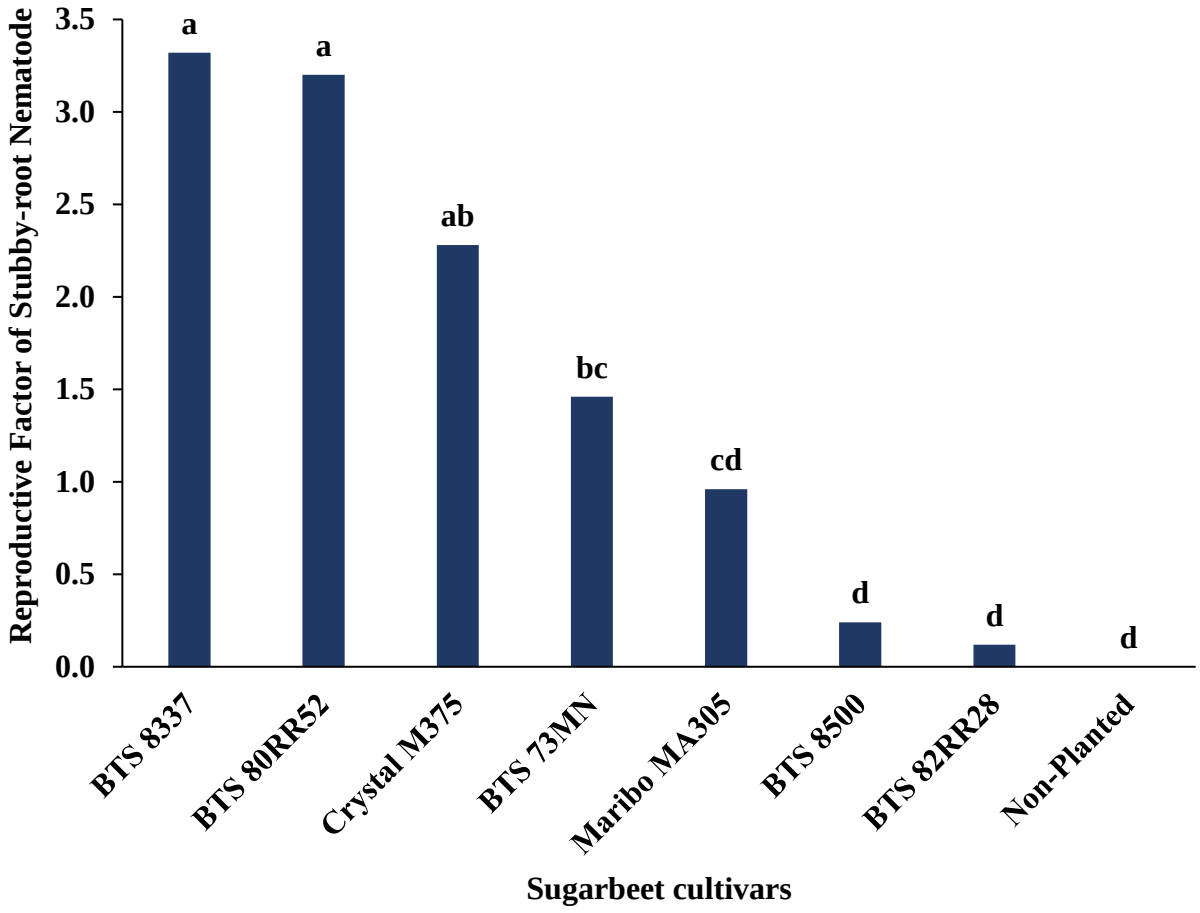


Fig. 4.5. Reproductive factor (RF) values (final nematode population/initial nematode population) of *Paratrichodorus allius* on seven sugarbeet cultivars in greenhouse. Naturally infested field soil with 67 *P. allius* / 200 gm of soil was used at planting in greenhouse conditions. Means of five replications was analyzed to calculate average RF for each cultivar. According to F-protected least significant difference test ($P < 0.05$), RF values with same letters are not significantly different.

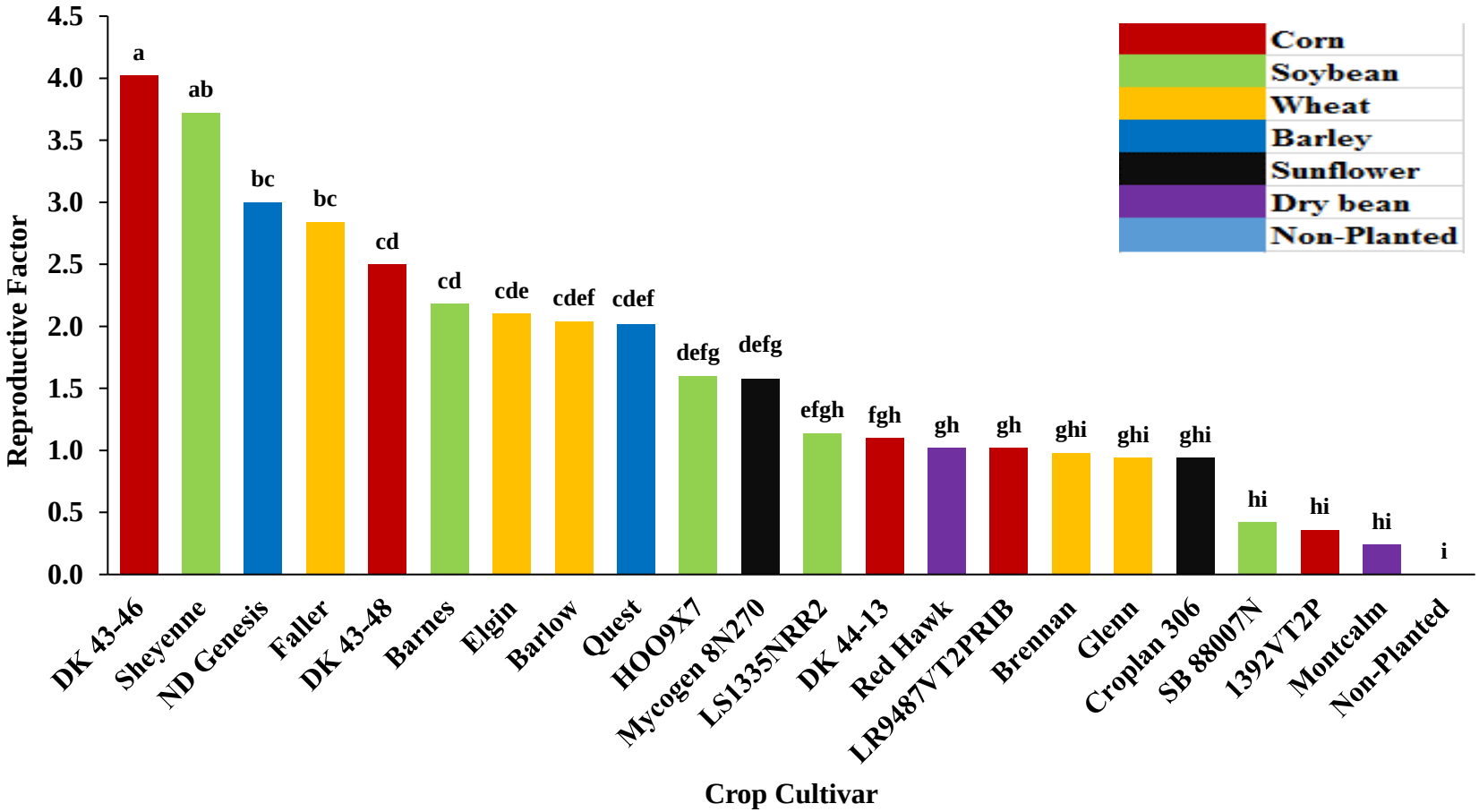


Fig. 4.6. Reproductive factor (RF) values (final nematode population/initial nematode population) of *Paratrichodorus allius* on 21 common crop cultivars grown in rotation with sugarbeet under greenhouse conditions from the second experiment. Naturally infested field soil with 67 *P. allius* / 200 gm of soil was used at the time of planting in greenhouse conditions. For, each cultivar, means of five replications was analyzed to calculate average RF. According to F-protected least significant difference test ($P < 0.05$), RF values with same letters are not significantly different.

Table 4.3. Host ranking of sugarbeet and rotational cultivars to stubby-root nematode, *Paratrichodorus allius*

| Crop | Cultivar | Experiment 1 [a] | | Experiment 2 [a] | | Combination of two experiments | |
|---|---|---|---|---|---|---|---|
| | | RF [b] | Host Ranking [c] | RF | Host Ranking | RF | Host Ranking |
| **Sugarbeet** | BTS 73MN | 1.06 | SH | 1.46 | SH | 1.26 | SH |
| | BTS 80RR52 | 3.40 | SH | 3.20 | SH | 3.30 | SH |
| | BTS 82RR28 | 0.30 | PH | 0.12 | PH | 0.21 | PH |
| | BTS 8337 | 3.62 | SH | 3.32 | SH | 3.47 | SH |
| | BTS 8500 | 0.72 | PH | 0.24 | PH | 0.48 | PH |
| | Crystal M375 | 2.32 | SH | 2.28 | SH | 2.30 | SH |
| | Maribo MA305 | 2.64 | SH | 0.96 | PH | 1.80 | SH |
| **Corn** | DK 43-46 | 4.14 | SH | 4.02 | SH | 4.08 | SH |
| | DK 43-48 | 2.54 | SH | 2.50 | SH | 2.52 | SH |
| | DK 44-13 | 2.32 | SH | 1.10 | SH | 1.71 | SH |
| | LR9487VT2RIB | 1.60 | SH | 1.02 | SH | 1.31 | SH |
| | 1392VT2P | 0.64 | PH | 0.36 | PH | 0.50 | PH |

[a] Experiment 1 and 2 were conducted to evaluate host ranking of sugarbeet and common crops grown in rotation with sugarbeet to stubby-root nematode. [b] RF (Reproductive Factor: final nematode population/initial nematode population) is the mean Reproductive factor of replication (n=5) for each sugarbeet and common crop grown in rotation with sugarbeet. [c] Host ranking based on Reproductive factor categorized into 3 classes: Suitable Host = SH ($RF \geq 1$), Poor-Host = PH ($0.1 < RF < 1$), and Non-Host = NH ($RF \leq 0.1$) as described by Mojtahedi et al. (2003).

Table 4.3. Host ranking of sugarbeet and rotational cultivars to stubby-root nematode, *Paratrichodorus allius* (Continued)

| Crop | Cultivar | Experiment 1 [a] | | Experiment 2 [a] | | Combination of two experiments | |
|---|---|---|---|---|---|---|---|
| | | RF [b] | Host Ranking [c] | RF | Host Ranking | RF | Host Ranking |
| **Soybean** | Sheyenne | 3.92 | SH | 3.72 | SH | 3.82 | SH |
| | Barnes | 2.86 | SH | 2.18 | SH | 2.52 | SH |
| | HO9X7 | 2.30 | SH | 1.60 | SH | 1.95 | SH |
| | LS1335NRR2X | 1.02 | SH | 1.14 | SH | 1.08 | SH |
| | SB 88007N | 0.74 | PH | 0.42 | PH | 0.58 | PH |
| **Wheat** | Glenn | 0.30 | PH | 0.94 | PH | 0.62 | PH |
| | Faller | 0.94 | PH | 2.84 | SH | 1.89 | SH |
| | Elgin | 3.00 | SH | 2.10 | SH | 2.55 | SH |
| | Brenan | 1.64 | SH | 0.98 | PH | 1.31 | SH |
| | Barlow | 3.36 | SH | 2.04 | SH | 2.70 | SH |
| **Barley** | Quest | 2.28 | SH | 2.02 | SH | 2.15 | SH |
| | ND Genesis | 2.70 | SH | 3.00 | SH | 2.85 | SH |
| **Sunflower** | Croplan 306 | 1.32 | SH | 0.94 | PH | 1.13 | SH |
| | Mycogen 8N270 | 1.12 | SH | 1.58 | SH | 1.35 | SH |
| **Dry Bean** | Montcalm | 0.88 | PH | 0.24 | PH | 0.56 | PH |
| | Red Hawk | 1.62 | SH | 1.02 | SH | 1.32 | SH |

[a] Experiment 1 and 2 were conducted to evaluate host ranking of sugarbeet and common crops grown in rotation with sugarbeet to stubby-root nematode. [b] RF (Reproductive Factor: final nematode population/initial nematode population) is the mean Reproductive factor of replication (n=5) for each sugarbeet and common crop grown in rotation with sugarbeet. [c] Host ranking based on Reproductive factor categorized into 3 classes: Suitable Host = SH ($RF \geq 1$), Poor-Host = PH ($0.1 < RF < 1$), and Non-Host = NH ($RF \leq 0.1$) as described by Mojtahedi et al. (2003).

## Discussion

This is the first report on detailed examination of the host preference for the stubby-root nematode species *P. allius* in ND and MN. Limited host preference screenings for *P. allius* have been done previously in other states and the association of corn and wheat (Mojtahedi et al., 2002a; Lopez-Nicora et al., 2014), beans (Ayala et al., 1970; Norton et al., 1984), sunflower (Ayala et al., 1970), and barley (Mojtahedi and Santo, 1999) to *P. allius* has been previously reported. *P. allius* has also been detected from soybean and sugarbeet fields from surveys in our region (Yan et al., 2015; 2016b; 2016c). However, detailed host preference screening of *P. allius* with twenty-eight cultivars used in this study has not been reported before. This study was conducted to determine the host status of sugarbeet and common crops in rotation with sugarbeet for the stubby-root nematode, *P. allius.* The reproduction of *P. allius* occurred on most of the sugarbeet and rotational crop cultivars, demonstrating nematodes ability to successfully develop and reproduce in sugarbeet and rotational crop cultivars. Among tested crop cultivars, 79 % (22/28) acted as suitable hosts whereas, and 21 % (6/28) acted as a poor-hosts. This result suggests that the specific cultivars of sugarbeet and rotational crops have an influential role in determining the reproduction potential of *P. allius.* Based on the RF values from two experiments combined, two sugarbeet cultivars examined were PH for *P.* allius. The range of nematode reproduction was (RF = 0.21 to 3.47) for the seven cultivars of sugarbeet tested. It demonstrates that *P. allius* has the capacity to survive on sugarbeet but poor reproduction on some sugarbeet cultivars does not make them a most suitable host. Sugarbeet cultivars, particularly BTS 8337 and BTS 80RR52 had higher reproduction of *P. allius* and they were significantly different ($P < 0.05$) when compared among tested sugarbeet cultivars.

The RF values obtained from two experiments were comparable to previous study on *P. allius* (Mojtahedi et al., 2003). Unlike research work reported by Mojtahedi et al. (2003), which used autoclaved soil and artificial inoculation of *P. allius*, our study examined the reproductive ability of *P. allius* under natural infested soil conditions. One of our other experiments to determine the reproductive ability of *P. allius* was carried out under greenhouse condition using autoclaved soil and artificial inoculation of *P. allius.* Unfortunately, using autoclaved soil condition and artificial inoculation, there was no reproduction of *P. allius.* The exact reason for this was unclear. It might be due to changes in several abiotic factors such as soil temperature, soil pH, soil texture, and other physical and chemical soil properties in autoclaved soil which is not the hospitable environment for culturing the ectoparasitic nematode, stubby-root nematode. which is considered to play a major role in the reproduction of nematode. However, successful reproduction of *P. allius* using naturally infested soil funder our experimental condition shows the ability of nematode development and reproduction and resemblance of such nematodes to grow under natural field soil conditions. Optimal conditions for artificial inoculation of this nematode need to be established to conduct further research experiments to analyze the effect of *P. allius* on plant growth and yield and to determine its economic threshold level. Our findings provide useful information to farmers of our region to choose appropriate poor-hosts identified in our study using naturally infested soil because all those sugarbeet and rotational cultivars are grown in the natural field conditions in our region.

This is the first detailed examination of host status of the most common crops grown in rotation with sugarbeet in eastern ND and MN for *P. allius.* This study confirmed that for *P. allius*, corn cultivar DK 43-46 was consistently a better host in two trials. In comparison, this nematode has also been recently reported in the corn fields in Ohio (Lopez-Nicora et al., 2014).

Overall, four out of five corn cultivars were an excellent host for *P. allius* with RF values ranging from 1.31 to 4.08. *P. allius* has also been reported in wheat fields (Mojtahedi et al., 2002a) and our results also suggested that four out of five wheat cultivars tested served as SH for *P. allius*. When stubby root nematode was first identified from a sugarbeet field in MN, the field had sugarbeet cv. BTS 8337 and was in rotation with wheat (Yan et al., 2016a). Therefore, our results demonstrated that high reproduction of *P. allius* is possible when sugarbeet and wheat crops are in rotation. Soybean (RF = 1.08 to 3.82) was also an excellent host for *P. allius* with varying RF values among the tested cultivars. Under the experimental conditions, barley cultivars (Quest and ND Genesis), sunflower (Croplan 306 and Mycogen 8N270), and dry bean (cv. Red hawk) were rated as SH for *P. allius* ( RF $\geq$ 1). These results agree with other studies that barley (Mojtahedi and Santo, 1999), sunflower (Ayala et al., 1970), and dry bean (Norton et al., 1984; Ayala et al., 1970) are good hosts for *P. allius*.

The hosting abilities of sugarbeet and rotational crops (soybean, wheat, corn, sunflower, dry bean, and barley) to *P. allius* were assessed in this study using naturally infested field soil under greenhouse conditions. Our results suggest us with the higher possibility of *P. allius* to reproduce on sugarbeet, corn, soybean, wheat, dry bean, barley, and sunflower but the response of different cultivars to *P. allius* suggest variability in reproduction ability of *P. allius. P. allius* creates a small wound on the epidermis of the plant root for their survival and can severely damage the host roots (Back et al. 2002). Later, this wound can act as an entrance for other fungal pathogens. Such wound can act as an entrance and promote the fungal growth within the seedling even after the fungal establishment in the root (Polychronopoulos et al., 1969). Therefore, it is better to avoid SH crops for rotation with sugarbeet to manage these group of nematodes but dry bean (cv. Montcalm), wheat (cv. Glenn), soybean (cv. SB 88007N), and corn

(cv. 139VT2P) identified as the PH from our experiments can be used as a better crops in rotation with sugarbeet. Among the sugarbeet cultivars tested, BTS 82RR28 and BTS 8500 can be a better choice for sugarbeet production to prevent them from *P. allius* infestation because they seem to act as PH in both of our experiments. Thus, farmers need to avoid the rotation of SH crops for *P. allius* and look for alternative non-host and poor-host crops. Furthermore, validation and follow-up field research are to be done from us before making any important suggestions at the farmers level.

The RF values obtained from our experiment suggest that *P. allius* has a wide host range making them difficult to remove from soil. Thus, nematode population cannot be completely eradicated but numbers can be lowered by regular rotation between host and poor-host species. Therefore, it is also required to estimate the damage threshold level of *P. allius.* Research at Kansas State University reported 50-100 stubby-root nematode per 100 cc of soil acted as an economic threshold level in different crop species such as corn, soybean, and wheat (Todd et al., 1993). Our initial stubby-root population was 50-67 stubby-root nematode per 200 gm of soil and has shown good reproduction of *P. allius* for different rotational crop and sugarbeet cultivars, suggesting *P. allius* possibility to affect the yield and production of different crop cultivars. The determination of the economic threshold level is needed as it helps implement timely and appropriate management strategies. Stubby-root nematodes have a wider host range including weeds, grasses, cereal crops, and potatoes (Hafez, 1998). Therefore, detailed study on the impact of *P. allius* for these crops is important because wheat, barley, corn, and soybean are widely cultivated and rotated with sugarbeet in our region. As per previous research work, the presence of *P. allius* in sunflower, dry bean, wheat, and corn, (Ayala et al., 1970; Lopez-Nicora et al., 2014; Mojtahedi et al., 2002a; Norton et al., 1984; Yan et al., 2015) as well as such crops

acting as a good hosts in our experiments, experiments considering crop-nematode interactions must not be neglected as they are rotated repeatedly in our region and can serve as an appropriate bridge for *P. allius* when rotated with sugarbeet. Finally, the results from our experiment also supports the identification of *Paratrichodorus* as a polyphagous species (Decraemer, 1995; Hooper, 1977; Rohde and Jenkins, 1957).

This study provides basic information of *P. allius* reproductive ability on sugarbeet and its rotational crops. It provides us with a piece of useful information for integrated pest management such as crop rotation, and/or use of poor or non-hosts. The finding from this research suggests us to further screen more crops cultivated in this region and use poor-hosts identified from our study. The use of such poor-hosts under crop rotation regime, along with some good management techniques can help prevent further infestation. Rotation of sugarbeet with the appropriate non-host crops will help lower nematode population. Thus, while planning for crop rotation in the sugarbeet-based cropping system, only those crops should be included which have lower or no reproduction of *P. allius* from our findings and can serve as a poor-hosts. Therefore, diverse cultivar screening using tested and non-tested cultivars is necessary for studying the effect of *P. allius* on different cultiavrs. Such diverse cultivar screening will help identify better rotational crops with lower reproduction of *P. allius*. Furthermore, this study can further help us assess damage incurred to plants in presence of *P. allius* and such damage assessment will help determine the impact of *P. allius* on present crop rotation system in our region.

In conclusion, the current study provides information on reproductive ability of *P. allius* on sugarbeet, corn, soybean, wheat, barley, dry bean, and sunflower cultivars commonly rotated in the eastern ND and MN. However, further studies on *P. allius* is warranted as the results

shows the nematodes survival and reproductive ability differed within crop cultivars. Among tested crop cultivars, 79 % (22/28) acted as SH whereas, 21 % (6/28) acted as PH. Finally, in addition to those tested crop cultivars, it is necessary to determine the reproductive ability of *P. allius* for other sugarbeet cultivars and different rotational crops which are not tested in our experiments but are grown in our region to find out more alternative non-hosts or poor-hosts cultivars for effective pest management.

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# CHAPTER 5. SUMMARY

Eight genera of PPNs including *Heterodera, Helicotylenchus, Tylenchorhynchus, Paratylenchus, Pratylenchus, Paratrichodorus, Hoplolaimus,* and *Xiphine*ma were identified from sugarbeet production fields in ND and MN in 2016 and 2017. *Heterodera, Helicotylenchus,* and *Tylenchorhynchus* were the top three nematode genera based on average population densities whereas, *Helicotylenchus* and *Tylenchorhynchus* were the top two nematode genera based on incidence. Nematode genera *Heterodera, Helicotylenchus,* and *Tylenchorhynchus* had the highest prominence as well as relative prominence values during the two-year surveying period. Sugarbeet cyst nematode was identified from western ND and eastern MT. But, sugarbeet cyst nematodes were not identified from the surveyed counties of eastern ND and western MN. One of the important species *Paratrichodorus allius* was also identified from eastern ND. Most of the cultivars of sugarbeet and their rotational crops have shown good reproduction abilities of *P. allius*. Out of seven cultivars tested for *P. allius*, two sugarbeet cultivars (BTS 82RR28 and BTS 8500) were ranked as poor-hosts, whereas five other cultivars (BTS 73MN, BTS 80RR52, BTS 8337, Crystal M375, and Maribo MA305) were ranked as suitable hosts. Twenty-one rotational crops were tested for reproduction ability of *P. allius* of which, corn cv. 1392VT2P, soybean cv. SB 8807N, wheat cv. Glenn, and dry bean cv. Montcalm were ranked as poor-hosts.

Eastern ND and western MN contribute for more than 51% of the national total sugarbeet production but has limited study on its interaction with PPNs. Therefore, this comprehensive survey and host ranking results can be an critical first step for identifying these groups of nematodes at the species level and their distribution across the region to determine the effective pest management strategies for improved sugarbeet production.

# APPENDIX. ACTIVITIES DURING SOIL SAMPLING AND GREENHOUSE TRIAL SETUP

Fig A1. Collecting soil samples from sugarbeet fields across different counties in North Dakota and Minnesota.

Fig A2. Sugarbeet field near Cavalier city, ND (Pembina County) where the stubby- root nematode inoculum (*Paratrichodorus allius)* was collected.

Fig A3. Host range experiment of different sugarbeet cultivars grown in ND for *Paratrichodorus allius.*

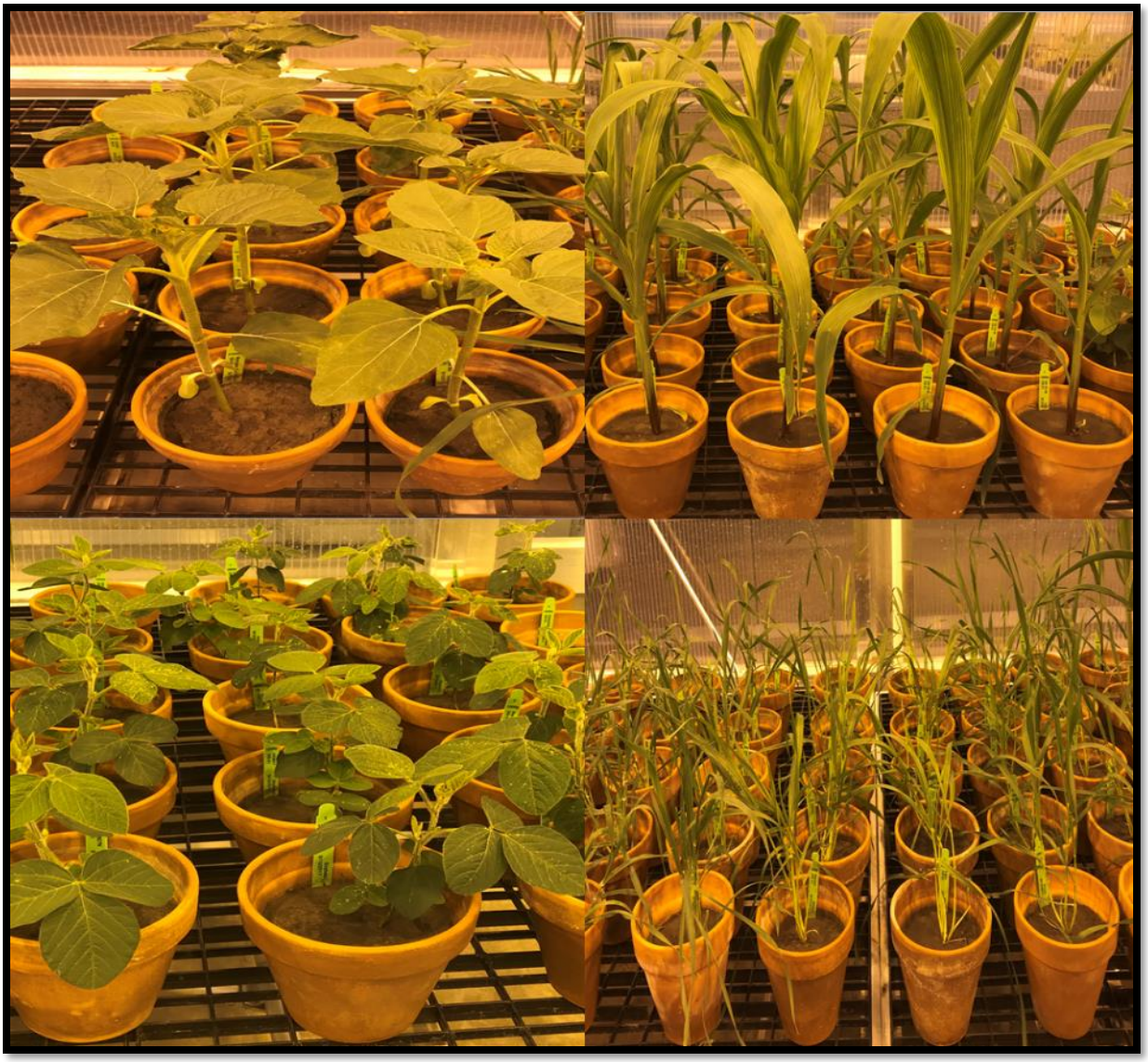

Fig A4. Host range experiment of rotational crops for sugarbeet grown in ND for *Paratrichodorus allius.*