PLANT-PARASITIC NEMATODES ON SUGARBEET IN NORTH DAKOTA AND MINNESOTA

[1]Guiping Yan, [2]Addison Plaisance, [3]Krishna Acharya, and [4]Richard Baidoo

[1]Assistant Professor of Nematology, [2]Research Specialist, [3]Graduate Student, and [4]Postdoctoral Research Associate
Department of Plant Pathology, North Dakota State University, Fargo, ND

INTRODUCTION AND OBJECTIVES

Plant-parasitic nematodes are one of the important groups of pests on sugarbeet. The sugarbeet cyst nematode (*Heterodera schachtii*) is a major pest affecting sugarbeet production in the world (Khan et al. 2016a). This nematode was identified to be the major cause of "beet weariness" which was responsible for the closure of many sugarbeet processing factories in Germany (Harveson and Jackson 2008). Sugarbeet cyst nematode (SBCN) was first discovered in the U. S. in Utah in 1895 and has spread to at least 17 states (Stewart et al. 2014). In 2012 the SBCN was first reported officially in the Yellowstone Valley of western North Dakota (Nelson et al. 2012). Sugarbeet production in Utah and Washington has been terminated largely due to heavy infestations of SBCN which has made growing of sugarbeet unprofitable. In Michigan, this nematode significantly lowered sugarbeet yield and quality, and the estimate of the annual economic loss caused by SBCN to the Michigan Sugar Cooperative is about 5-10 million dollars (Stewart et al. 2014).

Apart from the SBCN, several other nematodes such as stubby root, sting, needle, spiral, sheath, stem and bulb, root knot, false root not, and potato rot nematodes have been reported as pests on sugarbeet in California, Idaho, Colorado, and other parts of the world. However, they are not known to be a factor for sugarbeet production in North Dakota and Minnesota. Infection with plant-parasitic nematodes often enhances infection by *Rhizoctonia* and other root disease pathogens, which increases the overall effect of the nematode damage.

In June 2015, we received approximately 50 samples from the agriculturists at American Crystal Sugar Company and other extension people. Some of the samples looked like injury from stubby root nematode, needle nematode, or sting nematode. In general these plants were stunted compared to the rest of the field and the roots had very short necrotic lateral roots. Some of the samples were pulled from "sand syndrome" fields in certain areas of the Red River Valley. Six groups of plant-parasitic nematodes were detected including soybean cyst nematode, stubby-root, root-lesion, pin, spiral, and stunt nematodes. In one field with sand syndrome, stubby root nematodes were detected from the area of small and stunted plants but were not detected in the area with healthy plants, which led to the first detection of the stubby root nematode *Paratrichodorus allius* on sugar beet in Minnesota (Yan et al. 2016, Khan et al. 2016b). However, the incidence, distribution and species of the plant-parasitic nematodes across North Dakota and Minnesota are unknown. The effects of the vermiform plant-parasitic nematodes on sugar beet plant growth and crop yield are also not known.

The objectives of this research were to 1) conduct a survey of sugarbeet fields in North Dakota and Minnesota to determine the incidence, abundance and distribution of cyst nematodes and vermiform plant-parasitic nematodes; 2) determine the effect of vermiform plant-parasitic nematodes on plant growth of five sugarbeet cultivars commonly grown in ND and Minnesota; 3) determine the possible host range of stubby root nematode (*P. allius*), especially for those crops such as wheat, corn, barley, soybean, sunflower and edible beans grown in rotation with sugar beet; and 4) determine whether sugar beet varieties with the resistance to sugar beet cyst nematode are also resistant to *P. allius*.

MATERIALS AND METHODS

Soil and root samples were collected three times once during spring, once in late summer, and once during harvest from sugarbeet fields, factory districts and piling stations in the Red River Valley of ND and MN. We worked in collaboration with sugar beet company representatives and other sugarbeet producers to identify fields which might be infested with SBCN. Fields with poor sugarbeet growth possibly due to plant-parasitic nematodes and tare soil were targeted for sampling. A total of 108 soil samples were collected from sugarbeet fields in eight counties in ND and MN, and 48 soil samples were collected from sugarbeet piling stations in 13 counties in ND and MN. A soil sample consisted of 15-20 soil cores each in 2.5 cm in diameter by 30 cm deep.

Standard laboratory protocols were used in our lab for extracting nematodes from all of the samples and plant-parasitic nematodes including cyst nematodes and vermiform plant-parasitic nematodes were quantified using microscopy. Molecular procedures were optimized and utilized to differentiate SBCN from soybean cyst nematode that were found in sugarbeet fields (Ye 2012). Economically important vermiform plant-parasitic nematodes or nematode pathogens in high densities were attempted to be identified to species using molecular and morphological methods. A panel of nematode control species were requested and obtained from the USDA-ARS Nematology Laboratory in Beltsville MD.

Vermiform plant-parasitic nematode populations were extracted from soil from a naturally infested field to evaluate their effects on plant growth of five sugarbeet cultivars (BTS 8337, Crystal M375, BTS 80RR52, BTS 8233, BTS 73MNRP). The nematode inoculum were used to inoculate sugarbeet plants under controlled conditions in the greenhouse. At harvest, plants were assessed for emergence rate, plant height, shoot dry weight, root dry weight, and final nematode density. The nematode reproductive factor will be determined by dividing the final nematode populations by the initial populations inoculated into each pot.

Host crops of the stubby root nematode will be determined. Crops, including wheat, corn, barley, soybean, sunflower and edible beans, which are commonly grown in rotation with sugarbeet were evaluated as hosts for the stubby root nematode. Two cultivars for each crop were included; wheat cultivars: Faller and Glenn; corn: DK 43-46 and DK 43-48; barley: Quest and ND-Genesis; soybean: Sheyenne and Barnes; sunflower: Croplan 306 and Mycogen 8N270; and dry edible bean: Montcalm and Red Hawk. Two sugarbeet cultivars (BTS 8233, BTS 73MNRP) with resistance or tolerance to sugarbeet cyst nematode were included in evaluation for resistance to stubby root nematode.

RESULTS AND DISCUSSIONS

In 2016, soil samples (108) were collected from sugarbeet fields in 8 counties (Richland, Walsh, Cass, Pembina, Traill, Grand Forks, Clay, Norman) in ND and MN. Seven groups of plant-parasitic nematodes were detected including cyst nematode, stubby-root, root-lesion, pin, spiral, stunt, and dagger nematodes. Fifty-seven soil samples were infested with spiral nematodes ranging from 15 to 1,530/100 cc of soil. Ten samples contained root-lesion nematodes at 15-66/100cc soil, 43 samples had stunt nematodes at 18-600/100 cc of soil, 25 samples had pin nematodes at 20-320/100 cc of soil, five samples had dagger nematode at 20-60/100 cc of soil, and five samples had stubby root nematodes at 20-60/100 cc of soil. Twelve samples were found to have cyst nematodes at 20-8,600/100 cc of soil (Table 1). The average population densities of these seven groups of plant-parasitic nematodes were calculated and listed in Table 1, and the incidence (occurrence frequency) was shown in Figure 1.

In 2016, soil samples (48) were collected from sugarbeet piling stations in 13 counties (Swift, Stearns, Marshall, Polk, Clay, Norman, Wilkin, Cass, Richland, Walsh, Pembina, Grand Forks, Traill) in MN and ND. Four groups of plant-parasitic nematodes at low densities were detected including spiral, pin, dagger and cyst nematodes. Thirty-seven soil samples (77%) had spiral nematodes ranging from 20 to 320/100 cc of soil, three samples (6%) had pin nematodes at 20-80/100 cc of soil, one sample (2%) had 20 dagger nematodes/100 cc of soil, and one sample (2%) was found to have 20 cyst nematodes/100 cc of soil.

Soybean cyst nematode was first detected in ND in 2003 and in MN in 1978 (Bradley et al. 2004, Porter and Chen 2005). Infestation of soybean cyst nematode has spread to many soybean fields in which soybean is a rotational crop of sugarbeet. The soybean cyst nematode and the SBCN have very similar morphology and distinction between them is difficult and time consuming based on morphology using microscopic methods. Molecular procedures were optimized and utilized to identify the cyst nematodes to the species level. The cyst nematodes in 10 soil samples were tested using species-specific PCR assays. These samples showed PCR bands specific for soybean cyst nematode using soybean cyst nematode-specific primers but didn't produce amplification using sugarbeet cyst nematode-specific PCR primers, indicating these cyst nematodes were soybean cyst nematode but not sugarbeet cyst nematode. Likewise, the stubby root nematode we found was identified as *Paratrichodorus allius* using species-specific PCR. This is the first detection of the stubby root nematode *P. allius* in a sugarbeet field in ND.

To determine the species identity of other plant-parasitic nematodes, PCR products from these samples were purified and sequenced. The root-lesion nematode in one sample was identified as *Pratylenchus neglectus*, and the one in another sample was identified as *P. scribneri*. The spiral nematodes in two samples were identified as *Helicotylenchus microlobus*, and the pin nematode in one sample was determined as *Paratylenchus nanus*. The species identity of the dagger nematodes in two samples and the stunt nematodes in one sample were unknown based on the DNA sequence information, and more work is needed to confirm their species identity.

In September 2, one composite soil sample with 80 stubby root nematodes/100 cc soil along with 280 pin, 330 stunt and 40 spiral nematodes, collected from a field (Cavalier, ND) with "sand syndrome", was used to inoculate five sugarbeet varieties and 6 crops (wheat, corn, soybean, barley, sunflower, dry bean) each having 2 varieties. Each of these entries plus one unplanted control were planted in 5 replicates. This set of experiments was harvested in mid-December and the nematodes are being extracted, identified and counted.

In November 23, one soil sample with 55 stubby root nematodes/100 cc soil along with 345 pin and 240 stunt nematodes from the field with "sand syndrome" was planted to the six crops each having 2 varieties. Stubby root nematodes with other vermiform nematodes were extracted from 110 subsamples of the soil for inoculum. The soil was pasteurized to plant the five sugarbeet varieties for determining the effect of nematodes on plant growth by comparing with the plants inoculated with the vermiform nematodes extracted. This set of experiments will be harvested in early March.

Table 1. The population densities of plant-parasitic nematodes in 100 cc of soil from 108 soil samples collected from sugarbeet fields in 8 counties in ND and MN.

Nematode Common Name	Nematode Scientific Name	Lowest Density	Highest Density	Average Density
Spiral	*Helicotylechus*	15	1,530	88
Stunt	*Tylenchorhychus*	18	600	62
Pin	*Paratylenchus*	20	320	27
Lesion	*Pratylenchus*	15	66	3
Dagger	*Xiphinema*	20	60	2
Stubby root	*Paratrichodorous*	20	60	2
Cyst nematode	*Heterodera*	20	8,600	179

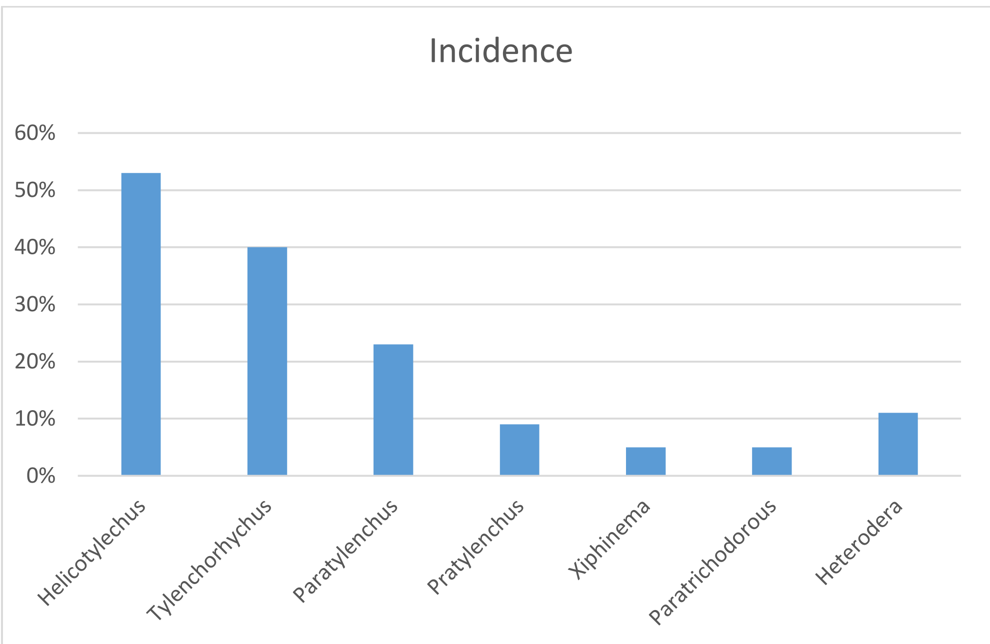

Figure 1. The occurrence frequency (incidence) of plant-parasitic nematodes in 108 soil samples collected from sugarbeet fields in 8 counties in ND and MN.

REFERENCES

Bradley, C. A., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Disease 88:1287.

Harveson, R. M. and Jackson, T. A. 2008. Sugar beet cyst nematode. NebGuide, University of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resources, Lincoln, NE. 4 p.

Khan, M., Arabiat, S., Chanda, A. K., and Yan, G. P. 2016a. Sugar beet cyst nematode. North Dakota Extension Bulletin PP1788, North Dakota State Univ., Fargo, ND. 2 p.

Khan, M., Arabiat, S., Yan, G. P., and Chanda, A. K. 2016b. Stubby root nematode and sampling in sugarbeet. North Dakota Extension Bulletin A1821, North Dakota State Univ., Fargo, ND. 4p.

Nelson, B. D., Bolton, M. D., Lopez-Nicora, H. D., Niblack, T. L., and Mendoza, L. del Rio. 2012. First confirmed report of sugar beet cyst nematode, *Heterodera schachtii*, in North Dakota. Plant Disease 96:772.

Porter, P. M. and Chen, S. Y. 2005. Sugarbeet cyst nematode not detected in the Red River Valley of Minnesota and North Dakota. Journal of Sugar Beet Research 42:79-85.

Stewart, J., Clark, G., Poindexter, S., and Hubbell, L. 2014. Sugarbeet cyst nematode (BCN) management guide. Michigan Sugarbeet REAch, Research & Education Advisory Council, Bay City, MI. 4 p.

Yan, G. P., Khan, M., Huang, D., Lai, X., and Handoo, Z. A. 2016. First report of the stubby root nematode *Paratrichodorus allius* on sugar beet in Minnesota. Plant Disease 100:1022.

Ye, W. 2012. Development of primetime-real-time PCR for species identification of soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) in North Carolina. Journal of Nematology 44:284-290.