PLANT-PARASITIC NEMATODES ON SUGARBEET IN NORTH DAKOTA AND MINNESOTA

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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 information on incidence, distribution and species of the plant-parasitic nematodes across North Dakota and Minnesota is limited. The host range of northern-grown crops to the stubby root nematode and effect 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 MN; 3) determine the host range of stubby root nematode (*P. allius*), especially for those crops such as wheat, corn, barley, soybean, and sunflower grown in rotation with sugar beet; and 4) evaluate sugarbeet varieties in ND and MN for resistance 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 in the Red River Valley of ND and MN. We worked in collaboration with sugar beet company representatives, sugarbeet producers and extension personnel to identify fields which might be infested with SBCN. Fields with poor sugarbeet growth possibly due to plant-parasitic nematodes were targeted for sampling. A total of

109 soil samples were collected from sugarbeet fields in eight counties in ND, four counties in MN, and one county in Montana. 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, Maribo MA305, BTS 73MNRP). The nematode inoculum were used to inoculate sugarbeet plants under controlled conditions in the greenhouse. At harvest, plants are 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 population by the initial population inoculated into each pot.

Hosting abilities of sugarbeet and rotational crops to the stubby root nematode will be determined. Northern-grown crops, including wheat, corn, soybean, barley, and sunflower, which are commonly grown in rotation with sugarbeet were evaluated as hosts for the stubby root nematode. Seven sugarbeet cultivars and five rotational crops were included; sugarbeet cultivars: BTS 8337, Crystal M375, BTS 80RR52, BTS 73MNRP, BTS 82RR28, Maribo MA305 and BTS 8500; wheat cultivars: Faller, Glenn, Elgin, Barlow and Brenan; corn cultivars: DK 43-46, DK 43-48, DK 44-13, 1392VT2P and LR9487VT2PRIB; soybean cultivars: Sheyenne, Barnes, HO9X7, SB-88O7N and LS-1335NRR2X; barley: Quest and ND-Genesis; and sunflower: Croplan 306 and Mycogen 8N270. A sugarbeet cultivar (BTS 73MNRP) with resistance/tolerance to sugarbeet cyst nematode were included in evaluation for resistance to stubby root nematode.

RESULTS AND DISCUSSIONS

In 2017, soil samples (109) were collected from sugarbeet fields in 8 counties (73 samples from Richland, Walsh, Pembina, Grand Forks, Cass, Traill, Benson, Williams) in ND, 4 counties (34 samples from Clay, Norman, Carver, Aitkin) in MN, and one county (2 samples from Richland) in Montana. Nine groups of plant-parasitic nematodes were detected including cyst nematode, stubby-root, root-lesion, pin, spiral, stunt, dagger, ring and lance nematodes. Thirty-eight soil samples (35%) were infested with stunt nematodes ranging from 20 to 620/100 cc of soil (Table 1, Figure 1). Thirty-five soil samples (32%) contained pin nematodes from 15 to 500/100 cc of soil. Twenty-six soil samples (24%) had spiral nematodes at 15 - 720/100 cc of soil, 11 soil samples (10%) had stubby root nematodes at 15 - 100/100 cc of soil, four samples had root-lesion nematodes at 20 - 60/100cc soil, one sample (1%) had ring nematode at 23/100cc soil, one sample (1%) had dagger nematode at 20/100cc soil, and one sample (1%) had lance nematode at 20/100cc soil (Table 1, Figure 1). Twenty soil samples (18%) were found to have cyst nematodes at 100-8,560/100 cc of soil. The average population densities of these nine groups of plant-parasitic nematodes were calculated, ranging from 20 to 1,196 (Table 1).

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 nine soil samples were tested using species-specific PCR assays and DNA sequencing. Seven of the samples from ND and MN showed PCR bands specific for soybean cyst nematode using soybean cyst nematode-specific primers but did not produce amplification using sugarbeet cyst nematode-specific PCR primers, indicating these cyst nematodes were soybean cyst nematode but not sugarbeet cyst nematode. Two of the samples from Montana close to the border of ND showed PCR bands specific for sugarbeet cyst nematode using sugarbeet cyst nematode-specific primers but did not produce amplification using soybean cyst nematode-specific primers, indicating these cyst nematodes were sugarbeet cyst nematode. DNA sequencing results confirmed that the samples from ND and MN sugarbeet fields are soybean cyst nematode and the samples from MT sugarbeet fields are sugarbeet cyst nematode.

Likewise, the stubby root nematode we found was identified as *Paratrichodorus allius* using species-specific PCR. This confirms the presence of the stubby root nematode *P. allius* in sugarbeet fields in ND and MN. To determine the species identity of other plant-parasitic nematodes, PCR products from these samples were purified and sequenced. The root-lesion nematode in two samples was identified as *Pratylenchus neglectus*. The spiral nematode in one sample was identified as *Helicotylenchus pseudorobustus*, and pin nematode in one sample was determined as *Paratylenchus nanus*. The stunt nematodes in three samples were identified as a new species that haven't been reported in any literature. More work is needed to further validate the species identity of these plant-parasitic nematodes.

On September 15, 2017, one composite soil sample with 67 stubby root nematodes/100 cc soil along with 160 pin, 160 stunt and 220 spiral nematodes, collected from a field (Cavalier, ND) with a history of "sand syndrome", was used to inoculate seven varieties of sugarbeet, five varieties of each of wheat, corn and soybean crops, and two varieties of each of barley and sunflower. Each of these entries plus one unplanted control were planted in 5 replicates. This set of experiments was harvested on December 22, 2017 and the nematodes are being extracted, identified and counted to determine the resistance reactions of the sugarbeet varieties and hosting abilities of the crop species and varieties.

A soil sample was collected from a field infested with stubby root nematodes. This field (Cavalier, ND) has a history of "sand syndrome". Stubby root nematodes with other vermiform nematodes were extracted from 138 subsamples of the soil for obtaining enough inoculum. The soil was pasteurized to plant the five sugarbeet varieties for determining the effect of nematodes on plant growth by comparing to the plants inoculated with the vermiform nematodes extracted. Two sets of experiments were set up on November 1, 2017 and November 27, 2017, and will be harvested in February.

Table 1. The population densities of plant-parasitic nematodes in 100 cc of soil from 109 soil samples collected from sugarbeet fields.

Nematode Common Name	Nematode Scientific Name	Lowest Density	Highest Density	Average Density
Spiral	*Helicotylechus*	15	720	133
Stunt	*Tylenchorhynchus*	20	620	83
Pin	*Paratylenchus*	15	500	104
Lesion	*Pratylenchus*	20	60	40
Dagger	*Xiphinema*	20	20	20
Stubby root	*Paratrichodorous*	15	100	36
Cyst nematode	*Heterodera*	100	8,560	1,196
Ring	*Mesocriconema*	23	23	23
Lance	*Hoplolaimus*	20	20	20

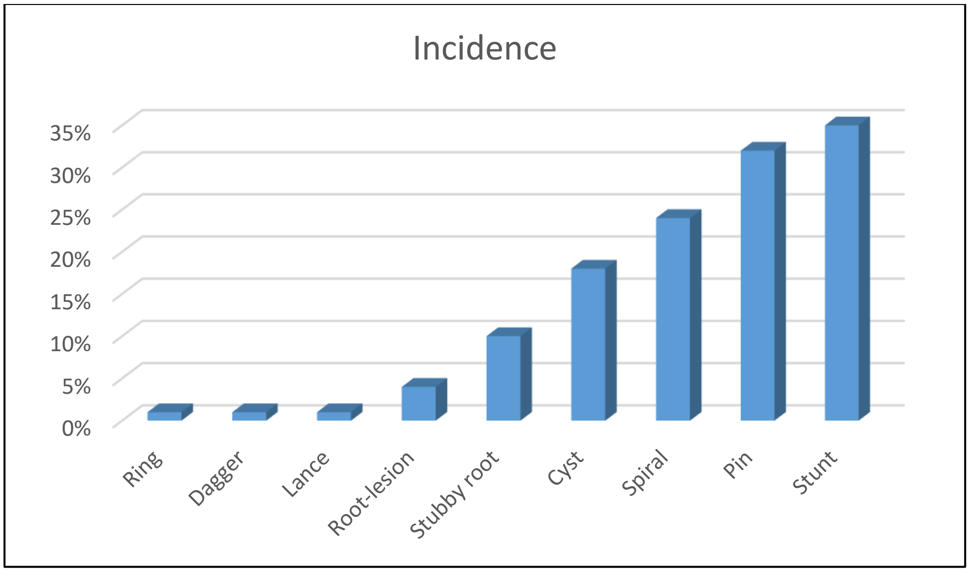

Figure 1. The occurrence frequency (incidence) of plant-parasitic nematodes in 109 soil samples collected from sugarbeet fields.

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