IMPACT OF THE SOYBEAN CYST NEMATODE ON SEEDLING DISEASES OF SUGARBEET

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Soybean cyst nematode (SCN; *Heterodera glycines*) was first reported in North Dakota in 2003 (Bradley et al. 2004) and was recently discovered in the Red River Valley (RRV) of Minnesota. SCN is now well established in Cass and Richland Counties of North Dakota and Wilkin, Clay, Norman and Red Lake counties in Minnesota, and is likely to continue to progress northward as long as soybean production continues. The nematode is easily moved from field to field in soil on farm equipment and in harvesting operations. The nematode survives from year to year as eggs within cysts. Although SCN is the most important pathogen of soybean in the United States (Niblack 2005), it is not reported to reproduce on sugarbeets. However, SCN is very closely related to the sugarbeet cyst nematode (SBCN). Indeed, the two species can hybridize to form fertile offspring (Colgrove et al. 2006).

When SCN eggs hatch, second stage juveniles (J2) emerge and maneuver through the soil to find a host root (Kim et al. 1987). Within hours of arriving at the surface of a susceptible soybean root, the nematode has penetrated through epidermal cells and migrated intracellularly to the vascular cylinder of the root. The female juvenile then initiates a permanent feeding site called a syncytium from which it draws nutrients from the plant root for its growth and development. However, when the juvenile arrives at the root surface of a non-host plant, penetration of the root can occur, but the syncytium is not established. These larvae either will die or may exit the non-host root.

SCN can build to high populations in field soil in the RRV. For example, fields have been measured that had average egg densities of over 10,000 eggs/100 cm^3 soil, and spots within fields had levels of 30,000 eggs/100 cm^3 soil in Richland county. When sugarbeet is planted into SCN-infested soil, the nematode may attempt to penetrate and establish itself in the sugarbeet roots. Such penetration attempts are likely to create lesions on the root surface. In addition, wounded roots may have an altered production of root exudates that attract sugarbeet pathogens. Since wounding of the sugarbeet root is known to increase disease severity for several sugarbeet diseases, the lesions made from entry by SCN might offer entry points for several sugarbeet pathogens. If penetration by SCN were to occur at high levels, there is a possibility it could increase susceptibility of sugarbeet roots to root pathogens such as *Rhizoctonia solani*, *Aphanomyces cochlioides*, *Verticillium dahliae*, or *Fusarium* species, especially in the seedling stage when plants are more susceptible to pathogens. In soybean, SCN is well known to increase root diseases caused by fungal pathogens. Therefore, the objective of this study were to determine if (i) juveniles can penetrate sugarbeet root tissue under field conditions, (ii) sugarbeet age influences nematode penetration, and (iii) SCN causes increased susceptibility to soil-borne pathogens.

Materials and Methods:

SCN penetration under field conditions. To determine if SCN can penetrate sugarbeet under normal field conditions, two locations in the Minn-Dak growing region were investigated. Site #1 was located 11 miles east of Wheaton, MN. Site #2 was located 7 miles east of Herman, MN. Two sugarbeet cultivars (SBCN-resistant and SBCN-susceptible) were planted at each location. Plants were collected at the 2-4 leaf stage in 10 locations within the field. Once plants were collected, they were taken back to the laboratory where they were washed to remove any excess soil. Roots were freeze-dried and ground to a homogenous powder. Genomic DNA was isolated using the CTAB method (Stewart and Via 1993). A second collection was taken two weeks after the first.

Influence of sugarbeet age on SCN penetration. To determine the age at which sugarbeet is most susceptible to SCN penetration, the SBCN susceptible cultivars M832224 and SES-SBCN-SUS and the SBCN resistant cultivars 0957-22 and SES-SBCN-RES were planted weekly in the greenhouse in Sunshine mix #1 (Sungro Horticulture, Canada). After 5 weeks, approximately 10,000 SCN eggs were added to the soil of each pot near the sugarbeet hypocotyl. After inoculation, the plants were kept in the greenhouse for 14 days maintained at 27°C. After the incubation period, roots were removed from soil, cleaned of excess potting soil, freeze dried, and ground to a homogenous powder. DNA was isolated using a CTAB method described above. Semi quantitative polymerase

chain reaction (PCR) was performed as described by Thomma et al (2006) with primers that were specific to SCN and sugarbeet actin. PCR was conducted using GoTaq Flexi DNA Polymerase kit (Promega, Madison, WI) following the manufacturer's instructions with a final $MgCl_2$ concentration of 2.5 mM. PCR conditions were as follows: an initial 95°C denaturation step for 3 min followed by denaturation for 30 s at 95°C, annealing for 30 s at 62°C and extension for 30 s at 72°C for 32 cycles. PCR amplicons were sequenced directly (McLab, South San Francisco, CA). This experiment was repeated.

Influence of SCN on *R. solani* disease severity. To determine if pre-infection by SCN increases disease severity from *R. solani* AG 2-2 IIIB, seeds of the cultivar M832224 (SBCN susceptible) were planted in Sunshine Mix #1 (Sungro Horticulture, Canada) in 10 cm pots. At 18 days post emergence, half of the total plants in the experiment were inoculated with 10,000 SCN eggs as described above. Twenty-four hours later, two barley kernels infested with *R. solani* AG 2-2 IIIB were placed approximately 2-3 cm from the hypocotyl and < 1cm beneath the surface of the soil. The plants were then incubated for 10 days in the greenhouse where the temperature was maintained at 27°C. After 10 days, plants were pulled from the soil and were rated for *Rhizoctonia* disease using a 0-7 rating scale (Bolton et al. 2010). This experiment was repeated twice. Each repeat had 40 replications per treatment.

Results

SCN penetration under field conditions. Amplification with SCN-specific primers revealed that SCN was present in both sugarbeet cultivars in both fields. Semi-quantitative PCR results revealed SCN infected both cultivars with no clear cultivar preference (not shown).

Influence of sugarbeet age on SCN penetration. Semi-quantitative PCR was utilized to quantify the amount of SCN in sugarbeet roots. In cultivar M832224, SCN was found only in plants inoculated at 1 and 2 weeks post emergence. In cultivar SES-SBCN-SUS, SCN was found in all four plant ages, with the 2 weeks post emergence time point showing the most SCN present. In cultivar 0957-22, SCN was found in 1 and 4 week post emergence plant ages, while in SES-SBCN-RES, SCN was found in weeks 2, 3, and 4 post emergence plant ages. The repeat of this experiment produced similar results. Taken together, plants inoculated at 2 weeks post emergence appeared to be most susceptible to SCN penetration.

Influence of SCN on *R. solani* disease severity. Repeat 1 and 2 showed significant difference between the mean root necrosis of plants that were inoculated with SCN and *R. solani* compared to plants that were only inoculated with *R. solani* (Fig. 1). While there was no significant difference in repeat #3, there was still an elevated amount of mean necrosis.

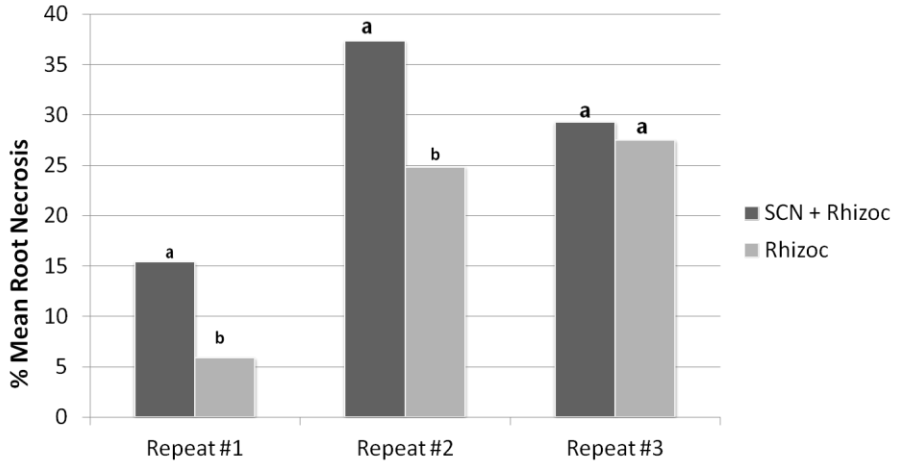

Figure 1. Influence of pre-infection by SCN on *Rhizoctonia solani* disease severity.

Discussion

In this study, we determined that SCN could penetrate the roots of sugarbeet seedlings under a normal field environment. Moreover, SCN was shown to penetrate both SBCN-susceptible and -resistant varieties equally. Nematode resistance is typically defined by the inability to complete a life cycle on the host root (Kim et al. 1987). Therefore, it may not be surprising that SBCN nematode resistance did not impart penetration resistance to SCN.

We determined that plants at two weeks post emergence were most susceptible to SCN penetration. Therefore, we utilized that time point to assess whether pre-infection by SCN would increase susceptibility to *R. solani*. In two of the three repeats of this study, there was a significant increase in the amount of disease in plants pre-infected with SCN (Fig 1). To determine whether SCN can break down *R. solani* tolerance, SCN infection of *R. solani* tolerant cultivars will be utilized in future studies. Since we show that SCN can increase susceptibility to *R. solani*, we are also interested if this is the case with other important soil pathogens of sugarbeet.

Literature cited

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