

## SOYBEAN CYST NEMATODE SOIL SAMPLE METHODOLOGY AND TEST RESULTS MEMO

Date: Tuesday, June 30, 2015

Project: Big Stone South to Ellendale (BSSE) 345kV Transmission Line

Subject: SCN Memo #3: BSSE SCN Soil Sample Methodology and Test Results

### BACKGROUND INFORMATION

The Public Utilities Commission of South Dakota (the Commission) granted Montana-Dakota Utilities Co. and Otter Tail Power Company (jointly, the Owners) a permit to construct and operate a 160- to 170-mile-long 345 kV transmission line from a new substation to be built near Ellendale, North Dakota to the Big Stone South Substation near Big Stone City, South Dakota (the Project). Condition 17 of the Permit, as amended by the Commission's August 22, 2014 Order, states:

*Applicant shall develop and implement a mitigation plan to minimize the spread of soybean cyst nematode, consistent with Exhibit 23, in consultation with a crop pest expert. After Applicant has finished the soil sample field assessment in accordance with the specifications for such assessment prepared in consultation with an expert in the proper methodology for performing such a sampling survey, Applicant shall submit to the Commission a summary report of the results of the field assessment and Applicant's specific mitigation plans for minimizing the risk of the spread of soybean cyst nematode from contaminated locations to uncontaminated locations. At such time and throughout the construction period, one or more Commissioners or Staff shall have the right to request of Applicant confidential access to the survey results to enable the verification of the survey results, assess the appropriateness of the mitigation measures to address such results, and monitor the execution of the plan during construction.*

Soybean Cyst Nematode (SCN) scientifically known as *Heterodera glycines*, is a species of roundworm that infects the roots of soybeans. When present in sufficient numbers in a field, it can dramatically reduce soybean yields. Minimizing and managing the spread of SCN is important to the State of South Dakota and to the landowners along the Project route.

The Owners, in consultation with South Dakota State University (SDSU), have developed this sampling protocol as part of its SCN mitigation plan. This memo details the SCN sampling methodology, including the process for determining sample patterns, collecting samples, and



the communication and collaboration procedures used by the Project to address Condition 17 of the Permit.

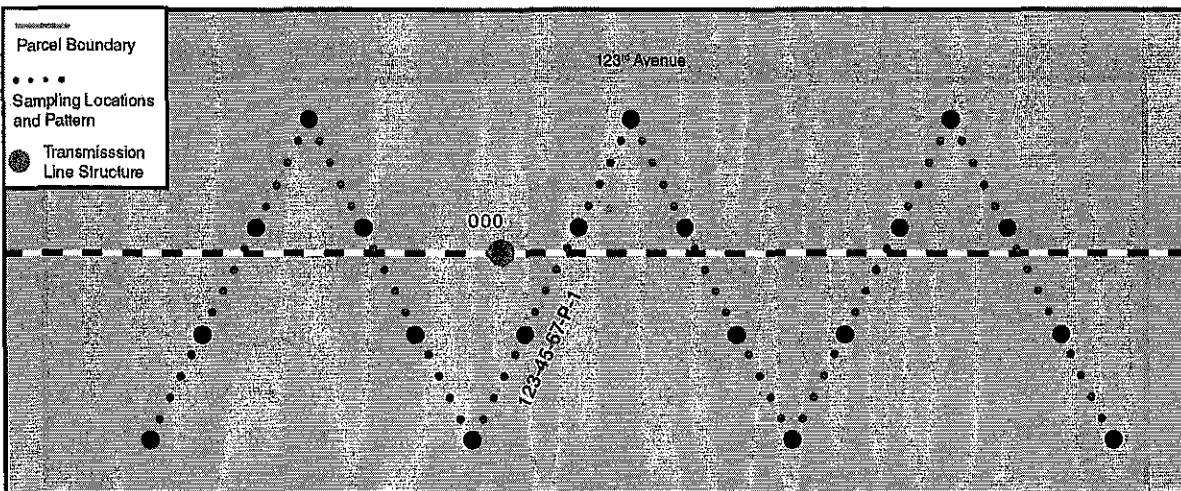
## **SCN SAMPLING METHODOLOGY**

Field staff received training from Dr. Emmanuel Byamukama at SDSU in Brookings, South Dakota in October 2014. The first portion of the training consisted of a 30-minute informational session on the history, current threats, and dangers of the spread of SCN. Dr. Byamukama provided an overview of the sample collection procedures as outlined in more detail below. Field staff received an overview of the Project and importance of the field work they would conduct. Additionally, the mitigation measures to use in the field were reviewed to minimize the likelihood of spreading SCN during field sampling.

Following the training, Dr. Byamukama and the field staff drove to the Project area for field work orientation. Dr. Byamukama demonstrated the proper technique for extracting soil samples for SCN testing. This technique included pushing a soil probe at least six inches into the soil and then extracting the soil from the probe using a utensil into a re-sealable plastic bag labeled with a pre-defined sample ID. If necessary, plant residue was brushed aside to expose the soil. Dr. Byamukama explained it was important to brush vegetation away because the soil sample needed to include enough soil to test and be deep enough reach the soybean root zone in the sample since that is where SCN would be found in a contaminated field.

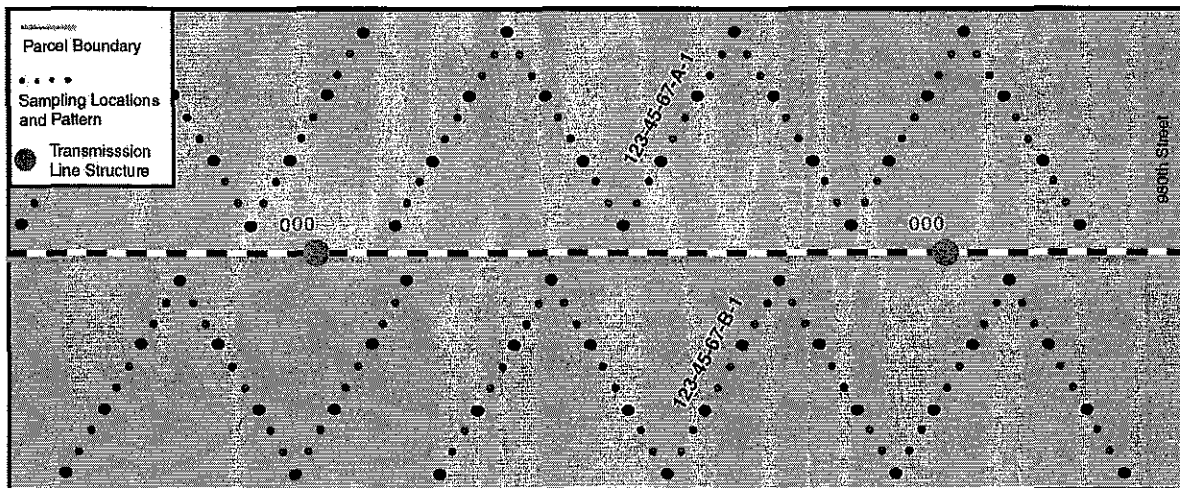
Soil sample locations were planned and mapped for the field crews prior to the start of field work. Dr. Byamukama facilitated and approved this process. A zig zag pattern composed of 19 sampling points was used to cover an area of about 0.25-mile-long by 500-foot-wide (see Figure 1 below).

**Figure 1: Zig Zag Pattern Identifying Soil Sample Locations**



The zig zag pattern was placed within most parcels of cultivated land along the entire Project route. A 500 foot buffer (250 feet to each side of the permitted route centerline) was used to determine boundaries of the sampling patterns ("Sampling Area"). For parcels greater than 0.25 miles in length, the sample area was divided into 0.25 mile long segments, or in equal parts, and each segment was sampled separately with a unique soil sample ID. Where the 500 foot buffer intercepted multiple parcels, additional sample patterns were placed so each parcel had its own sample (see Figure 2). Each zig zag pattern, even where multiple patterns were within the same parcel, had its own unique soil sample ID. The soil collected from the 19 sample points were placed within the same zip-lock bag, mixed, and labeled with their soil sample ID for testing. The goal in obtaining 19 sample points was to obtain a representative sample of each parcel. Some parcels of land narrowly intersected the 500 foot buffer or did not contain cultivated land and staff used professional judgment in determining if a soil sample was needed for those parcels or not.

Figure 2: Zig Zag Pattern Where the 500 Foot Buffer Intersects Two Parcels



The field staff used GIS information provided by the local and state agencies along with aerial imagery to identify cultivated lands and sample patterns. Staff assumed and communicated that not all land identified as cultivated would actually be cultivated, and some areas could potentially be missed due to the outdated aerial photographs and agency data. To account for the changing landscape, field staff used GPS units loaded with the data points to find sample locations. Other modifications to the sample patterns included soil samples placed on wetlands or non-cultivated land of the same sample pattern. Where this occurred, staff still extracted soil from 19 sample points, but did so outside the pre-defined pattern. When a sample pattern was altered, the staff created points on the GPS equipment noting the new sample point locations.

SCN soil sampling took four weeks in fall 2014 and about 2 weeks in the spring 2015 to complete.

Dr. Byamukama met with field staff 1-2 days per week to collect the zip-lock bags of soil samples to transfer to SDSU to test for SCN. SDSU and field staff had ongoing communication during this time to ensure samples were correctly labelled, collected and transported to SDSU for testing.

SDSU measured 100 cm<sup>3</sup> of soil for each sample and elutriated it for SCN. To verify that a negative sample is truly negative, first time SCN-negative samples were elutriated twice. SCN was assessed as present or absent for each parcel for the purposes of the Project and the SCN population density for each positive sample was also measured. A soil sample was considered positive for SCN if at least one egg was found in one or more replicates. SDSU reported these results to the Project on the spreadsheet they used to communicate sample patterns collected by field staff and received by SDSU.

## SCN TEST RESULTS

Initially, 719 samples were identified to test for SCN. During the 2014 field work 526 sample were collected, 5 additional samples were identified in the field as needing testing, and 29 were removed because testing was not needed (reasons included the land was not cultivated or was out of the Sampling Area). During the 2015 field work 88 samples were collected, 23 additional samples were identified as needing testing, and 41 were removed because testing was not needed. In total, 63 samples (9.3%) remained uncollected due to the Project's lack of access to the parcel of land

Table 1: Sample Pattern Collection Counts

	Number	Percent (%)
<b>Samples (2014)</b>	<b>526</b>	<b>77.7</b>
<i>Pre-sampling Soil Sample Patterns</i>	719	
<i>Soil Sample Patterns ID'd in field</i>	(+)5	
<i>Soil Sample Patterns Removed</i>	(-)29	
Soil Samples Uncollected Due to Weather and Lack of Access	169	25.0
<b>Samples (2015)</b>	<b>88</b>	<b>13.0</b>
<i>Pre-sampling Soil Sample Patterns Remaining</i>	169	
<i>Soil Sample Patterns ID'd for testing</i>	(+)23	
<i>Soil Sample Patterns Removed</i>	(-)41	
<b>Soil Samples Uncollected Due to Lack of Access</b>	<b>63</b>	<b>9.3</b>
<b>TOTAL PATTERNS</b>	<b>677</b>	<b>100.0</b>

Egg counts were provided by SDSU in test results as they are an indicator of level of infestation and are used in determining management strategy. Lower egg counts indicate impacts from an infection that is more manageable by the producer. Although each parcel of land is unique, an egg count of 1-2,000 eggs/100 cm<sup>3</sup> is considered a low infection, 2,001 – 12,000 is considered a medium infection and egg counts over 12,000 eggs/100 cm<sup>3</sup> is considered highly infected (Byamukama & Tande, 2013).

In 2014, 526 sample patterns were successfully collected and brought to SDSU for testing. A total of 22.2% of the samples tested positive for SCN. The results of the SCN testing are summarized with egg counts in Table 2.

Table 2: SCN Testing Results (2014)

	Number	Percent (%)	
<b>Total Samples (2014)</b>	<b>526</b>	<b>100.0</b>	
<b>Total Positive for SCN</b>	<b>117</b>	<b>22.2</b>	
# Eggs per 100 cc of soil		Percent (%) of Positive Samples	Percent (%) of All Samples
<b>Total</b>	<b>117</b>	<b>100.0</b>	<b>19.1</b>
1-50 Eggs	61	52.1	9.9
51-2,000 Eggs	55	47.0	9.0
2,001-3,000 Eggs	1	0.9	0.2
3,001+ Eggs	0	0.00	0.00

In 2015, 88 samples were successfully collected and brought to SDSU for testing. A total of 35.2% of the samples tested positive for SCN. The results of the SCN testing are summarized with egg counts in Table 3.

Table 3: SCN Testing Results (2015)

	Number	Percent (%)	
<b>Total Samples (2015)</b>	<b>88</b>	<b>100.0</b>	
<b>Total Positive for SCN</b>	<b>31</b>	<b>35.2</b>	
# Eggs per 100 cc of soil		Percent (%) of Positive Samples	Percent (%) of All Samples
<b>Total</b>	<b>31</b>	<b>100.0</b>	<b>5.1</b>
1-50 Eggs	4	12.9	0.6
51-2,000 Eggs	25	80.7	4.1
2,001-3,000 Eggs	1	3.2	0.2
3,001+ Eggs	1	3.2	0.2

A total of 614 samples were successfully collected and brought to SDSU for testing between 2014 and 2015. The cumulative number of samples that tested positive for SCN was 24.1%. The result of the SCN testing is summarized in Table 4.

Table 4: SCN Testing Results (All samples)

	Number	Percent (%)	
<b>Total Samples (All)</b>	<b>614</b>	<b>100.0</b>	
<b>Total Positive for SCN</b>	<b>148</b>	<b>24.1</b>	
# Eggs per 100 cc of soil		Percent (%) of Positive Samples	Percent (%) of All Samples
<b>Total</b>	<b>148</b>	<b>100.0</b>	<b>24.1</b>
1-50 Eggs	65	43.9	10.6
51-2,000 Eggs	80	54.1	13.0
2,001-3,000 Eggs	2	1.3	0.3
3,001+ Eggs	1	0.7	0.2

The Project chose to treat all infected fields equally, but it is important to note that 43.9% of the SCN positive fields tested in the low infection range, with the highest SCN egg count result being 3,950 eggs/100 cm<sup>3</sup>. SCN test results conducted by the Project may not be a complete reflection of a field's SCN infection because samples were only collected in the 500-foot Sampling Area. SCN is known to affect some areas more than others in a cultivated field including areas of higher pH, low spots, spots prone to flooding, and field entrances (Byamukama & Tande, 2013). Because construction and maintenance of the Project is not expected to impact an entire field crossed by the Project, and after consultation with SDSU, the Project determined not to test outside of the Sample Area. SCN may in fact be present on other areas of the landowner's parcel, even if the test is negative. The Project intends to do no further testing prior to construction. Landowners are encouraged to continue to test their land for SCN including outside of the Project's Sample Area to better understand and develop their own SCN management plans.