Comparison of Two Soil Sampling Methods for Estimating Population Densities of *Heterodera glycines* Cysts

Ethan Smrtnik and Terry Niblack, The Ohio State University, Columbus 43210; Pierce Paul and Anne Dorrance, Department of Plant Pathology, The Ohio State University, Wooster 44691; and Dain Bruns, Syngenta Crop Protection, Marysville, OH 43040

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**ABSTRACT**


Soybean cyst nematode (SCN) is a significant economic pest of soybean in Ohio and the Midwest, in which yield losses are directly linked to the population density and distribution of cysts within a field. Advances in automation technology have been used to expedite soil fertility sampling for large-scale and high-density field maps. In this study, we explored the use of this technology as an option for sampling for SCN. There was a significant positive correlation for the number of SCN cysts between soil samples collected with an Automated Precision Soil Sampler and those collected using the traditional soil sampling method from three separate fields ($P < 0.05$, $r$ values of 0.79 to 0.93). These results suggest that expedited soil sampling in fields with high population densities could be an added benefit for soybean farmers to encourage more sampling for SCN to monitor population levels as part of their overall management program.

**INTRODUCTION**

Soybean cyst nematode (*Heterodera glycines*) is one of the most devastating pests of soybean and presents a major economic burden to soybean farmers in Ohio, the north-central region, and throughout the world (Koenig and Wrather 2010; Niblack 2005). Soybean cyst nematode (SCN) was first reported in soybean fields in Ohio in 1987 (Riedel and Golden 1988), and by the mid 1990s, SCN was found in 73% of the soybean acreage within the state (Wilson et al. 1996). In a recent survey of 143 soybean fields, 81.8% were positive for SCN; only 6 of the 143 soybean fields sampled had population densities exceeding an economic threshold of greater than 2,000 eggs/100 cm$^3$ soil (Lopez-Nicora et al. 2016). However, a large number of individual fields in the state do have very high population densities of SCN (Dorrance and Niblack, unpublished data) and some populations in the region have adapted to the most widely deployed resistance derived from PI88788 (Niblack, unpublished data; Niblack et al. 2008).

Among all soybean pathogens, SCN is the greatest contributor to economic loss (Koenig and Wrather 2010; Wrather et al. 2001). The presence of a single SCN cyst is a cause for concern for growers (Niblack 2005) as it may be present in a field without any aboveground symptoms on soybean plants (Wang et al. 2003). When SCN populations are high, the most common symptoms include chlorosis, stunting, and lack of vigor. Decreases in yield of 30 to 50% have been noted in the absence of symptoms (Dorrance, unpublished data; Niblack 2005). Three cysts per 100 cm$^3$ soil has become an economic threshold across the Midwest (Niblack 2005).

Management of SCN is accomplished through crop rotation with non-host crops and rotation of soybean cultivars with different sources of resistance (Long and Todd 2001; Niblack 2005). However, rotation to crops other than soybean has not always been economical for some production regions. To manage SCN, farmers are advised to sample to avoid planting susceptible cultivars when populations reach a level of 200 eggs/100 cm$^3$ soil (Wheeler et al. 1997). Planting resistant cultivars in a field with a high SCN population can result in significantly higher yields of up to twice that of susceptible cultivars (Wang et al. 2003).

Quantifying SCN population densities is crucial for management, as continual yield declines and plateaus present a major economic hurdle to soybean production. When population...
densities are high, females (cysts) can be observed directly on the roots when the body is still white (Fig. 1). However, estimates of whole-field population densities can only be made through soil sampling. Traditional sampling with a hand-held soil probe can be difficult, tedious, time-consuming, and costly. Methods are needed to increase sampling efficiency and reduce sampling cost, while at the same time maintaining the accuracy and precision of SCN population estimates.

Adoption of GPS technology, computer-controlled variable-rate applications, and automation advanced exponentially in production agriculture during the 1990s (Bullock et al. 2002). Efficiency and return on investment have been the main drivers of these modern advances. Soil sampling density is directly proportional to the investment in the required labor (Francl 1986), hence the attractiveness of automation. Various methods of fertility sampling automation have gained attention in the agricultural community. Adaptation of automated fertility technology to sample for soilborne organisms like SCN has yet to be tested. Therefore, the objective of this study was to compare the accuracy and precision of traditional SCN soil sampling with a hand held soil probe to that of an automated machine with GPS capability.

SITE SELECTION

Three sites in Ohio with a history of SCN were selected for comparison of a traditional sampling method to an automated sampler. Approximately four hectares of each site were chosen in the spring of 2015, prior to crop establishment. Site one (Clark) was on a producer’s field located in Clark County, and was characterized by low-lying ground with a history of a two-year rotation between corn and soybean. This was the only site to have any tillage performed at the time of sampling. Site two (Western) was situated at the Ohio Agricultural Research and Development Center Western Research Station in Clark County, and was a field with a multiple-year history of soybean production. Site three (Mont) was also located on a producer’s field in Montgomery County, and was in a two-year rotation between corn and soybean production. All three sites were sampled between 29 April and 15 May 2015. Field conditions relating to residue and soil type were fairly uniform across the three sites, with no weeds at the time of sampling.

COMPARISON OF SAMPLING METHODS

Sampling was conducted in a grid pattern (Fig. 2), with 30.5 × 30.5-m spacing. For the automated machine samples, a patented Automated Precision Soil Sampler (APSS) (Integrated Agricultural Services, Milford Center, OH) was used to collect the samples. This machine has been used previously to sample large numbers of hectares in Ohio for soil fertility, thus presenting the possibility of adoption for sampling of SCN. The APSS machine is a 3-point mounted implement that collects a soil sample by means of a knife mounted to a shank. The knife

**FIGURE 2**

Field sampling protocol to compare two soil sampling methods, an Automated Precision Soil Sampler (APSS) and a hand-held soil probe. Red arrows depict the direction and length of the APSS soil sampler and small green circles indicate the locations for the hand-held soil probe.
contacts the ground, collecting soil when the shank is lowered to a preset depth. Compressed air cleans the knife between each collection, sending the soil for storage in an individually marked container. At each grid location, the APSS collected a composite sample, continuously for approximately 3 m, while traveling at a speed of 0.93 to 1.24 km/h. The amount of soil collected for each sample ranged from 30 to 100 cm³. Each automated sampling location was geo-referenced, followed by a sample with a hand probe at the same geo-referenced point. Hand samples were taken with a stainless steel hinged soil probe, 2.54 cm in diameter. Eight hand cores were taken around the machine sampling line to form a composite sample of 100 cm³ of soil placed in a labeled bag. Both the APSS soil samples and hand samples were collected to a depth of 17.8 cm. A total of 78, 74, and 46 sites were sampled at Clark, Western, and Mont, respectively, resulting in 396 samples (198 for each sampling method). All soil samples were placed in cold storage at 4°C until further processing.

**DATA COLLECTION**

Due to the high clay content in these soils, 30 to 100 cm³ of each sample was placed in a 1000-ml beaker with 400 ml of water for approximately 24 h to allow soil particles to separate. Following this suspension, the sample was elutriated for 4.75 min in a semi-automatic soil elutriator (University of Georgia, Athens, GA), following standard elutriation procedures (Byrd et al. 1976; Niblack et al. 2009). Excess soil and debris remaining after this initial step were removed by hand-sieving each sample. Each sample was mixed with water in a 400-ml beaker and decanted through a #60 sieve. Since the aim of this comparison was to differentiate between sampling methods and not viable SCN egg population densities, cyst counts were evaluated rather than egg counts. The number of cysts was counted under a dissecting scope at ×10 magnification and recorded. Final cyst counts for each sample were adjusted to the soil volume and reported as number of cysts/100 cm³ soil.

**STATISTICAL COMPARISONS BETWEEN THE TWO SAMPLING METHODS**

For each site, a data matrix was created with each pair of cyst count data from the APSS- and hand-collected samples along with a reference number corresponding to each sample location within the field. Agreement between cyst population density estimates for the two sampling methods then was quantified as described by Madden et al (2007), Lin (1989), and Carrasco and Jover (2005), based on concordance correlation coefficient (CCC). CCC ranges from –1 to 1, with –1 representing perfect inverse agreement and 1 perfect agreement. A value of 0 represents perfect disagreement. Cyst population densities varied among sites and among sampling methods within a given site (Fig. 3 and Table 1). Cyst population densities varied among sites and among sampling methods within a given site (Fig. 3 and Table 1).

**FIGURE 3**

Boxplots summarizing the distribution of SCN cyst population densities following APSS and hand-sampling methods at the Mont (A), Clark (B), and Western (C) locations in Ohio, 2015.

**TABLE 1**

Summary statistics for the number of cysts of *Heterodera glycines* (soybean cyst nematode) from soil sampling with the Automated Precision Soil Sampler (APSS) or a hand-held soil probe to assess population density and distribution of SCN at three locations in Ohio, spring 2015.

<table>
<thead>
<tr>
<th>Variable</th>
<th># Samples</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Med</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clark APSS</td>
<td>78</td>
<td>80.4</td>
<td>65.2</td>
<td>66.3</td>
<td>0</td>
<td>325</td>
</tr>
<tr>
<td>Clark Hand</td>
<td>78</td>
<td>38.1</td>
<td>30.7</td>
<td>32.4</td>
<td>0</td>
<td>169</td>
</tr>
<tr>
<td>Western APSS</td>
<td>74</td>
<td>46.1</td>
<td>109.8</td>
<td>10.0</td>
<td>0</td>
<td>528</td>
</tr>
<tr>
<td>Western Hand</td>
<td>74</td>
<td>37.3</td>
<td>96.2</td>
<td>8.0</td>
<td>0</td>
<td>640</td>
</tr>
<tr>
<td>Mont APSS</td>
<td>46</td>
<td>73.7</td>
<td>76.5</td>
<td>36.0</td>
<td>3.33</td>
<td>254</td>
</tr>
<tr>
<td>Mont Hand</td>
<td>46</td>
<td>59.0</td>
<td>65.5</td>
<td>25.5</td>
<td>4</td>
<td>233</td>
</tr>
<tr>
<td>Total APSS</td>
<td>198</td>
<td>66.1</td>
<td>87.7</td>
<td>(Sum) 13085</td>
<td>0</td>
<td>528</td>
</tr>
<tr>
<td>Total Hand</td>
<td>198</td>
<td>42.5</td>
<td>69.4</td>
<td>(Sum) 8406</td>
<td>0</td>
<td>640</td>
</tr>
</tbody>
</table>
80% of all samples collected using the two methods had cyst counts below 30 cysts/100 cm³ soil. At all sites, mean cyst count was higher for the APSS sampling method than the soil probe method (Table 1), but the difference between the two methods was only statistically significant ($P < 0.001$) at Clark (Fig. 3B).

The Clark field varied the most from the other two locations sampled in this study. Half of this site was previously in corn, with standing crop residue, and the other half previously in soybeans that had been prepared for spring planting with tillage. The soil samples collected with the APSS had significantly ($P < 0.0001$) greater numbers of cysts compared with those collected with a hand soil probe (Table 1). The Western location has been in continuous soybean production for several years. There was no significant difference ($P = 0.6069$) in the number of cysts recovered from the soil between the APSS and hand sampling with a soil-probe (Table 1). The greatest number of cysts per soil sample was recovered from this location. Lastly, the Mont location was in no-till production and the previous year the crop was corn. All of the samples had cysts present. In addition, there was no significant difference ($P = 0.335$) between the number of cysts recovered from the soil using the APSS or hand sampling with a soil probe (Table 1).

**SUMMARY AND FUTURE WORK**

Data from the three sites were first analyzed individually, then pooled and reanalyzed. The hand sampling method was used as the standard, and the machine sampling was viewed comparatively, as an alternative. Concordance correlation coefficients (CCC) were estimated for each dataset as a measure of agreement between the two sampling methods. For Mont and Western the CCC values were closer to 1 (perfect agreement), 0.84 and 0.92, respectively, compared with the Clark (CCC = 0.45). Correspondingly, the data points were closer to the line of perfect agreeing at Mont and Western than at Clark, indicating much better agreement between the two sampling methods at the two former sites than at the latter. At all three of the sites, the number of cysts found per 100 cm³ of soil with the automated precision soil sampler was higher than that of the hand sampling method (most of the points above the diagonal line were of perfect agreement in Fig. 4), suggesting a bias towards overestimation with the APSS. At the Clark County location, the bias varied with cyst count. There was greater bias (greater departure from the diagonal line) observed at higher cyst counts than at lower levels. It may be important to note that this site had slightly higher soil moisture levels than the other sites when

**FIGURE 4**

Plots showing associations between SCN cyst counts collected with a soil probe (hand) and an automated sampling machine (APSS) at Mont (A), Western (B), and Clark (C), then pooled data from the three sites (D). Solid 45° lines are lines of perfect agreement between the two methods. CCC = concordance correlation coefficient, with a value of 1 representing agreement and 0 perfect disagreement.
samples were collected, and was the only site to have been sampled after the ground had been tilled. Determining whether these differences affected the estimates should be a priority to assess the accuracy of the machine prior to adjustments in soil sampling recommendations.

For the pooled data, CCC was 0.80. Overall, cyst counts from the APSS were slightly higher than from hand-collected samples. Although comparisons of the different methodologies were reasonably parallel, automation resulted in a slight tendency to overestimate cyst counts when higher populations were observed, when compared with sampling by hand. Further comparisons of the machine and hand sampling methods are necessary to determine accuracy of detection across a wider range of cyst population densities, soil types, and tillage practices.

The ability to accurately sample for *Heterodera glycines* on a large scale has the potential for more accurate understanding of pathogen population and variability in Ohio and on a field level. Implications include increased knowledge of pathogen spread, population levels and regional variability. Management decisions can also be made from understanding SCN populations within individual fields, and growers can manage their fields accordingly. As new products are developed to help manage SCN, different applications and or cultivars could be used in different parts of the field according to SCN population densities. This would be especially feasible when coupled with GPS guidance and product control technology. Additionally, differences in yield can be compared within fields based on the distribution of SCN if cyst population densities are known. Sampling with an automated sampler could be practically applied to a production setting with a larger number of soil samples collected in a more efficient period of time. In the future, comparisons of sampling techniques will be conducted to evaluate optimum frequency of sampling resolution for number of samples per hectare and whether modifications of this technique are needed to detect SCN at low population densities.

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**LITERATURE CITED**


