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Intra- and interplot variability of *Heterodera glycines* population densities in experimental settings to soybean variety evaluations in Nebraska

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ABSTRACT

The intra- and interplot variability of soybean cyst nematode (SCN; *Heterodera glycines*) population densities was characterized in three experimental areas in Nebraska in 2014. Through single-core cluster sampling of 4-m-long plots, a random pattern of SCN incidence (number of SCN-positive soil cores out of 10 per plot) within plots was suggested by the binomial distribution in one location. The detected randomness, however, was associated to the high SCN infestation level and tillage in the experimental area. In the other two experimental areas, the beta-binomial distribution adequately described SCN incidence (index of aggregation $\theta > 0$; index of dispersion $D > 1.0$), suggesting the presence of an aggregated pattern within plots. Based on the properties of the fitted distributions, the intraplot aggregation of incidence affects the probability of cyst recovery during sampling and thereby the estimation of SCN population densities. At the interplot scale, correlogram analysis of 36 transects of 28 and 35 m showed that discrete foci of SCN population densities were spatially dependent with foci located up to 15 m away. This was confirmed by spatial autocorrelation, which indicated that SCN population densities in the 4-m-long plots were positively correlated with population densities of up to two contiguous plots. Interplot aggregation of SCN population densities suggests lack of independence among plots or experimental units. Altogether, the results of this study recommend that researchers conducting field experiments with *H. glycines* consider SCN spatial pattern, particularly, if rigorous comparisons among soybean varieties are sought. Consideration may comprise both the diagnostics of spatial dependence of SCN population densities in a prospective variety evaluation site, prior to spring sampling, and the subsequent allocation of varieties to plots based on their degree of spatial correlation. While limited to a single-year assessment, the information on SCN spatial pattern from the present study, coupled with that on soybean plant populations, row spacing, and soil conditions, can be useful to determine site suitability for a variety trial. The information could also be incorporated into statistical analysis to determine the number of replications needed to detect differences among varieties for their response to SCN.

1. Introduction

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) is a significant and widespread pathogen of soybean in the major soybean producing regions of the world (Noel et al., 1994; Liu et al., 1997; Riggs, 2004; Doucet et al., 2008). In the United States of America (U.S.), since its discovery in North Carolina in 1954 (Winstead et al., 1955) SCN has remained the number one pathogen limiting yield of soybean (Doupnik, 1993; Wrather and Koenning, 2006; Wrather, 2008). Yield suppression due to SCN was estimated at 35 million metric tons during 2006 (Wrather, 2008), a much higher yield reduction than that caused by any other soybean disease in the country (Wrather and Koenning, 2006).

The use of SCN-resistant soybean varieties is an effective strategy to manage SCN (Niblack, 2005; Noel, 2008; Tylka, 2008). In support to that strategy, in the major soybean growing areas of the U.S. hundreds of varieties are evaluated every year for agronomic

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performance and response to this pathogen (Chen et al., 2001; Donald et al., 2006; Giesler et al., 2012; Orf et al., 2013; Tylka et al., 2014). The methodology for variety evaluations is fairly standard in most U.S. regions and is described in detail by other researchers (Giesler et al., 2012; Tylka et al., 2014). Normally, varieties are planted to four-row plots (5- to 8-m-long), SCN population densities (eggs/100 cm^3 of soil) are determined for each plot or variety replication, and the sustained soybean yield and SCN reproduction factor are compared among varieties (Donald et al., 2006; Giesler et al., 2012; Tylka et al., 2014). The SCN population densities are determined at planting and harvest from composite samples of 10–12 soil cores, which are withdrawn arbitrarily along the center rows of each plot; yield is also determined from center rows (Chen et al., 2001; Orf et al., 2013; Tylka et al., 2014). In the collection of samples, it is the cysts recovered from the soil that constitutes SCN sampled entities in surveyed areas (Niblack et al., 2002; Niblack, 2005).

Researchers generally recognize that in field experiments the analysis of the spatial variability (heterogeneity or pattern) of nematode population densities can be an important tool to enhance sampling schemes and to interpret nematode population dynamics (Noe and Campbell, 1985; Campbell and Madden, 1990; Shaukat and Khan, 1993). In the case of SCN, the spatial attributes of field populations have been described in a few studies (Francl, 1986; Alston and Schmitt, 1987; Avendaño et al., 2004; Gavassoni et al., 2007). However, the spatial variability of population densities in experimental settings for SCN-resistant soybean variety evaluations remains uncharacterized. Specifically, how SCN sampled entities (cysts) arrange within and among plots and how the resulting SCN egg population densities in plots correlate with each other remain unknown. Understanding such variability may provide significant insights on the relationship between the number of soil cores retrieved within plots at sampling and the estimated SCN population densities. Also, understanding of such variability can provide clues on the pathogen dispersal at that spatial scale. Ultimately, combined with additional host and pathogen information, such understanding could serve to improve the current methodology or to develop new methods to evaluate soybean varieties for their response to SCN, and more importantly, to understand how the observed variability affect the estimation of SCN population densities and how population densities relate directly with yield. The objective of this research was to characterize the intra- and interplot variability of SCN population densities in standard experimental settings for evaluation of SCN-resistant soybean varieties in Nebraska.

2. Materials and methods

2.1. Experimental areas

Three experimental areas were established in the state of Nebraska, U.S. in 2012 in fields with a history of SCN. The field locations are herein referred to as Bellwood, Plattsmouth and Waterloo. The Bellwood field had a loamy sand-textured soil and was under central-pivot irrigation. The Plattsmouth field had a silty clay loam-textured soil and was non-irrigated, and the Waterloo field had a sandy loam soil type and was under central-pivot irrigation. Bellwood and Plattsmouth were conventional-till fields and Waterloo was a no-till field.

In each experimental area a soybean variety trial was established adopting the standard plot size for variety evaluations in the major soybean growing regions of the U.S. (Giesler et al., 2012; Tylka et al., 2014). The experimental area was stratified into six blocks, each block consisting of six plots separated by 0.60-m alleys, and each plot consisting of four 5.2-m-long rows spaced at 0.76 cm. Two varieties were assigned in a generalized randomized complete block design and were planted on May 22 and 23, 2012 in the three locations. The main purpose of this study was to characterize the intra- and interplot variability of SCN population densities. Therefore, SCN population densities at harvest and yield comparison between the tested varieties are not discussed in this manuscript.

2.2. SCN sampling

SCN sampling in each location was carried out within a few minutes after planting and consisted of a composite soil sampling followed by a single-core soil sampling. Before collecting the samples, a 4-m inner segment of the center rows of each plot was delimited. Within the marked segment, five 2.5-cm-diameter 15- to 20-cm-deep soil cores (one at every 1.0 m interval) were collected along each of the center rows, and the 10 cores obtained were mixed to a composite sample. After collecting all composite samples in each location, 10 additional soil cores were collected along the same center rows of each plot in a similar manner as before, yet in this sampling each core constituted an individual sample. Each core was collected within a 10-cm radius from where the cores for the composite samples were removed. The soil core collection was repeated if a soil core was <15-cm long, as determined visually. Soil probes were washed and rinsed in a bucket between each core extraction to avoid SCN contamination between individual samples and plots. In total, 360 individual- and 36 composite samples were collected in Bellwood and Plattsmouth, and 300 individual samples and 30 composites in Waterloo.

2.3. Sample processing and determination of SCN population density

In the laboratory, each composite and single-core soil sample was thoroughly mixed to obtain a homogenous sample. For the composite samples, a 100 cm^3 subsample, measured by water volumetric displacement, was processed to determine the number of SCN eggs/100 cm^3 of soil. For the single-core samples, the whole soil sample was processed in the same manner as the composites. Volumetric displacement of each sample was determined before processing, and the obtained SCN population density was adjusted to the standard 100 cm^3 of soil. Processing was done using standard sieving techniques for cyst nematodes: decanting the sample content onto a 710-μm-pore sieve stacked over a 250-μm-pore sieve to collect the cysts and then decanting and grinding the collected cysts on a 125-μm-pore sieve stacked over a 25-μm-pore sieve to extract the eggs (Khan, 2008). The extracted eggs from each sample were collected in a 100-ml beaker, rinsing with water and maintaining the volume of the collecting solution at <20 ml for all the samples. One ml of a staining solution based on acid fuchsin stain was added to each sample, and the sample was microwaved for 70–90 s (at 700 W power) to stain the eggs (Hooper, 1986). After the sample was raised to a standard volume of 20 ml, 1 ml from each sample was transferred to a counting dish, observed under a dissecting microscope at 35× magnification, and the number of eggs was recorded. Two additional 1-ml subsamples were examined (three in total) to obtain an average egg number per sample. The identity of SCN eggs, second stage juveniles and cysts was confirmed by morphometrics (Golden, 1986).

2.4. Intraplot variability of SCN incidence

Distribution theory has been widely used in biology and ecology to study relationships between disease entities (Smith, 1983; Madden and Hughes, 1994, 1995). In this study, the binomial and beta-binomial distributions were used to determine the intraplot