Establishment of *Heterodera glycines* in Three Soil Types

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Abstract: The establishment of *Heterodera glycines* race 3, from soil plugs infested with population densities ranging from 0 to $10^6$ eggs and second-stage juveniles per 10 cm$^3$ soil, was compared in three soils (Haynie sandy loam, Eudora silt loam, and Chase silty clay loam) that were either pasteurized or unpasteurized. Final population densities of *H. glycines* in soil and on soybean (*Glycine max* cv. Williams 82) roots were affected by soil type but not by soil pasteurization ($P = 0.05$). Higher numbers of *H. glycines* females and cysts were recovered from the sandy loam than from the silty loams after 8 weeks. The relationships between initial populations in infested soil plugs and the levels of recovery in the previously uninfested soils were described by sigmoidal Gompertz growth models. Estimated threshold levels for establishment were approximately 75% lower in the sandy loam than in the silty loams.

Keywords: establishment, *Glycine max*, *Heterodera glycines*, soil type, soybean cyst nematode, soybean.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is a relatively new problem for growers in northeastern Kansas (13). To date, the nematode has been recovered from Doniphan, Atchison, Leavenworth, and Wyandotte counties, which border the Missouri River, and Johnson County, which borders Wyandotte County and the Kansas River. Infestations are found generally in the sandy soils of the river flood plain.

Movement of cyst-infested soil by farm implements and machinery, as well as by natural agents such as wind and water, serves to spread *H. glycines* locally (7,14). Also, the passage of viable cysts, juveniles, and eggs through the digestive tracts of blackbirds may disseminate the nematode over long distances (4). The spread of *H. glycines* into new areas through the movement of contaminated soil is, however, dependent upon establishment of the nematode. Establishment ultimately is dependent upon parameters such as migration and root penetration, which for some nematodes have been shown to be influenced by edaphic factors (6,16). Edaphic conditions may further impact the risk of crop damage due to the nematode following establishment (10). Therefore, information on the effect of edaphic factors on the establishment of *H. glycines* is essential for evaluating the potential for further spread of this nematode.

The objectives of the current study were to determine 1) the establishment of *H. glycines* as influenced by three soil types, 2) the effect of reducing native soil biota through soil pasteurization on establishment of the nematode, and 3) the relationship between population levels of *H. glycines* in nematode-infested soil plugs and the level of successful infection of soybean roots following migration into uninfested soil.

Materials and Methods

Three soils, differing in texture but all free of *H. glycines* infestation, were collected from the plow layer of commercial soybean (*Glycine max* (L.) Merr.) fields in Riley County, Kansas. Soil types were Haynie sandy loam (60% sand, 30% silt, 10% clay, 1.1% organic matter; pH 8.1), Eudora silt loam (30% sand, 46% silt, 24% clay, 2.3% organic matter; pH 7.9), and Chase silty clay loam (14% sand, 60% silt, 26% clay, 3.1% organic matter; pH 7.6). Half of each soil was steam pasteurized for 2 hours at 74 C and then aerated for 3 days before planting. Nematodes were extracted from 100-cm$^3$ subsamples of each soil type before pasteurization using a modified Christie–Perry technique (2). Densities of phy-
TABLE 1. Analysis of variance for *Heterodera glycines* total populations (Pt) and root population densities (Pr) in three soil types with and without pasteurization.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Pt</th>
<th>Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>0.87</td>
<td>0.08</td>
</tr>
<tr>
<td>Soil type (S)</td>
<td>1.48*</td>
<td>0.82*</td>
</tr>
<tr>
<td>Error A</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>Pasteurization (P)</td>
<td>0.42</td>
<td>0.16</td>
</tr>
<tr>
<td>S × P</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Error B</td>
<td>0.29</td>
<td>0.16</td>
</tr>
<tr>
<td>Initial population (Pi)</td>
<td>56.06*</td>
<td>36.93*</td>
</tr>
<tr>
<td>S × Pi</td>
<td>0.33</td>
<td>0.18</td>
</tr>
<tr>
<td>P × Pi</td>
<td>0.21</td>
<td>0.11</td>
</tr>
<tr>
<td>S × P × Pi</td>
<td>0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>Error C</td>
<td>0.27</td>
<td>0.14</td>
</tr>
<tr>
<td>CV (%)</td>
<td>27.41</td>
<td>25.85</td>
</tr>
</tbody>
</table>

*P < 0.05.

Logt0 (total females and cysts from roots and soil - 1).
Logt0 (females and cysts/g root + 1).

Heterodera glycines race 3 was collected from plants grown in naturally infested Sarpy loamy sand (82% sand, 4% silt, 14% clay, 0.8% organic matter; pH 8.0) in Doniphan County, Kansas. Cysts were recovered by spraying roots with pressurized water over a 150-μm-pore sieve. Eggs and second-stage juveniles (J2) were extracted by macerating cysts and then separating them from debris by centrifugal-flotation as described previously (17). Nematode inoculum was incorporated into pasteurized Sarpy loamy sand to produce stock population densities (Pi) of 0, 10², 10³, 10⁴, and 10⁵ eggs and J2 per 10 cm³ soil.

The experiment was designed as a split-split plot with whole plots arranged in a randomized complete block with three replications. Whole plots consisted of 11-cm-d x 12-cm pots containing Haynie sandy loam, Eudora silt loam, or Chase silty clay loam soil. Subplots consisted of pasteurized or unpasteurized soils and sub-subplots consisted of five levels of *H. glycines* added as 10-cm³ soil plugs (1.5 cm d x 6.0 cm) of infested Sarpy loamy sand. Four soybean (*Glycine max* cv. Williams 82) seeds were planted in pots containing 600 cm³ soil. An infested soil plug was placed with a plastic syringe on the soil surface of each pot immediately after seeding. Each pot was watered lightly with a sprinkler nozzle to maintain the integrity of the soil plug.

Plants were grown for 8 weeks under greenhouse conditions with ambient temperatures of 30 °C day and 24 °C night and uniformly top watered as needed. The level of successful root penetration and nematode development was measured by collecting cysts and females of *H. glycines* from roots and soil (17) at the termination of the experiment. Data were subjected to analysis of variance and nonlinear regression procedures (9). Nematode population data were log-transformed (log₁₀ [x + 1]) prior to analysis.

RESULTS AND DISCUSSION

No differences (P = 0.05) in *H. glycines* total root and soil population densities (Pt) or root population densities (Pr) were detected between pasteurized and unpasteurized soils (Table 1). This suggests that native soil biota had little influence on establishment of the nematode under our experimental conditions. Alterations in soil chemical properties due to pasteurization were not measured, but these were assumed to have minimal effects on the nematode.

Higher total populations and root population densities were observed in the Haynie sandy loam than in the Eudora silt loam or Chase silty clay loam soils (P = 0.05) (Table 1, Fig. 1). These differences may have resulted from enhanced migration or root penetration of *H. glycines* in coarse-textured soil, a phenomenon documented for *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven and *P. neglectus* (Rensch) Filipjev and Schuurmans Stekhoven (18). Soil type may also affect the survival and hatch of *H. glycines* (16), but since the nematode inoculum used in this study was initially placed in soil plugs of a single type (Sarpy loamy sand), and since
experimental conditions were not adverse, differences in survival or egg hatch are unlikely.

Final population densities of *H. glycines* in pots were primarily a function of initial population densities in the soil plugs. In each case, the relationship between initial population (Pi) and final population means (Pt or Pr) of *H. glycines* was described by an asymmetrical sigmoid curve derived from the integrated form of the Gompertz growth model (1). In Haynie sandy loam, final population data were described by the equations:

\[
Pt = 4.40e^{-14.22e^{-1.04Pi}}, \quad R^2 > 0.99 \text{ (Fig. 1A)}
\]

\[
Pr = 3.96e^{-11.96e^{-0.87Pi}}, \quad R^2 > 0.99 \text{ (Fig. 1B)}
\]

Populations of *H. glycines* in Eudora silt loam and Chase silty clay loam were described by the equations:

\[
Pt = 3.38e^{-198.38e^{-1.83Pi}}, \quad R^2 > 0.99 \text{ (Fig. 1A)}
\]

\[
Pr = 3.22e^{-126.68e^{-1.60Pi}}, \quad R^2 > 0.99 \text{ (Fig. 1B)}
\]

Models for the sandy loam and for the silty loams differ primarily in their response to the Pi range between 1.0 and 3.0. For example, at Pi = 2.0 (100 eggs and J2), Pt = 0.7 (five females and cysts) in sandy loam, whereas in the silty loams, the same level of Pt required a Pi of 2.6 (400 eggs and J2). This difference indicates that the establishment threshold was lower in sandy loam, suggesting that a higher frequency of establishment may occur in coarser soil types.

In the sandy loam and silty loam soils, the curves for Pt and Pr were more responsive in the Pi range of 2.0–4.0 than at the highest inoculum level. This decrease in response at high Pi is similar to that described for other systems and may be a function of resource limitations (5,11). A higher ceiling level (12), however, was predicted for the sandy loam than for the silty loams. At the highest Pi level, root population densities averaged 3.4 (2,512 females and cysts per gram) in sandy loam and 3.1 (1,259 females and cysts per gram) in silty loams. The lower ceiling level observed in the silty loams may indicate differences in resource limitations for the nematode due to soil type. Localized infection of *H. glycines* was observed in the undifferentiated region of soybean roots (3), but root morphology, specifically root
elongation, can be influenced by soil type (8). Altered root elongation, therefore, may influence the suitability of the root as a substrate and modulate ceiling limits.

Movement of infested soil is considered to be a major factor in the widespread dissemination of *H. glycines* (7,14). In this study, the number of nematodes that developed on soybean roots following migration from infested soil plugs into uninfested soil varied with soil type but was not affected by soil pasteurization. Based on the predictive models in Figure 1, successful infestation of sandy loam soil (as measured by the recovery of cysts from roots) resulted from as little as 25% of the inoculum potential required to establish an infestation in the silty loam soils. Our data indicate that soil type can influence establishment, although precise thresholds for establishment in the field cannot be predicted. In the field, cysts of *H. glycines* on contaminated equipment or in soil peds may be subjected to adverse environmental conditions which have been shown to reduce viability of the nematode (15). The nematode inoculum used in this study consisted of eggs and J2 of recent production that were not subjected to desiccation or temperature extremes. Future research should focus on the establishment of the nematode under field conditions.

**LITERATURE CITED**