2022 Final Report: Delaware Soybean Board

Visualization of Economic Damage Thresholds and Interaction of Nematode Populations following use of Winter Cover Crops

Dr. Alyssa Koehler, Assistant Professor and Field Crops Extension Specialist, Plant Pathology
Department of Plant and Soil Sciences, University of Delaware, Georgetown, DE
akoehler@udel.edu | (302)242-9056 | @UDPlantPath

Project Overview:

Soybean cyst nematode (*Heterodera glycines*) is considered the most damaging pathogen of soybeans across the US and is the most significant nematode pest affecting soybeans in Delaware and the eastern shore of Maryland (Allen et al. 2018). Following closely is root knot nematode (*Meloidogyne incognita*). SCN females establish permanent feeding sites on roots and turn into cysts that each contain hundreds of eggs. There may be several generations within a single growing season and recalcitrant cysts and eggs can persist in the soil for long periods of time. SCN has been present in Delaware since 1979 and Maryland since 1980. Growers have relied heavily on resistant varieties, primarily using the PI88788 resistance source. Long-term exposure to this resistance gene can select for SCN populations that are able to overcome this source of resistance. Detailed SCN surveys were conducted across Delaware and Maryland in 1993 (Sindermann et al. 1993). Surveys in DE were conducted again in 2009 and it was observed that populations shifted from predominately race 3 to race 1. During the 2009 survey, elevated reproduction on PI88788 was observed at rates of 44-80%. Surveys were continued in 2019-21 and high populations with reproduction rates >65% were observed. In these surveys, RKN populations ranging from 4,000-9,000 nematodes per 500 cc of soil were also observed, particularly in fields with a history of vegetable rotation. A high level of infestation is considered >170 RKN per 500 cc, meaning many local fields are dealing with populations 25-53 times above threshold. The survey work in 2019-2021 demonstrated that SCN and RKN are widely prevalent in the region, though the extent of their damage may be unnoticed by farmers (Kessler and Koehler 2022). Visualization of nematode effects is a valuable extension tool to demonstrate stunting and yields effects for soilborne pathogens that are difficult to visualize in the field without destruction of plants. Fumigants such as methyl bromide and ethylene dibromide once provided excellent control to soilborne pathogens like RKN, but were phased out due to human and environmental health and safety concerns. Safer, non-fumigant chemical control options have not been as effective and integrated approaches to nematode management are needed. Many growers are already planting winter cover crops, so there are consistently questions regarding the effect of these crops on nematode populations the following season.

Cover crops are commonly used as a method to slow erosion, control weeds and pests, and improve water availability and soil health during the non-growing season for future crops. A common question regarding cover crops is their ability to suppress nematode population densities by breaking pest cycles or via suppression. Cover crops such as winter rye, Daikon radish, and annual ryegrass have reduced SCN populations when used in consecutive years (Acharya et al.,

2021). Greenhouse trials comparing the use Daikon radish, annual ryegrass, and crimson clover as cover crops, followed by SCN susceptible soybeans also demonstrated reduced SCN females on soybean roots (Harbach and Tylka, 2021). There was no evaluation for the effects of these cover crops on Root Knot Nematode, a common species within the region. In a previously funded project, Dr. David Owens established plots to examine the role of cover crops on slug populations. Leveraging these already planted plots, this proposal aimed to sample for nematodes within the cover crop choices of rye, barley, and crimson clover to monitor nematode populations in grower fields and increase observations of nematode response to cover crops in our region. Much of the work conducted in the Midwest is in soils with high organic matter. In sandier, low organic matter soils, nematode damage can be more severe and interactions with cover crops can differ. The ability to use common cover crops within the region as a tool to manage nematodes would be very helpful, but little is known regarding these complex interactions at this time.

The objectives of this project were to: 1) Utilize rhizoboxes to visualize economic damage thresholds for Soybean Cyst Nematode (SCN), Root Knot Nematode (RKN), and the interaction of SCN and RKN. 2) Track in-season nematode populations following the use of winter cover crops. 3) Share research findings through extension events and use findings to inform future management trials. This project established initial methods to view nematode damage in rhizoboxes that will be expanded to demonstrate nematode interactions and nematode effects in different soil types. Cover crop effect on nematodes is difficult to obtain due to field variability of nematode populations. This work provided an initial observation for the region and will be complimented by future studies.

Project Activities and Methods:

Objective 1. Utilize rhizoboxes to visualize economic damage thresholds for Soybean Cyst Nematode (SCN), Root Knot Nematode (RKN), and the interaction of SCN and RKN in local soil types.

Figure 1: Example of corn roots growing in a Rhizobox

The Koehler lab received funding to build rhizoboxes to aid in extension demonstrations. These boxes were based off of rhizoboxes developed for corn trials (Figure 1) with a clear Plexiglas front that allows for visualization of root systems. Two soybeans were planted into each box. Using RKN populations maintained at the Carvel Research and Education Center Greenhouses, RKN eggs were extracted from roots. Eggs were induced to hatch and infective stage J2 nematodes were used to inoculate the rhizoboxes. Soybeans planted into our 2022 SCN trial field were dug and SCN females were extracted following a root blast protocol where females are washed from the roots and collected in a sieve for enumeration. SCN females were used to inoculate SCN boxes. Photos were captured to preserve visualization of symptoms.

Objective 2. Track in-season nematode populations following the use of winter cover crops.

This portion of the trial was conducted in field trials set up by Dr. David Owens as part of a previously funded DSB proposal "Cover Crop Selection and Termination Implications for Slugs". Briefly, large field plots were planted at a cooperator farm. In October, 2021, 50' x 100' plots were planted with rye, barley, crimson clover, or no cover. In the spring, plots were terminated. When plots were sampled in the spring and fall for slugs, select nematode soil samples were also collected to observe nematode populations following each cover crop at the sprain and end of season once the field was planted in soybeans.

Objective 3. Share research findings through extension events and use findings to inform future management trials.

Findings from this project will be shared through the University of Delaware's Weekly Crop Update, which reaches over 700 growers, consultants, and stakeholders and provides a platform to discuss disease concerns and other production issues. Data was shared through training events and extension presentations including the 2022 Carvel Research and Education Center Field Day, 2022 Carvel Nematode Field Day, Mid Atlantic Crop School, and the Nematodes in Atlantic Soybean Production Webinar.

Results and Discussion:

This project was the first time using the soybean rhizoboxes and optimization of the process was required. Rhizoboxes were built scaling down from a model established for corn trials. While this model allowed for robust root expansion (Figure 2), this model was not as portable/user friendly for extension events. We aim to create a smaller set that will aid in extension portability (Figure 3). However, the larger boxes will serve as a useful set for replicated greenhouse trials to observe nematode effects. Additionally, the soybean rhizoboxes were built with Plexiglas on both the front and the back. Many of the root systems drifted to the back of the box and in future boxes a solid back will be used with Plexiglas only on the front.

Figure 2: Soybean roots expanding to the bottom of a newly created soybean Rhizobox

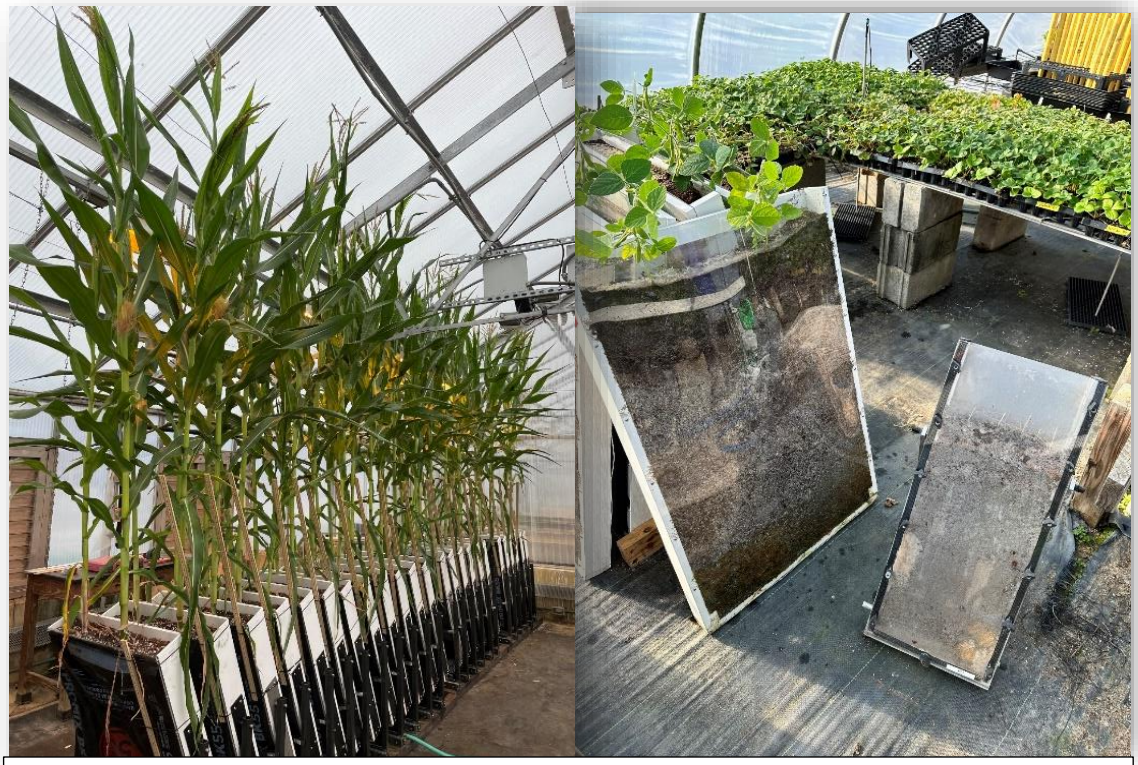

Figure 3: Corn rhizoboxes (left) and soybean rhizoboxes (right). Original soybean rhizoboxes were reduced in size from the corn boxes, but further reductions in size were needed for portability for in-person extension demonstration.

For the 2022 season, emphasis was on refining boxes and making initial observations. While replicated trials were not yet conducted, preliminary observations were interesting. In boxes with SCN, plants were notably shorter in visual observations (Figure 4) and the number of pods along with development of pods were lower than the control boxes and the RKN boxes (Figure 5). RKN boxes did not grow as well as the controls, but growth was not as impacted as the SCN boxes. We will continue to optimize inoculation rates and experiment with different soil types in the boxes to develop an inventory of images and time-lapse videos displaying a range of nematode population/soil type combinations.

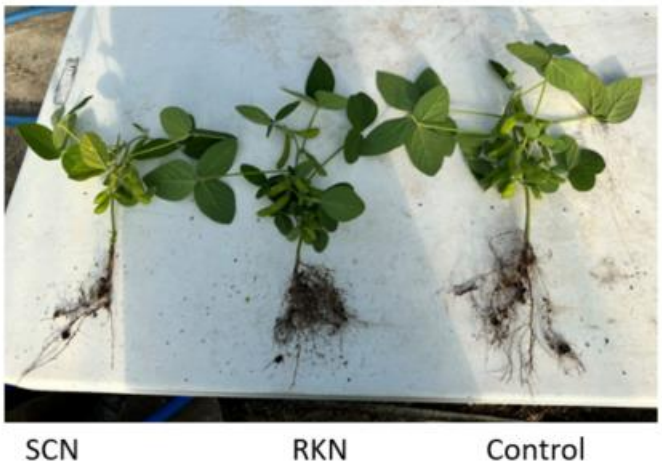

Figure 4: Growth in soybean plants inoculated with SCN (left), RKN (middle), or no nematodes (right).

Nematode samples were collected from cover crop plots on May 25, 2022. Cover crop plots included clover, rye, barley and no cover. Each plot types was replicated four times. No SCN or RKN were recovered at the spring sampling. Lance nematode was present in all plots, but at a low population level. End of season nematode soil samples were collected from the soybean cash crop on October 14, 2022. Samples were submitted to the NCDA Nematode Assay Lab. Due to the increased expansion of the Guava root knot nematode (a new nematode of regulatory concern present in neighboring states) samples have been delayed and results have not yet been received (press release attached). An updated report will be uploaded once the samples are received.

Figure 5: Number and development of soybean pods in plants inoculated with SCN (left), RKN (middle), or no nematodes (right).

This project initiated the first demonstration rhizobox testing for soybeans. Preliminary results have been very promising that these boxes will allow for important visualization and quantification of nematode damage at specific nematode thresholds across different soil types to assess if current nematode thresholds are accurate for our region and newer soybean varieties. The use of cover crops to manage soybeans is complex and the findings from the current study will be expanded in future projects and on-farm observations.

References:

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FOR IMMEDIATE RELEASE
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CONTACT:
Colleen Hudak-Wise, director
NCDA&CS Agronomic Services Division
919-733-2655

Turnaround times for routine nematode testing expected to be long; molecular diagnosis of guava root-knot nematode will be given priority

RALEIGH – The N.C. Department of Agriculture and Consumer Services' Nematode Assay Lab is warning North Carolina growers to expect turnaround times of 15 weeks or longer for routine (predictive) nematode samples submitted this year.

Samples submitted for diagnostic testing, including molecular diagnosis of guava root-knot nematode species, will be given priority and those results should be available in around five working days. Currently, there are around 15,400 samples waiting analysis.

The Nematode Assay Lab can assay soil samples for the presence of at least 46 different plant-parasitic nematodes, which are microscopic threadlike worms that live in the soil. The results of a nematode assay help growers make important management control decisions for the upcoming crop season.
Increased demand for testing and staffing shortages have led to the long turnaround times, said Dr. Weimin Ye, NCDA&CS nematologist.

"This is highly detailed work, requiring up to a year of specialized training for nematology technicians," Ye said. "Even with three experienced nematology technicians and a lab manager qualified to identify and count nematodes, only about 200 samples can be completed daily."

Management is working to address the issue but does not expect efforts will alleviate the bottleneck of samples in the short-term. Two new staff members are currently in training and five temporary employees have been hired to help extract nematodes from samples in preparation for analysis by trained staff.

"We are estimating that samples received before Dec.1 should be completed by the end of March 2023. Samples are being processed in the order that they are received," Ye said. "Given the anticipated delays, growers should consider whether or not assay results will be ready in time to make management control decisions before submitting additional samples to the Nematode Assay Lab."

Long storage times in the lab are not expected to impact assay results, as the nematodes are at the overwinter stage and do not feed and reproduce, Ye said. The nematode soil sample in the sample box is in similar condition as in the field.

You can check estimated turnaround times at https://www.ncagr.gov/agronomi/PALS/Default.aspx. Growers with nematode-specific concerns or questions can contact Ye at 919-664-1600 or by email at weimin.ye@ncagr.gov.

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Funded Budget:

Graduate Student Stipend (17% effort) ($26,677/yr)	=$4,535
Fringe benefits 8%	=$363
Greenhouse supplies (sand, fertilizer, tags, etc.)	=$120
Nematode soil samples (40 samples, $20 per sample)	=$800
Total Budget	**=$5,818**

Research Dissemination and DSB Recognition:

-August 10, 2022: Carvel Research and Education Center Field Day
-August 18, 2022: Carvel Nematode Field Day
-November 15, 2022: Mid-Atlantic Crop School
-December 5, 2022: Nematodes in Atlantic Soybean Production Webinar

Public Summary:

Soybean cyst nematode (SCN) (*Heterodera glycines*) and Root knot nematode (*Meloidogyne* spp.) consistently rank as top destructive pathogens of soybeans (*Glycine max* (L.) Merrill) across the United States. In surveys conducted across DE and MD from 2019-2021 SCN and RKN were widely prevalent across the state, particularly in Sussex County. Nematodes often go undiagnosed, but can be very damaging to soybean production reducing both yield and quality. Visualizing stunting and other silent symptoms can assist recognition of unnoticed yield impacts. The PI88788 resistance gene once effectively managed soybean cyst, but populations are now able to reproduce at high levels, reducing the effectiveness of this resistance source. Growers are in need of additional tools to manage nematode populations and often ask questions about the impact of cover crop decisions on nematode populations. Project objectives included: 1) Utilize rhizoboxes to visualize economic damage thresholds for Soybean Cyst Nematode (SCN), Root Knot Nematode (RKN), and the interaction of SCN and RKN. 2) Track in-season nematode populations following the use of winter cover crops. 3) Share research findings through extension events and use findings to inform future management trials. In rhizobox trials, effect of SCN and RKN on reduced growth and pod set were visualized and optimizations to the rhizobox system will be made to continue to develop photo and video resources at varying nematode population levels. Nematode soil samples were collected from different cover crops to provide insight on the nematode species present and their relative abundance among plots after winter cover crops. No notable nematode populations were recovered in any of the plots in the spring, fall results are pending analysis and final conclusions will be updated upon their arrival.

Please contact Alyssa Koehler (akoehler@udel.edu) with any additional questions.