# EFFECTS OF SOURCE, A NOVEL FOLIAR-APPLIED CHEMISTRY, ON PLANT PARASITIC NEMATODE POPULATIONS AND SOYBEAN PRODUCTION IN THE DELMARVA REGION.

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Major Project/ Report submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Online Master of Agricultural and Life Sciences in Plant Science and Pest Management

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#### ABSTRACT

Plant parasitics pose a unique challenge to soybean producers despite crop rotation, variety resistance, and use of chemicals. Soybean nematodes cause an average 10-15% loss in yield with reductions being much higher in fields with high nematode population. Soils with low organic matter as is found in much of the Mid-Atlantic region can create challenges to managing nematode populations, even with crop rotation. With hybrid selection, this may eventually give way to heavy selection pressure that can create nematodes that can evolve resistance to cultivars over time. Chemical use of nematicides such as seed treatments can be costly and can negatively impact people and the microbiome.

This project focused on the impact of using SOURCE, a novel NSS (novel synthetic strigolactone) on nematode populations, soybean plant health, and yield in the Mid-Atlantic for the 2023 season. Trials were conducted in two locations in the Delmarva area identified as having high nematode populations. Replicated treatments with and without NSS were mapped and sampled for nematodes. Soil samples were collected in treatment strips throughout the season to measure nematode populations and species variation. Additionally, yield data was collected in test strips to determine the effect of NSS on yield. Results showed no significant differences of nematode populations between treated and untreated, however the large plot trial did have an increase of 4.7 bu/A in the treated area versus the untreated. Future large and small plots trials should be considered with the addition of tissue samples taken in season.

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#### **INTRODUCTION**

#### **BACKGROUND & SETTING**

Soybeans (*Glycine max*) are one of the major row crops in the United States with an estimated 82.79 million acres to be harvested in 2023 (*USDA ERS - Market Outlook*, 2023). 30% of Maryland's land is dedicated to soybean production and is valued at \$298 million (*USDA/NASS 2022 State Agriculture Overview for Maryland*, n.d.). Similarly 30% of Delaware's land is also for soybean production valued at \$92 million (*USDA/NASS 2022 State Agriculture Overview for Maryland*, n.d.). Similarly 30% of Delaware's land is also for soybean production valued at \$92 million (*USDA/NASS 2022 State Agriculture Overview for Delaware*, n.d.). Virginia has 35% or 680,000 acres of soybeans planted as of 2022, and is the number one crop for the state valued at \$225 million (*Agriculture Facts* | *Virginia Farm Bureau*, 2022). The overall production of soybeans accounts for over \$660 million in revenue in the Delmarva area, however the impact of parasitic nematodes on yield can cause significant challenges to production and profitability.

SCN (Soybean cyst nematodes, *Heterodera glycines*) are the most yield limiting nematode species in the Delmarva area (Kessler & Koehler, 2023; *USDA ERS - Related Data & Statistics*, 2023). Overall, the impact from parasitic nematodes is substantial with SCN, causing 30% of soybean disease losses in North America or \$1.5 billion dollars in annual losses to the United States (Haarith et al., 2021; Pokharel, 2018). RKN (Root Knot Nematode, *Meloidogyne* spp.) is second for causing yield reductions in soybean (Koehler, 2023). Farmers continue to look for options to suppress and overcome these nematodes to protect against yield losses.

#### STATEMENT OF THE PROBLEM

Each year farmers are faced with threats of weather, diseases (including nematodes), weeds and insects that can affect the profitability for the farm. Disease caused by nematodes for both Maryland and Delaware are caused by: soybean cyst, root knot, and root lesion, stem and bulb and dagger nematodes (Pokharel, 2018). There has been increased interest within the scientific community to use biologicals to control parasitic nematodes on soybeans. Biological controls have been studied to understand the impact on nitrogen-fixing bacteria and phosphate-solubilizing bacteria, both of which are activated by an NSS ((a novel synthetic strigolactone, brand name SOURCE<sup>TM</sup>) (Sound Agriculture, Emeryville, CA)) have been documented, however, the effects of an NSS have not been measured for reducing nematode populations or yield loss in soybean (Quintanilla & Yazdani Fazlabadi, 2023; *SOURCE*, 2023).

With SCN being the number one major threat for soybean production globally, research on biological control has shown they have limited efficacy, possibly due to a lack of understanding of how biological control products interact in the soil ecosystem to affect nematode populations (Carter et al., 2018; Haarith et al., 2021; Quintanilla & Yazdani Fazlabadi, 2023). Biological seed treatments are the most common delivery method for biological controls but have limited efficacy against plant-parasitic nematodes as soil biology such as pH and soil type can reduce efficacy (Burns, 1971). The number and type of native microbial communities also pose competition between a biological agent and the native microbes in the soil for resources, thus limiting the effectiveness of certain biological controls (Haarith et al., 2021).

#### PURPOSE OF THE PROJECT

Soil ecology and microbial communities work together and can have an effect on plant-parasitic nematode populations as well as plant health (Haarith et al., 2021). SOURCE<sup>™</sup>, an NSS, is a novel foliar applied chemistry that stimulates the native microbial communities in the soil that may offer a benefit for defense against plant-parasitic nematodes in soybeans by

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providing nutrients while increasing beneficial microbial and fungal communities (*SOURCE*, 2023). NSS works by translocating down the plant into the root zone, to stimulate nitrogen fixing and phosphorus solubilizing microbes (*SOURCE*, 2023). Functioning as a synthetic strigolactone, NSS works by activating the soil by mimicking a signal that plants naturally make to increase microbial activity in order to provide nutrients to the plant (*SOURCE*, 2023).

#### **PROJECT OBJECTIVES**

The goal of this project is to evaluate NSS in soybean fields that have a history of high populations of SCN, RKN and other identified plant-parasitic nematode species on soybean plant health and yield in the Delmarva region for 2023. Soybean fields with documented evidence of nematode damage were selected for trial locations. The first location was the Everett Farm in Delaware which has had a history of nematode pressure throughout the field. The Everett Farm location was laid out as a large plot strip trial where soybeans were planted and harvested with farm-scale equipment. Everett Farm had a replicated strip of NSS and non-NSS mapped out for soil sampling. In addition to the areas marked for soil samples, additional check areas or non-NSS were mapped out to compare NSS for yield comparison (see Figure 2 in the Appendix).

The second trial location was at TAREC (Tidewater Agriculture Research & Extension Center, Holland Rd., Suffolk, VA). This trial was conducted as a small plot, consisting of 12 other treatments in addition to NSS to compare control of nematodes. For purposes of this report only the NSS results will be discussed.

At the beginning of the season two additional fields in Maryland were also selected for large plot trials: Baker and Todd Farms. These soil sample site locations did not line up for a proper side-by-side comparison and therefore the data from those trials is not discussed. Soil

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sample results from the Baker and Todd fields are noted in Tables 6-9 in the Appendix.

At each location, NSS was applied to the field while leaving a control or non-NSS check strip. Throughout the growing season soil samples were collected in the NSS-treated strips and the check strip location to see if there is variation in nematode populations or species between NSS and non-NSS at each farm. The soil samples were processed by Virginia Tech's TAREC Nematode Diagnostic Lab to determine the number and species of nematodes present in each sample. Plant health or performance was assessed by yield. Photos of plant size and developed roots were taken at the Everett Farm in July (see Figure 1 in the Appendix). Plant vigor, nematode damage, the number of cysts on 3 randomly selected roots, and yield were also collected at TAREC (see Table 5).

The first objective is to determine whether NSS had a positive impact on plant health given the history of known nematode pressure in the field. The primary metric for this will be yield data across both large plot and small plot trial locations. The second objective focused on whether NSS had an impact on the number of nematodes and species over the growing season. These comparisons were made between NSS and the non-NSS areas in the same field through soil samples taken throughout the season.

After completion of the trials, yield data along with a summary of nematode populations and species count was summarized to identify if NSS provided plant health benefits that were able to outperform non-NSS soybean plants with similar nematode pressure. Although NSS does not make any product claims to protect or combat against nematode pressure, NSS does provide additional key nutrients like nitrogen and phosphorus in season (*SOURCE*, 2023). Access to more nutrients through increased microbial activity over non-NSS plants could provide an advantage to soybeans under stress from pressure such as parasitic nematodes.

### **REVIEW OF LITERATURE**

Signaling compounds such as strigolactones and isoflavones impact plant development through biosynthesis at the roots, affecting the growth and production of rhizobia and arbuscular mycorrhizal fungi (AMF) (Sugiyama, 2019; Sun et al., 2022). Strigolactones are phytohormones, a signaling hormone that plants use to mitigate abiotic and biotic stress as well as trigger germination of parasitic plants (the former is not covered in this paper) (Sun et al., 2022). Strigolactones are released during times of environmental stress such as periods of drought or from lack of macronutrients like nitrogen and phosphorus (Nasir et al., 2020; Sun et al., 2022; Tariq et al., 2023). Strigolactones signal the regulation of shoot branching which thereby increases plant biomass and yield (Sun et al., 2022; Zwanenburg & Pospíšil, 2013). This can be of particular benefit when plants are under attack from parasitic nematodes. The combination of strigolactones ability to mitigate stress and increase plant biomass production in nutrient deficient environments can allow plants to partially overcome nutrient deficits. Strigolactones also stimulate hyphal branching in AMF which allows plants to get more access to nutrients and water (Sun et al., 2022).

Soybeans have a unique symbiotic relationship with AMF and microbial communities in the soil and produce a natural dominant metabolite, isoflavones, a subgroup of flavonoids (Sugiyama, 2019). Similar to strigolactones, flavonoids play an important multifunctional role in biological communication with soil microbes through the rhizosphere, the soil area immediately surrounding the root zone (Cesco et al., 2012; Sugiyama, 2019). Plants releasing flavonoids serve multiple roles where they can impact plant growth and overall health such as combating environment stress of disease or pests, or by influencing biosynthesis of nutrients, or resistance to nematode pathogens (Carter et al., 2018; Cesco et al., 2012; Gupta et al., 2016). Flavonoids are released when plants are under stress and biosynthesis is increased in the rhizosphere to help mitigate a negative impact to plant health (Cesco et al., 2010). Evidence for how flavonoids and other signaling compounds such as strigolactones are still under way as well as research on their impact on plant-microbial communication (Cesco et al., 2012).

NSS in the treatments currently have two unique molecules manufactured by Sound Agriculture as a co-pack formulation SOURCE XC: a polyphenolic flavanol and a maltol lactone (*SOURCE Application and Mixing Instructions*, n.d.). NSS's polyphenolic flavanol functions similarly as the naturally producing flavonoids produced in plants. NSS's maltol lactone functions as a synthetic strigolactone, mirroring a plant's natural signaling compounds. As NSS provides additional synthetic and organic signaling through its chemical formulations, plants should respond by increasing root branching, availability to nutrients, increase in AMF (not covered in this paper) and ability to rebound from abiotic and biotic stress. This will be helpful when plants are under pressure from parasitic nematodes in the field.

#### **PROJECT OVERVIEW**

#### TARGETED POPULATION AND PARTICIPATING AUDIENCE

Farms which participated in the trials were located in the Delmarva area. Everett Farm is located in Seaford, Delaware and has known nematode pressure throughout the field. Everett Farm typically rotates between corn and full season soybeans. Group 3.7, 3.9, and 4.1 soybeans were planted in this field in 2023. The soil is a loamy sand to sandy loam where the test strips were located (*Soils Hydric Sussex*, n.d.). This field was under pivot irrigation. Peking SCN

hybrids which are bred for high resistance to nematodes were not planted in this trial (Mourtzinis & Conley, 2017; Skorupska et al., 1994).

A small plot replicated trial, 8-ft alleys with 30-ft rows, was conducted at the TAREC (Tidewater Agriculture Research & Extension Center, Holland Rd., Suffolk, VA). SOURCE<sup>™</sup> was one of thirteen conventional in-furrow and seed treatments to see whether there was control of nematodes in soybeans. This trial location has a history of high nematodes and is a Nansemond loamy fine sand soil type.

#### PROGRAM METHODOLOGY

The Everett Farm trial contained one replicated strip containing both low and high nematode pressure. Each strip was 1000' long and 120 ft wide. NSS strips were located on either side of a non-NSS or check strip. The first NSS strip was located at (38.6075214, -75.5508485), noted as ES1. The second NSS strip was located at (38.6069773, -75.5510745), noted as ES2. The non-NSS strip was located at (38.6072180, -75.5509400), noted at ENS. A map of the GPS coordinates can be found in Figure 2. Everett Farm's soybeans were planted with a Xitavo soybean variety 3922. The field was sprayed with SOURCE SC (NSS), a product which Sound Agriculture has since been discontinued for the 2024 season, but has the identical molecule of polyphenolic flavanol which is in the SOURCE XC co-pack. (NSS) was applied during the vegetative (V4) stage of growth with a tank mix containing Roundup, Anthem Max, Enlist One, Crop Max, and Fulvic Acid. Soil samples were collected and sent to Tidewater AREC Nematode Diagnostic Lab. As mentioned earlier, photos were taken in July to compare plant and root health between the NSS and Non-NSS strips (see Figure 1). Spray maps and yield data were collected via yield monitor as raw files and uploaded on SMS. The small-plot, replicated trial at TAREC trial utilized 5 randomized complete blocks with 8-ft alleys between each block. There were two, 30-ft rows per plot with 36-in. row spacing. 5 of the 13 treatments shown in Tables 4 & 5 (Appendix). Soybeans were planted on June 2nd 2023 with SCN susceptible AG54FX0 variety. NSS was applied during the reproductive (R1) stage of growth via a tractor-mounted boom sprayer. Soil samples were collected preemergence and nematode cysts were collected on August 4th from three roots of randomly selected plants (see Table 5). The block trial also included an untreated strip to include a control.

#### TIMELINE

Trials were conducted over the 2023 growing season. Soil samples at the Everett Farm were collected in February, June, July, September and October to assess nematode populations and species. GPS transects were first determined in February 2023, along with the initial collection of soil samples in order to establish a baseline for nematodes. The planting date of the Everett Farm was April 13th for group 3.7, 3.9, and 4.1 full season soybeans. Soil samples were taken at TAREC prior to pre-plant to establish a baseline for nematode pressure. TAREC was planted on June 2nd with AG54FX0 variety.

Everett Farm was sprayed with NSS's polyphenolic flavanol on May 15th when the plants were at V4 vegetative stage. July samples were taken at the Everett Farm at the same time as the photos were taken (Figure 1). August soil samples were taken at the Everett Farms at R1, reproductive stage.

TAREC was sprayed on July 6th with NSS's XC combination, SOURCE DC 0.5 fl oz + SOURCE SC 1.25 fl oz/ac at R1 reproductive stage. It should be noted that both application windows are within the label of NSS's product recommendations. Plant vigor was assessed at

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TAREC on July 1st, prior to the NSS application. Nematode damage was assessed on July 28th and a count of nematode cysts collected from the roots of each block of 3 randomly selected plants on August 4th. TAREC was harvested on November 8th.

Final soil samples were collected in October 2023 at approximately R8 (or full maturity) across all farms. All test strips were harvested in October - November 2023. Final analysis of harvest data and nematode samples were completed in November 2023.

#### DATA COLLECTION

Soils tests were taken using a soil probe throughout the season. At each GPS transect, sampling was done in a Z-pattern at a depth of no less than 6 inches deep, with a least 8-10 core samples taken at each spot (*How to Effectively Sample for Nematodes*, 2018). The composites were then bagged, kept in a cool, dry location and immediately sent to the Virginia Tech Tidewater nematode Assay lab in Suffolk, VA where nematode identification and enumeration was conducted (Arjoune et al., 2022). Once results were returned via emailed in PDF format, data was presented in Tables 1-7 (see <u>Appendix</u>). For the Everett, Todd and Baker fields, nematodes were categorized into three areas as per the Tidewater facility 1.) no thresholds are established for this crop 2.) production of this crop the following growing season should not be affected by nematodes and 3.) nematodes may cause damage to this crop if growing conditions are unfavorable. Only the impact of soybeans were used in categorizing the nematode intensity for purposes of this report. Nematode soil samples were collected at TAREC plots during pre-plant, mid-season and harvest.

Planting data, spray maps and harvest data were collected at Everett Farm farms through digital harvest data provided in raw data via a USB file. Once the spray maps were received, a

composite side by side of NSS and non-NSS was analyzed (see Figure 2).

### SUMMARY OF OUTCOMES

#### **PROGRAM OUTCOMES**

There were no numerical trends between treatments for nematode populations or species, although the NSS strips did have a lower overall count of nematodes. At the Everett Farm ENS, the non-NSS strip contained 6 species of nematodes with 3 samples showing threshold levels of SCN juveniles (2,180) and cysts (100). ES1 had 7 species of nematodes with 4 samples coming back as high pressure including SCN juveniles (800) and male cyst (20). ES2 had 6 species of nematodes with 3 samples showing threshold levels of SCN juveniles (1000) and male cysts (20). The non-NSS strip had a total of 7,100 identified specimens as compared to the two NSS strips ES1 at 3,160 and ES2 at 1,920 respectively.

The NSS strips had an average of 64% fewer total nematodes compared to the non-NSS check strip. These data are presented in Tables 1-3 in the Appendix. Yield data was collected perpendicular to the NSS strips approximately 500 ft away in the field in order to provide a side by side. NSS yielded an average of 97.3 bu/A compared to the check 92.6 bu/Ac (see Figure 2 in the Appendix). This area was analyzed over the NSS strips as it was difficult to get a side by side comparison within such small parameters given how the raw data was received.

Pre-plant soil samples at TAREC showed no differences in nematode populations among plots. None of the plots had any significantly different SCN counts pre-plant as compared to untreated plots. Nematode damage, nematode cysts and yield results also showed no significant differences among treatments compared to the untreated plot.

#### CONCLUSIONS

The Everett Farm's untreated non-NSS strip did show higher populations of nematodes over the season compared to the two NSS-treated strips. However since this was a large plot trial there was variability in nematode pressure throughout the field (see Figure 2). Although soil samples were taken as composites within each strip, sample results varied throughout the season. The NSS-sprayed area of the field had a yield higher of 4.7 bu/A as compared to the non-NSS untreated area. It appears that the early application of NSS at V4 helped soybeans establish greater root mass and overall vigor (see Figure 1).

In the TAREC trial, there were no significant differences in nematode damage or yield as compared to the untreated. Although the NSS plot was sprayed at R1 which is within the label guidelines for optimal nutrient availability for the product, this may have had minimal impact compared to earlier applications at the Everett Farm.

#### RECOMMENDATIONS

The increase in yield in the NSS treated area at the Everett Farm, in addition to observations of increased plant health and root development in season, show a benefit of applying the NSS product at V4. More evidence of the impact of the NSS product could have been collected through tissue tests taken in season, comparing the NSS strips to the non-NSS strips. Moving forward tissue tests should be conducted on large plot trials.

The comparison of a large plot trial at Everett Farm to the small plot trial at TAREC is something to be considered moving forward for additional research. Large plot trials provide growers a means of greater visibility and acceptance since these more closely resemble field conditions; however, consistent nematode pressure is absent in large test srips. More vigorous soil sampling could be implemented in order to obtain more thorough composite samples within strips. The Baker and Todd Farms were initially large plot trials that were set up to have the NSS and non-NSS transects directly through the middle of a documented high pressure nematode area in the field. Over the course of the 2023 growing season, the logistics of aligning the spray map up with the nematode location proved to be too challenging given the logistics of the grower's operation.

Problems associated with on-farm strip trials emphasizes the need for additional small plot trials where nematode pressure is less variable. At TAREC, where treatments were arranged in randomized blocks, future trials should include an early application of NSS at V4 as compared to R1. The V4 application could ensure NSS is present in the plant early on to provide additional nutrients, reducing the effect of nematode damage earlier in the season which may impact yield. More controlled greenhouse trials may also provide further insight into the effectiveness of NSS in reducing damage caused by nematodes.

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## APPENDIX

The below includes tables of the soil samples taken for nematode analysis, spray and

yield maps of the site locations, and pictures of the Everett Farm.

**Table 1.** Everett Farm analysis of ENS, GPS transects (38.6072180, -75.5509400) or Non-NSS check strip. (# Nematodes/500 cc soil).

Species	Feb	June	July	Sept	Oct	Total
Juveniles (Root-knot)						0
Eggs (Root-knot)						0
Males (Root-knot)						0
Galls/Females (Root-knot)						0
Juveniles (Cyst)	160	1920	20	60	20	2180
Eggs (Cyst)						0
Males (Cyst)						0
Females/Cysts (Cyst)	20	60			20	100
Pratylenchus (Lesion)			20			20
Tylenchorhyncus (Stunt)	60					60
Helicotylenchus (Spiral)	680	1180		2120	120	4100
Hoplolaimus (Lance)						0
Mesocriconema (Ring)						0
Trichodurus (Stubby root)	180	200		260		640
Belonolaimus (Sting)						0
Xiphinema (Dagger)						0
					TOTAL	7100

Species	Feb	June	July	Sept	Oct	Total
Juveniles (Root-knot)						0
Eggs (Root-knot)						0
Males (Root-knot)						0
Galls/Females (Root-knot)						0
Juveniles (Cyst)	20	540	40	160	40	800
Eggs (Cyst)						0
Males (Cyst)		20				20
Females/Cysts (Cyst)					60	60
Pratylenchus (Lesion)						0
Tylenchorhyncus (Stunt)			720			720
Helicotylenchus (Spiral)	120	60			960	1140
Hoplolaimus (Lance)	140				60	200
Mesocriconema (Ring)						0
Trichodurus (Stubby root)				180	40	220
Belonolaimus (Sting)						0
Xiphinema (Dagger)						0
					TOTAL	3160

**Table 2.** Everett Farm analysis of ES1, GPS transects (38.6075214, -75.5508485) or first NSSstrip. (# Nematodes/500 cc soil).

Species	Feb	June	July	Sept	Oct	Total
Juveniles (Root-knot)						0
Eggs (Root-knot)						0
Males (Root-knot)						0
Galls/Females (Root-knot)						0
Juveniles (Cyst)	40			820	140	1000
Eggs (Cyst)						0
Males (Cyst)				20		20
Females/Cysts (Cyst)	120					120
Pratylenchus (Lesion)						0
Tylenchorhyncus (Stunt)						0
Helicotylenchus (Spiral)	20			60	80	160
Hoplolaimus (Lance)			80		200	280
Mesocriconema (Ring)						0
Trichodurus (Stubby root)	260				80	340
Belonolaimus (Sting)						0
Xiphinema (Dagger)						0
					TOTAL	1920

**Table 3.** Everett Farm analysis of ES2, GPS transects (38.6069773, -75.5510745) or second NSS strip. (# Nematodes/500 cc soil).

	Nematodes /500 cc soil <sup>y</sup>						
Treatment and rate/A <sup>z</sup>	Cyst juvenile	Cyst female	Stunt	Spiral	Stubby root		
Untreated	52	24	24	104	0		
Velum 6 fl oz/A (F)	40	20	40	88	4		
Avicta 500FS 0.15 mg ai/seed (S)	28	4	20	56	8		
AgLogic 15G 7 lb/A (F)	112	4	64	48	8		
SOURCE DC 0.5 fl oz + SOURCE SC 1.25 fl oz/A (FS at R1)	44	0	16	72	0		

**Table 4.** Pre-plant nematode populations (SOYSEEDNEMA423, Tidewater AREC, Suffolk,VA 2023).

<sup>z</sup> (F) In-furrow applied at planting on 2 Jun. (S) = seed treatment. (FS) foliar spray applied at R1 on 6 Jul.

<sup>y</sup> Plots were sampled on 2 Jun. Pre-plant data are the mean counts of nematodes in a sample from six reps of each designated treatment plot; data reflects pre-treatment nematode populations only.

**Table 5**. Effect of treatment on emergence, plant health, root cysts, and yield in soybean(SOYSEEDNEMA423, Tidewater AREC, Suffolk, VA 2023).

Treatment and rate <sup>z</sup>	No. plants /ft. <sup>y</sup> 29 Jun	Vigor (1-10) <sup>x</sup> 1 Jul	Nematode damage <sup>w</sup>	No. cysts/3 roots <sup>v</sup> 4 Aug	Yield (bu/A) <sup>u</sup>
Untreated	12.5	7.8	2.0	133	34.7
Velum 6 fl oz/A (F)	12.4	7.8	1.6	101	32.1
Avicta 500FS 0.15 mg ai/seed (S)	12.2	7.8	1.0	319	30.4
AgLogic 15G 7 lb/A (F)	11.4	7.6	1.6	150	31.3
SOURCE DC 0.5 fl oz + SOURCE SC 1.25 fl oz/A (FS at R1)	13.0	7.8	1.8	137	30.9
P(F) = 0.10	0.45	0.81	0.28	0.16	0.54

<sup>z</sup> (F) In-furrow applied at planting on 2 Jun. (S) = seed treatment. (FS) foliar spray applied at R1 on 6 Jul.

<sup>y</sup> Determined from counts of 1-meter of row ft in each row of plot.

<sup>x</sup> Vigor index rating scale: 10 = 100% vigor, 1 = no vigor.

<sup>w</sup> Index rating scale for damage by nematodes to plants above the surface: 0 = no damage, 10 = most severe.

<sup>v</sup> Mean count of nematode cysts collected from the roots of 3 randomly selected plants per plot.

<sup>u</sup> Yields are weight of soybean with 13.5% moisture. One bushel equals 60 lb. Soybean was harvested on 8 Nov.

	Baker Good				
Species	Feb	June	Sept	Oct	Total
Juveniles (Root-knot)	40				40
Eggs (Root-knot)					0
Males (Root-knot)					0
Galls/Females (Root-knot)					0
Juveniles (Cyst)			60	1040	1100
Eggs (Cyst)					0
Males (Cyst)				20	20
Females/Cysts (Cyst)					0
Pratylenchus (Lesion)				40	40
Tylenchorhyncus (Stunt)	200			60	260
Helicotylenchus (Spiral)	320	80	1140	1080	2620
Hoplolaimus (Lance)	60	40	220	20	340
Mesocriconema (Ring)					0
Trichodurus (Stubby root)	440		280	280	1000
Belonolaimus (Sting)					0
Xiphinema (Dagger)					0
				TOTAL	5420

**Table 6.** Baker Farm analysis of BG1, GPS transects (38.6511245, -75.8439197) or low nematode pressure. NSS was not applied to this area of the field. (# Nematodes/500 cc soil).

	Baker Bad				
Species	Feb	June	Sept	Oct	Total
Juveniles (Root-knot)					0
Eggs (Root-knot)					0
Males (Root-knot)					0
Galls/Females (Root-knot)					0
Juveniles (Cyst)	60	40	1040	780	1920
Eggs (Cyst)					0
Males (Cyst)			20		20
Females/Cysts (Cyst)				260	260
Pratylenchus (Lesion)	20			120	140
Tylenchorhyncus (Stunt)					0
Helicotylenchus (Spiral)	140	440		20	600
Hoplolaimus (Lance)	100	280	80	120	580
Mesocriconema (Ring)					0
Trichodurus (Stubby root)	160	80	40	100	380
Belonolaimus (Sting)					0
Xiphinema (Dagger)					0
				TOTAL	3900

**Table 7.** Baker Farm analysis of BB1, GPS transects (38.6505987, -75.8442641) or high nematode pressure. NSS was applied to this area of the field. (# Nematodes/500 cc soil).

	Todd Farm TG1				
Species	Feb	June	Sept	Oct	Total
Juveniles (Root-knot)					0
Eggs (Root-knot)					0
Males (Root-knot)					0
Galls/Females (Root-knot)		40			40
Juveniles (Cyst)			200	20	220
Eggs (Cyst)					0
Males (Cyst)					0
Females/Cysts (Cyst)					0
Pratylenchus (Lesion)			20		20
Tylenchorhyncus (Stunt)			40	120	160
Helicotylenchus (Spiral)	100	1820	700	2840	5460
Hoplolaimus (Lance)				20	20
Mesocriconema (Ring)					0
Trichodurus (Stubby root)				200	200
Belonolaimus (Sting)					0
Xiphinema (Dagger)					0
				TOTAL	6120

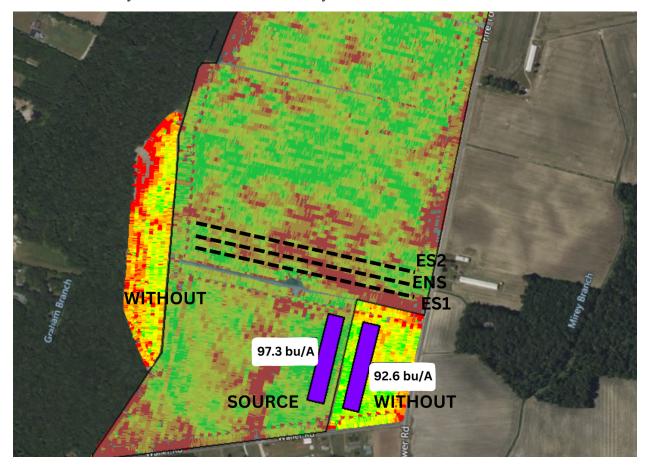
**Table 8.** Todd Farm analysis of TG1, GPS transects (38.6204320, -75.8691737) or low nematode pressure. NSS was not applied to this area. (# Nematodes/500 cc soil).

Table 9. Todd Farm analysis of TB1, GPS transects (38.6207102, -75.8697521) or high nematode
pressure. NSS was not applied to this field. (# Nematodes/500 cc soil).

	Todd Farm TB1				
Species	Feb	June	Sept	Oct	Total
Juveniles (Root-knot)	60			80	140
Eggs (Root-knot)					0
Males (Root-knot)					0
Galls/Females (Root-knot)					0
Juveniles (Cyst)			160	160	320
Eggs (Cyst)					0
Males (Cyst)					0
Females/Cysts (Cyst)					0
Pratylenchus (Lesion)		20		20	40
Tylenchorhyncus (Stunt)				40	40
Helicotylenchus (Spiral)	3480	20	1320	7120	11940
Hoplolaimus (Lance)					0
Mesocriconema (Ring)					0
Trichodurus (Stubby root)	60		40	80	180
Belonolaimus (Sting)					0
Xiphinema (Dagger)					0
				TOTAL	12660

**Figure 1.** Everett Farm side by side of NSS treated plants on left, non-NSS plants on the right. Photo taken in July 2023.





**Figure 2.** Everett Farm map of NSS area and non-NSS area, location of ES1, ENS, ES2, and location of where yield data was taken for side by side.