



SON2026 Abstracts

Characterization of a new dorsal gland protein secreted by adult female root-knot nematodes

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Abstract Root-knot nematodes (RKN, *Meloidogyne* spp.) represent the most damaging group of plant-parasitic nematodes affecting global crop production. RKN adult females secrete virulence proteins from specialized esophageal gland cells through a protrusible stylet, reprogramming host cells into feeding sites. Deciphering the identity and function of these secreted proteins is essential for developing new control strategies and advancing resistance breeding. A comparative glycoproteomic analysis of *M. incognita* adult female stylet secretions identified *Minc*s secreted by females, including several previously shown to be upregulated in dorsal gland transcriptomes. Further filtering yielded a subset of candidate secreted proteins with signal peptides, no transmembrane domains, and no *Caenorhabditis elegans* homology. Fourteen of these candidates were selected for *in situ* hybridization, with six confirmed as dorsal gland-expressed *Minc*s. We focused on one candidate, *MincA*, for detailed characterization. *MincA* expression was 24- and 649-fold higher in adult females than in juveniles and eggs, respectively. Orthologs were identified across six *Meloidogyne* species, and conserved dorsal gland expression was confirmed in the distantly related *M. hapla*. *MincA* was also detected in feeding-tube enriched samples, supporting its secretion into giant-cell cytoplasm and possible role in parasitism. Ongoing work involves generating tomato plants expressing RNAi constructs targeting *MincA* via virus-induced gene silencing to assess resistance to RKN infection. Because RNAi suppression can act across species, transformed plants will be tested against both *M. incognita* and *M. hapla*. These findings highlight promising new targets for nematode management.

Evaluation of soybean varieties for resistance to southern root-knot nematode (*Meloidogyne incognita*) under field conditions in Louisiana

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Abstract Soybean is an economically important crop in Louisiana, representing 21% of the state's crop value. The Southern root-knot nematode (SRKN), *Meloidogyne incognita*, causes approximately 2.25% yield loss annually, representing more than \$12 million dollars in lost revenue. Several management tactics are used to reduce nematode-associated yield loss in soybean, including the deployment of resistant varieties, application of nematicides, and the use of cover crops. This study aimed to evaluate the performance of seven soybean varieties labeled as resistant to SRKN relative to the performance of a susceptible variety. A field trial was conducted in a SRKN-infested field in Bossier City, Louisiana, to evaluate the influence of host resistance on SRKN densities in soil and roots, as well as soybean yield. Soil samples were collected at planting, mid-season, and harvest. Nematodes were extracted using an elutriator and sugar flotation technique. Root samples were collected at mid-season and harvest to assess root gall ratings (0–5 scale) and nematode egg production. At harvest, nematode soil population ranged from 4,240 to 16,960 nematodes per 500 cc of soil. Root galling indices ranged from 1.5 to 2.3 across all of the soybean varieties, and nematode egg densities ranged from 9,168 to 78,240 eggs per plant, indicating significant parasitism and nematode reproduction in all soybean varieties, regardless of labelled resistance. Despite this, six of the soybean varieties labelled as resistant produced higher yields than the susceptible variety, indicating host tolerance to SRKN. These results highlight the need to identify new sources of enhanced SRKN resistance as well as research into integrated nematode management to enhance nematode control on the existing soybean varieties.

Artificial intelligence for molecular and genomic discovery in nematology

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Abstract The rapid expansion of genomic and transcriptomic datasets has created new opportunities for molecular discovery in nematology, while also introducing major challenges in annotation, interpretation, and data integration. Artificial intelligence (AI) is emerging as a useful assistive framework for handling these increasingly complex datasets and for generating biologically meaningful hypotheses from large-scale molecular data. This presentation will examine practical applications of AI in molecular and genomic nematology, with emphasis on both its promise and its limitations. Topics to be discussed include AI-assisted genome annotation and gene model refinement, automated classification of gene families and protein domains, prediction of effectors and secreted proteins, comparative genomic analyses across nematode species and populations, and pattern recognition in transcriptomic datasets to identify stage-specific or condition-specific expression signatures. The presentation will also address the use of AI for detecting annotation inconsistencies, recognizing potential database-derived errors, and synthesizing high-dimensional molecular datasets into more interpretable biological patterns. Special emphasis will be placed on the responsible use of AI in nematology. Although AI can improve analytical efficiency and reveal patterns that may be difficult to detect manually, its outputs remain strongly influenced by reference database quality, model assumptions, taxonomic representation, and the underlying training data. Overreliance on AI-generated predictions may therefore lead to propagation of annotation errors, overconfidence in inferred gene function, and conclusions that are not adequately supported by biological evidence. This presentation will argue that AI is most valuable when used as a support tool rather than a replacement for expert interpretation. Integrating AI-assisted outputs with morphology, ecology, vouchered specimens, and experimental validation is essential to maintain biological relevance and taxonomic accuracy. Overall, this talk will provide a practical overview of how AI can support molecular and genomic nematology while highlighting the need for transparent, reproducible, and biologically grounded workflows.

Evaluation of *Pseudomonas* sp. JDE115 as a biological seed treatment for cotton growth promotion and suppression of reniform and root-knot nematodes

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Abstract Soil-borne plant-parasitic nematodes, including reniform nematode (*Rotylenchulus reniformis*) and root-knot nematodes (*Meloidogyne* spp.), are major constraints to cotton production and often reduce plant vigor, root health, biomass accumulation, and yield potential. Biological seed treatments that promote early plant growth while suppressing nematode infection and reproduction may provide a sustainable tool for integrated nematode management. In this study, we evaluated *Pseudomonas* ssp. JDE115, a plant growth-promoting and biocontrol bacterium previously characterized from soybean nodules, as a seed-applied biological treatment for cotton. Previous genomic and laboratory characterization indicated that JDE115 possesses multiple plant-beneficial and antagonistic traits, including siderophore production, phosphate solubilization, protease activity, biofilm formation, volatile-mediated antagonism, and nematocidal activity. Cotton seeds were treated with freshly cultured JDE115 and evaluated under greenhouse conditions against reniform and root-knot nematode pressure. Treatments included (i) JDE115 alone, (ii) untreated control, (iii) reniform nematodes alone, (iv) JDE115 + reniform nematode, (v) root-knot nematode alone, and (vi) JDE115 + root-knot nematode, with five replications per treatment. Plant growth parameters included germination, plant height, stem diameter, boll number, plant weight, and visual vigor ratings. Preliminary results showed that JDE115 improved cotton growth compared with the untreated control and nematode-only treatments. Mean plant height was greater in JDE115-treated plants and in JDE115 + nematode treatments than in plants treated with reniform or root-knot nematodes alone. JDE115-treated plants also produced more bolls than untreated and nematode-only plants. Preliminary analysis indicated significant differences in plant weight between JDE115-treated and control plants, and between JDE115 + reniform nematode and reniform-only plants. In contrast, nematode reproduction showed the opposite pattern, with higher reproduction in nematode-only treatments and reduced reproduction when JDE115 was applied. Laboratory bioassays further supported the nematocidal potential of JDE115. Direct-contact assays showed rapid reniform nematode mortality after exposure to JDE115, while media-only and water controls remained active. Three-chamber volatile assays demonstrated nematode mortality without physical contact, suggesting that volatile compounds produced by JDE115 contribute to nematode suppression. Together, these results indicate that JDE115 can function as both a plant growth-promoting bacterium and a biological nematode-suppressive agent in cotton. These results support further validation through replicated greenhouse trials, optimization of seed coating formulations, and field validation under commercial cotton production systems.

Mystery molecules from *Meloidogyne chitwoodi* glands: Functional characterization of novel effectors reveal candidate drivers of virulence and resistance activation

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Abstract The Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi*, is a major quarantine potato pathogen in the Pacific Northwest of the United States, where tuber blemishes caused by infection can severely reduce crop value and marketability. Root-knot nematodes rely on effectors secreted from their esophageal glands to manipulate host cells, suppress immunity, and establish feeding sites. However, the identities and biological functions of *M. chitwoodi* effectors are almost entirely unexplored. To address this gap, we generated the first esophageal gland-specific transcriptome for *M. chitwoodi* and identified candidate parasitism genes expressed in the nematode secretory glands. From this dataset, two highly expressed and previously uncharacterized candidates, *McGland1* and *McGland19*, were selected for detailed investigation. *In situ* hybridization confirmed gland-specific expression for both genes, supporting their classification as secreted effectors. Sequence analyses revealed that none of these proteins contain conserved domains or recognizable functional motifs, emphasizing the unique and poorly understood biology of *M. chitwoodi* parasitism. Comparative analyses identified limited similarity to genes from other plant parasitic nematodes, but no clear functional annotations were identified for any of the candidates. Expression profiling showed that all three effectors are highly expressed during the pre-parasitic second-stage juvenile (J2) stage, consistent with a role during early host invasion. Interestingly, the 3 effectors showed similar expression patterns between the avirulent Race 1 population and the resistance-breaking Roza population during early infection stages. *McGland1* and *McGland19* were significantly downregulated in the virulent Roza population during later infection stages of the resistant potato breeding line PA99N82-4, suggesting that transcriptional regulation of these effectors may contribute to adaptation during incompatible interactions. To investigate their biological roles, Arabidopsis transgenic lines constitutively expressing *McGland1* or *McGland19* were generated and challenged with *M. chitwoodi*. While overexpression of *McGland1* increased nematode parasitism of the roots, overexpression of *McGland19* resulted in increased host resistance. Consistently, *McGland1*-overexpressing plants displayed reduced elicitor-induced reactive oxygen species (ROS) accumulation, and *McGland19*-overexpressing plants displayed increased ROS accumulation. Collectively, this work represents the functional characterization of gland-secreted effectors from *M. chitwoodi* and reveals a set of enigmatic proteins that simultaneously contribute to parasitism and activate host defense responses. These findings provide new insights into the molecular dialogue between potato and CRKNs. It helps establish a foundation for future studies aimed at identifying durable resistance mechanisms and effector-informed nematode control strategies.

Nematode-enabled discoveries from niche adaptation to pangenomics

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Abstract For more than 60 years, *Caenorhabditis* nematodes have enabled numerous biological discoveries, including signaling pathways in development, programmed cell death, complete neuronal circuits, small RNAs, and RNA interference. Each of these fundamental advances used the laboratory-adapted strain of *C. elegans* called N2, found in Bristol, England in 1951. This reductionist approach is akin to studying the biology of one entire species by focused experiments using a single individual. I will present my lab's focus on bringing natural diversity to *C. elegans* to understand evolutionary principles and other aspects of fundamental biology. First, I will present my lab's efforts to investigate the natural niche for *Caenorhabditis* nematodes, including highly scaled ecological sampling approaches paired with genome sequencing. Then, I will describe quantitative and population genetics approaches to investigate how this species is adapted to its niche with a focus on the molecular mechanisms of plasticity. Finally, I will turn to our recent efforts to define hyper-diverse genomic regions that underlie adaptation to the environment. These regions harbor thousands of new genes that are hypothesized to enable host-pathogen and bacterial food interactions. We require pangenomic resources to investigate highly divergent gene contents, and I will explain our efforts to move our understanding of the genome and species beyond the single reference strain N2.

Plant-parasitic nematode communities in missouri soybean fields: Prevalence, richness, and implications for management

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Abstract A statewide survey was conducted during the 2024 and 2025 growing seasons to characterize the prevalence, richness, and composition of plant-parasitic nematode (PPN) communities in Missouri soybean fields. Soil and root samples were collected from 200 fields across 81 counties in all nine USDA-NASS crop reporting districts. Nematodes were extracted, identified to genus or diagnostic group, and quantified. Field-level presence was defined as detection in soil or root samples. Soybean cyst nematode (SCN; *Heterodera glycines*) populations were further characterized using egg density and virulence phenotyping. PPNs were widespread. The most prevalent taxa were spiral (*Helicotylenchus* spp.), root-lesion (*Pratylenchus* spp.), and SCN, detected in 85, 84, and 80% of samples, respectively. Stunt (*Tylenchorhynchus* spp. group) and lance nematodes (*Hoplolaimus* spp.) occurred in 25 and 14% of samples, while other taxa occurred at low frequencies (<5%). Fields contained 1 to 5 PPN taxa, with 47% of fields containing three taxa. This indicates that most Missouri soybean fields support multi-species communities. Population densities varied among PPN taxa. Spiral nematodes had a median density of 120 per 100 cm³ soil, while root-lesion nematodes had a median density of 60 per 100 cm³ soil and 6.8 per g of root. Root-knot nematode was identified in 3% of samples and was predominantly restricted to southeast Missouri, where median density reached 600 individuals per 100 cm³ soil and 82.3 per g of root among positive fields. SCN population densities were also highly variable across the state. Median egg density among SCN-positive fields was 2,100 eggs per 100 cm³ soil. Virulence phenotyping indicated widespread reproduction on PI 88788, with all populations tested to date ($n = 105$) having a female index (FI) ≥ 10 and a statewide median of 66. On Peking, 49% of populations exceeded this threshold, with a median of 10. Soybean fields in Missouri are characterized by diverse PPN communities. The widespread distribution and multi-species occurrences suggest that nematode management strategies in soybean should consider the broader PPN community rather than focusing on a single species.

Field strength and frequency-dependent effects of pulsed electric fields on nematodes, and weeds

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Abstract Pulsed electric field (PEF) technology is an emerging non-chemical approach for soil disinfestation, with potential applications in the management of plant-parasitic nematodes and weeds. This study evaluated how applicator design, electric field strength, pulse frequency, and energy density influenced PEF efficacy against *Meloidogyne chitwoodi*, *Pratylenchus neglectus*, and several weed species (*Cyperus esculentus*, *Digitaria sanguinalis*, and *Echinochloa crus-galli*). Two electrode configurations, vertical pins (VP) and parallel plates (PP), were compared. Numerical simulations demonstrated that the PP applicator generated a more uniform electric field distribution, whereas the VP configuration produced highly localized gradients. This difference translated into biological outcomes: the PP system consistently outperformed the VP applicator, providing more uniform and greater suppression of both nematodes and weeds. Plant-parasitic nematodes were highly susceptible to PEF treatments at relatively low energy inputs. At 25 J cm⁻³, *M. chitwoodi* second-stage juveniles were reduced up to 100% at frequencies ≥90 Hz. Suppression increased significantly with pulse frequency ($p < 0.008$) and field strength ($p < 0.03$). For *P. neglectus*, field strength was the dominant factor ($p < 0.001$), with reductions ranging from 74–93% at 200 V mm⁻¹ and exceeding 98% at 400 V mm⁻¹ across all frequencies. In contrast to *M. chitwoodi*, frequency had no significant effect on *P. neglectus* densities. These results indicate nematode species-specific responses, likely driven by differences in morphology and biophysical properties. Weed responses required substantially higher energy inputs and were strongly species-dependent. For *C. esculentus*, shoot biomass was reduced with increasing energy density, with marked reductions at ≥100 J cm⁻³ and complete suppression at >125 J cm⁻³ under specific field conditions. *D. sanguinalis* biomass reduction was gradual but consistent reductions began at 50–75 J cm⁻³, depending on field strength. In contrast, *E. crus-galli* was more tolerant, with significant biomass reduction observed only at the highest energy levels (150–200 J cm⁻³). Across species, energy density ($p < 0.001$), field strength ($p \leq 0.01$), and their interaction were significant drivers of weed suppression. Overall, nematodes were more sensitive to PEF than weed propagules, requiring approximately four- to eight-fold lower energy densities for effective control. The superior performance of the PP applicator highlights the importance of uniform electric field distribution for achieving consistent biological effects. These findings demonstrate that PEF can selectively target soilborne pests, with strong efficacy against plant-parasitic nematodes at moderate energy levels, while higher inputs are required for weed suppression.

From roots to results: Plant growth-promoting rhizobacteria for managing root-knot nematodes in cotton

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Abstract Root-knot nematode, *Meloidogyne incognita*, is a major biological constraint to cotton (*Gossypium hirsutum*) production across the southeastern United States, where infestations reduce root function, impair plant vigor, and ultimately lower yield potential. Biological alternatives to existing management strategies are increasingly sought to improve long-term soil and crop health. This study evaluates a diverse collection of plant growth-promoting rhizobacteria (PGPR), isolated from plants roots collected in La Luz, New Mexico, for their ability to suppress nematode populations while supporting cotton growth under controlled and field conditions. An initial *in vitro* screening of 235 bacterial isolates was conducted to identify strains exhibiting nematocidal activity. *In vitro* nematode bioassays targeting second-stage juveniles, evaluated using NaOH viability test after 48 hours, revealed significant mortality ranging from 0% to 36% among all the PGPR strains screened. *Chryseobacterium hilarii* (DT-3) induced the highest juvenile mortality at 36%, followed by *Sphingomonas asragali* (DT-204, 32%), *Bacillus velezensis* (DT-59, 32%), all higher than the untreated control ($P \leq 0.05$). Based on this preliminary assessment, 37 promising strains were selected for greenhouse evaluation, and seven for field testing. Both experimental units consist of no-treatment control (water), a chemical control (10 ppm Fluopyram), a biological control (Majestene®; containing heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media) as controls. All experiments were arranged in a randomized complete block design with five replications and were repeated. Mortality data were analyzed using a generalized linear mixed model (PROC GLIMMIX), and treatment means were compared with Tukey's HSD test ($\alpha \leq 0.05$). Under greenhouse conditions, DT-155 (*Pseudomonas turukhanskensis*) significantly reduced nematode density (6,348 eggs/g root), comparable to no-treatment control (26,395 eggs/g root) ($P = 0.0021$). In field trials, treatment effects on stand establishment ($P = 0.98$), plant height ($P = 0.98$) and plant biomass ($P = 0.46$) were not statistically different among treatments. However, DT-129 (*Arthrobacter nitrophenolicus*) produced the numerically tallest plant (25.75 cm), and greatest biomass accumulation (8.84 gram). Several strains demonstrated significant suppression of *M. incognita* populations; DT-87 (*Bacillus amyloliquefaciens*), DT-204 (*Sphingomonas asragali*), and DT-105 (*Bacillus velezensis*), which reduced egg densities to 2,710, 2,952, and 3,537 eggs/g root, respectively, compared with 14,166 eggs/g root in the water control ($P \leq 0.005$). Yield data revealed that DT-105 (*Bacillus velezensis*) produced the numerically highest lint yield among treatments (1,928 lb./A), whereas DT-14 (*Pseudomonas piscicola*) had lowest yield (1,538 lb./A); however, differences were not statistically significant ($P = 0.6341$). Several PGPR isolates significantly suppressed *M. incognita* under greenhouse conditions, with selected strains reducing egg densities by more than 50% relative to controls. However, these effects did not consistently translate to significant improvements in yield under the dry field conditions of 2025. These findings indicate that isolates DT-87 (*Bacillus amyloliquefaciens*), DT-204 (*Sphingomonas asragali*), and DT-105 (*Bacillus velezensis*) have potential as biological control agents. Further optimization under diverse field environments is needed to improve before integration into nematode management programs.

Virulence characterization of *Globodera* populations from Peru and the United States

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Abstract Potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, are among the most economically important plant-parasitic nematodes, causing substantial yield losses in potato production. While introduced PCN populations in regions like the United States exhibit limited virulence, native Andean populations possess broad, underexplored phenotypic diversity. Current commercial potato resistance relies on a narrow genetic base targeted at predominant pathotypes outside the Andes, leaving it vulnerable to the highly diverse native Andean populations. Developing broad spectrum resistance therefore requires a thorough understanding of the PCN diversity present at their center of origin. To address this, our study assessed the virulence of ten Peruvian populations alongside U.S. reference populations (*G. pallida* Idaho and *G. rostochiensis* New York) on a comprehensive panel of standard differentials, commercial varieties, and advanced breeding lines. By comparing these phenotypic profiles, we quantified the 'virulence gap' between introduced U.S. and native Peruvian populations and evaluated the efficacy of different resistance sources against this broader virulence spectrum. The species composition of Peruvian populations was identified as six pure *G. pallida*, one pure *G. rostochiensis*, and three mixed-species populations. While bioassays showed U.S. populations aligning predictably with standard *G. pallida* Pa2/3 and *G. rostochiensis* Ro1 pathotypes, Peruvian populations exhibited a substantially broader virulence spectrum. Most (five of six) Peruvian *G. pallida* matched Pa2/3 profiles, but some overcame Pa2/3 resistance in the variety 'Maria Huanca'. Furthermore, a unique 'Pa1-like' *G. pallida* population also compromised 'Maria Huanca' resistance despite avirulence on an *H2*-containing clone. The single *G. rostochiensis* population was controlled by the 'Performer' variety but showed higher virulence on certain clones (D47/11) than the U.S. reference. Notably, mixed-species populations displayed extreme virulence and variability, with one population breaking resistance in 'Performer'. These findings demonstrate that the virulence diversity maintained within the Andean reservoir poses a persistent and severe threat to global potato production. To mitigate this risk, enforcing strict quarantine measures against new introductions and pyramiding genes for broad-spectrum resistance will be vital.

Field to lab: Characterizing fitness costs in resistance-breaking nematode populations

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Abstract The success of California's processing tomato industry depends on the growers' ability to implement management strategies, including host resistance, effective pesticides, and non-host rotation crops to eliminate or control pathogens. Despite these efforts, root-knot nematodes (RKNs), *Meloidogyne* spp., cause an estimated 100 million USD in yield loss for processing tomatoes by suppressing the plant immune system, damaging root tissues, and creating entry points for secondary pathogens such as *Fusarium* species. For decades, the resistance gene *Mi-1* has effectively recognized and inhibited RKN feeding site formation in tomatoes, but the underlying mechanisms by which it recognizes these pathogens remains largely unknown. Additionally, resistance-breaking populations have been increasingly identified in both greenhouse and field settings, threatening the effectiveness of the *Mi-1* gene and consequently the tomato industry. To better understand how RKNs interact with *Mi-1* resistance and an impact on fitness, we utilized two closely related strains of *Meloidogyne javanica*: VW4, a wild-type strain, and VW5, a lab-generated resistance-breaking strain to perform comparative genomics to identify unique regions in the genome. Through this approach we identified a large deletion containing 50 genes. This platform allows us to establish VW4 and VW5 as a model to identify resistance breaking genes and virulence genes associated with fitness. Following infection of susceptible hosts (tomato, cucumber, and rice), VW5 showed a significant decrease in egg production. Further examination of nematode feeding sites at early and late infection stages revealed giant cell deformities in VW5. Transcriptional analysis at corresponding timepoints showed a lack of host plant manipulation for feeding site formation and a weaker host immune suppression for VW5 as well. Meanwhile, a survey for resistance-breaking populations was conducted in California where fourteen strains were collected and confirmed to be resistance breaking at stable temperatures. Strains were purified and sequenced for species identification. A PCA Plot generated based on SNPs showed diversity in the collected strains indicating independent events. The four most diverse populations of *M. incognita* were tested on current rotation crops and suggested crops for processing tomatoes (corn, alfalfa, wheat, cucumber, and susceptible tomato) to evaluate virulence and identify sources of management. With this research, we aim to improve understanding of how RKNs interact with *Mi-1* leading to resistance and develop alternate management strategies against resistance breaking populations, ultimately supporting California's tomato growers.

Freedom to farm: RinoTec™ technology, a novel nematicide/insecticide

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Abstract RinoTec™ nematicide/insecticide secured EPA registration in March 2025 with a tolerance exemption, has been successfully and extensively tested over the past five years by universities and third-party CROs, demonstrating strong activity against economically important plant parasitic nematodes including Columbia root-knot, northern root-knot, and root lesion nematode. RinoTec™ is available for use in both row crops and specialty crops under crop specific and geographic labels, NEOVO™, ARINO™, And BRONTE™. Its ease of use is highlighted by the absence of buffer zones for soil applications and a short 4-hour re-entry interval (REI). The product was also the recipient of the 2024 EPA Green Chemistry Award, underscoring its favorable environmental profile and low impact on beneficial insects. Made in the USA, RinoTec™ has established MRLs and will be available to US growers for the first time in 2026, providing a new, high-performance solution for mitigating losses from nematodes.

Neovo™ a novel liquid in-furrow nematicide/insecticide for corn and soybean (RinoTec™ technology)

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Abstract For row crop growers facing increasing losses from yield robbing nematodes, insect resistance and complex planting and harvesting logistics, NEOVO™ is a next-generation in-furrow liquid nematicide/insecticide. NEOVO™, powered by RinoTec™ technology, provides growers with the ultimate freedom to farm with a new mode of action, grower-friendly “Caution” label (4-hour REI, no herbicide interactions, and no buffer zones). This ensures that growers can maximize their seed/seedling protection while maintaining the flexibility to tank-mix with starter fertilizers and plant on their own terms. With multiple mechanisms of action including ingestion and repellency, NEOVO™ backed by five years of university and third-party CROs, provides sustained protection against economically important plant parasitic nematodes including root lesion nematode and soybean cyst nematode as well as corn rootworm.

Papaya seed extract for management of *Radopholus similis* on anthurium

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Abstract Anthurium cut flowers are an economically important ornamental crop and signature export in Hawai'i, but their production is threatened by the burrowing plant-parasitic nematode, *Radopholus similis*, which reduces yield and increases production costs. This study evaluated the nematocidal potential of crude extract from ground papaya seed (PGS CE), a local agricultural byproduct containing glucosinolates that hydrolyze into benzyl isothiocyanate (BITC), a compound active against *R. similis*. One *in vitro* trial and two shadehouse pot trials were conducted to compare the effects of PGS CE treatment at 0.5% and 1% concentration to commercially available AzaGuard® (neem oil) and unamended (NA) and uninoculated (UI) controls. In the *in vitro* bioassay, beakers containing 100 vermiform *R. similis* each were treated with 0.5 mM or 0.1 mM synthetic BITC, 1% or 0.5% PGS CE, 2 µl/ml AzaGuard®, or a sterile water control. Samples were counted first at 24 hours, rinsed and incubated in clean sterile water, then counted again at 48 hours to determine nematostatic versus nematocidal effects. Shadehouse trials were conducted using 'Leilani' anthurium transplanted in clean cinder media and inoculated with 500 vermiform *R. similis* per pot (excluding uninoculated pots). Pots were drenched with either 0.5% or 1% PGS CE, 0.2% AzaGuard®, or water only at the start of the experiment, and drenching was repeated every month for 6 months. *In vitro* results showed 1% PGS CE caused 95% mortality of *R. similis*, outperforming pure BITC (0.1 and 0.5 mM) and 0.2% AzaGuard® treatments. Shadehouse trials demonstrated that monthly drenching with 0.5 or 1% PGS CE significantly reduced *R. similis* population densities in both roots and cinder media, decreased root lesion severity, and increased leaf production without phytotoxicity or adverse effects on populations of free-living bacterivorous nematodes. While 1% PGS CE slightly reduced plant biomass, 0.5% PGS CE improved plant growth and flower production. Regression analysis supported the link between nematode suppression and enhanced anthurium health. Thus, PGS CE presents a sustainable, locally available, and effective biofumigant for nematode management in anthurium, warranting further optimization and future field-scale validation.

Re-evaluating root lesion nematode disease in pepper: The *Pratylenchus capsici*–microbiome disease complex, their interactions, and temperature-driven dynamics

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Abstract Rising soil temperatures associated with climate change are expected to alter rhizosphere microbial communities, with important consequences for plant–nematode interactions. Here, we investigated temperature-mediated changes in root- and rhizosphere-associated microbial communities in pepper (*Capsicum annuum*) in response to infection by the root lesion nematode *Pratylenchus capsici*, using high-throughput metabarcoding. Pepper plants were grown under controlled soil temperatures of 25°C, 30°C, and 33°C, and root tissues and rhizosphere soils were sampled separately. Fungal and bacterial communities were characterized using ITS and 16S rRNA gene sequencing, respectively. In parallel, nematode communities were analyzed using nematode-specific primers to assess plant-parasitic and free-living nematode assemblages. Distinct microbial assemblages were observed between roots and soils across temperatures. Root-associated fungi were dominated by *Rhizophagus*, with *Olpidium* consistently enriched in roots and showing higher abundance at 25°C. Co-occurrence analysis revealed an asymmetric interaction in which *Pratylenchus* was negatively affected by *Olpidium* occurrence. Soil fungal communities were more diverse, dominated by *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizophagus*, showing minimal temperature effects. Root-associated bacteria were dominated by rhizobia and *Pseudomonas*, with moderate abundances of other genera, while soil bacterial communities were more diverse, with *Bacillus* as the most prominent genus. Overall, increasing soil temperature reshapes microbial communities in a compartment-specific manner, and asymmetric nematode–fungus interactions may modulate disease development. These findings provide a foundation for climate-smart, microbiome-informed management of root lesion nematodes in pepper.

Efficacy of non-fumigant nematicides for sting nematode management in Florida potato production

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Abstract Sting nematode (*Belonolaimus longicaudatus*) is a major plant-parasitic nematode limiting potato (*Solanum tuberosum*) yield in Florida. Management of this nematode primarily relies on fumigation with 1,3-dichloropropene (1,3-D), but it can be costly and can have limited availability. Fluazaindolizine is a liquid nematicide that may be an alternative for managing sting nematode. Three field trials were conducted in 2024, 2025 and ongoing in 2026 to evaluate the efficacy of fluazaindolizine nematicide for sting nematode management. The experiment was arranged in RCBD design with seven treatments each replicated six times. Treatments included preplant broadcast applications of (i) fluensulfone at 1.96 kg a.i./ha, (ii) fluazaindolizine at 2.2 kg a.i./ha; preplant fumigation of 1,3-D at (iii) 66.2 kg a.i./ha and (iv) 33.1 kg a.i./ha; and combination treatments of 1,3-D (33.1 kg a.i./ha) followed by in-furrow (v) fluazaindolizine (2.2 kg a.i./ha) or (vi) oxamyl (1.1 kg a.i./ha); and (vii) an untreated control. Nematode soil samples were collected at midseason (6 weeks after planting) and harvest. Marketable potato yield and return on investment were measured at harvest, but 2026 yield is pending. Sting nematode pressure was low in 2025, so nematicide treatments did not affect sting nematode soil abundances that year. In 2024 and 2026, any treatments with 1,3-D generally provided effective control for sting nematode soil abundances. In 2024, any treatment with 1,3-D improved yield and return on investment relative to untreated. Any 1,3-D treatment improved yield relative to fluazaindolizine or fluensulfone in 2025, but 1,3-D treatments did not improve return on investment relative to untreated in 2025. Neither preplant fluazaindolizine nor fluensulfone significantly reduced sting nematode abundances or increased yield relative to untreated, except that fluensulfone managed final sting nematode abundances in 2025. Similarly, supplementing 1,3-D fumigation with in-furrow fluazaindolizine or oxamyl did not improve sting nematode control or potato yield relative to fumigation alone. In summary, non-fumigant nematicides were not an effective tool for managing sting nematode in potato.

The sweet solution for nematode monitoring: Quantification and detection of root-knot nematode eggs in soils

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Abstract Root-knot nematodes (RKN) are the most damaging plant-parasitic nematodes worldwide, posing major threats to many agricultural and horticultural crops. Quantifying second-stage juveniles (J2s) from soil is the most common diagnostic method to measure field infestation levels. However, this is often not a reliable indicator of actual RKN populations or potential crop damage, especially when pre-plant soil samples are collected from fallow fields. RKN eggs, the main survival stage, are far more abundant and better represent potential infestation levels, yet accurate soil-based egg quantification methods are limited. This study introduces a practical egg-quantifying method to help researchers and growers more precisely assess infestations, improve management decisions, and enhance crop yield and sustainability. Our research focuses on refining sugar flotation-based extraction methods, including classical sucrose centrifugation and the mini-FLOTAC technique (an apparatus for quantifying animal parasite eggs), across different soil types and environmental conditions to enhance consistency and sensitivity, particularly at low population densities. In parallel, we developed and optimized a quantitative PCR (qPCR) assay for highly sensitive and specific detection and quantification of RKN eggs in soils. By integrating optimized egg recovery protocols with molecular quantification, the project aims to establish a standardized and scalable diagnostic pipeline for predicting RKN population dynamics. The research aims to provide a new and more accurate method for predicting RKN damage in the field, which will enable future improvements in nematode management by making more informed recommendations for the application of nematicides and other nematode management practices.

Evaluation of antagonistic fungal isolates for the suppression of *Meloidogyne enterolobii* and *Neocosmospora falciformis* in Guava

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Abstract Guava (*Psidium guajava*) production in South Florida represents the majority of United States commercial production and is increasingly threatened by a disease complex involving the root-knot nematode *Meloidogyne enterolobii* and the soilborne fungal pathogen *Neocosmospora falciformis*. Interactions between these pathogens are considered synergistic, resulting in greater root damage, reduced tree vigor, and more severe yield losses than infections caused by either pathogen alone. Management strategies remain limited, and therefore identifying antagonists capable of suppressing both pathogens represents a promising approach for sustainable disease management. Two *N. falciformis* isolates (L7 and L12) and 63 possible biological fungal (PBF) isolates were obtained from four guava orchards in South Florida and identified morphologically and molecularly. Each PBF isolate was evaluated separately for antagonistic activity against both *N. falciformis* isolates (L7 and L12) and *M. enterolobii* under laboratory conditions using three independent assays. In dual-culture assays, each PBF isolate was paired individually with either L7 or L12 on potato dextrose agar (PDA), and percent inhibition of pathogen growth was calculated relative to the pathogen-only control. In culture filtrate assays, diffusible metabolites produced by each PBF isolate in potato dextrose broth (PDB) were tested by exposing *M. enterolobii* second-stage juveniles (J2) to 50% filtrates, and nematode mortality was assessed after 48 hours of exposure relative to PDB control. Water agar (WA) assays were conducted to evaluate nematode J2 parasitism by PBF isolates after 3 days of exposure. Each treatment consisted of four replicates, and the experiment was conducted twice as independent experimental trials. All assays were conducted under controlled environmental conditions at 25°C. Data from each assay were analyzed separately using ANOVA, followed by Tukey's HSD for mean separation. In the dual-culture assays, two morphologically distinct *Trichoderma* spp., C4 (white morphotype) and D1 (green morphotype), consistently exhibited the strongest antagonistic activity, significantly ($P < 0.0001$) reducing colony growth of *N. falciformis* by up to 74% and 80% after 3 and 6 days of incubation at 25°C, respectively, compared to the control. Culture filtrate assays using PBF isolates demonstrated that diffusible metabolites significantly affected nematode mortality ($P < 0.0001$). Several isolates, including *Exophiala* sp., *Purpureocillium* sp., and *Talaromyces* sp., caused high mortality (95–100%) of *M. enterolobii* compared to the PDB control (3.3%). In WA assays, isolates *Exophiala* sp. and *Purpureocillium* sp. exhibited strong parasitic activity, resulting in 100% and 83.3% parasitism of *M. enterolobii*, respectively, compared to the control (2.6%). These findings show variations in antagonistic activity among PBF isolates against the fungal and nematode pathogens. The suppression of *N. falciformis* growth and induction of *M. enterolobii* mortality underscore the potential of these biological control agents, warranting further evaluation under greenhouse and field conditions to assess their efficacy for sustainable management of guava decline in South Florida.

Synergistic interactions of *Meloidogyne enterolobii* and *Neocosmospora falciformis* in guava

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Abstract Guava (*Psidium guajava*) production in South Florida is associated with the presence of the root-knot nematode (RKN) *Meloidogyne enterolobii* and the soilborne fungus *Neocosmospora falciformis*, isolated from local orchards. While these pathogens are known to independently infect roots, the role of *N. falciformis* in guava disease under Florida conditions has not been clearly established. In Brazil, co-occurrence of *M. enterolobii* and *N. falciformis* has been associated with increased disease severity, suggesting a potential synergistic interaction. However, it remains unknown whether a similar interaction occurs with local isolates in South Florida. Therefore, this study evaluated the potential for synergistic effects between *M. enterolobii* and *N. falciformis* in guava under South Florida's conditions. Screenhouse experiments were conducted during two seasons: summer (June–September 2025) and winter (November 2024–February 2025). Each experiment included treatments of *M. enterolobii* alone, *N. falciformis* alone (isolates L7, L9, L12, and L14), co-inoculation, and an untreated control arranged in a completely randomized design. Plant growth, disease severity, fungal proliferation (CFU/g soil), and nematode reproduction were analyzed using ANOVA followed by Tukey's HSD test for mean separation. Plant growth was significantly affected by fungal isolate ($P = 0.026$), nematode presence ($P = 0.015$), and season ($P < 0.001$). Root growth was strongly reduced by nematodes ($P < 0.0001$), while above-ground growth was influenced by interactions among treatments ($P \leq 0.042$). Growth reductions were greatest under co-inoculation, with isolate L12. Fungal proliferation (CFU g⁻¹ soil) was significantly influenced by nematode presence ($P = 0.0128$), season ($P = 0.0128$), and their interaction ($P < 0.0001$). Under summer conditions, isolate L12 showed the greatest increase (~ 9-fold), followed by L7 and L9 (~ 7-fold), while L14 showed lower proliferation (~ 3-fold). In winter, all isolates showed lower increases (~ 2-fold). When evaluated independently, *M. enterolobii* alone resulted in a high reproduction factor (RF) and severe root galling, confirming its strong parasitic capacity on guava. Mean RF was higher in summer (5.85) than in winter (4.63). RF values were also more variable in winter. These results indicate that environmental conditions may influence nematode reproduction. Disease severity was greater in summer than winter ($P < 0.0001$), with increased canopy decline, root damage, and plant stress. Disease response varied among fungal isolates and between seasons ($P < 0.0001$), with L12 consistently associated with the most severe symptoms. Co-inoculated plants generally showed greater growth reduction and mortality than single-pathogen treatments. The most severe effects occurred with *M. enterolobii* and isolate L12, resulting in significant canopy reduction and up to 50% plant mortality; however, responses were not consistent across isolates. Overall, disease severity in guava was influenced by pathogen interactions and environmental conditions. Co-inoculation resulted in greater damage than single-pathogen treatments, but this response was isolate-dependent. These findings underscore the urgent need for management strategies targeting this disease complex in guava.

A DNA extraction procedure for *Meloidogyne enterolobii* directly from soil using Fe₃O₄ superparamagnetic nanoparticles

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Abstract Root-knot nematodes are an economically important plant pathogen that has severely impacted national and North Carolina (NC) agriculture. The guava root-knot nematode, *Meloidogyne enterolobii*, can infect vital crops in NC such as cotton, sweetpotato, and soybean causing severe galling on the roots which leads to a decrease in crop yields. Species identification of this nematode in a soil sample is crucial in combating yield loss and damage. Current methods of species identification include observing morphological characteristics under the microscope and molecular techniques such as PCR, yet to use these techniques, the nematode must first be separated from the soil matrix. Separating nematodes from the soil can be laborious. For example, a Whitehead tray extraction must typically incubate for 3 days before nematodes can be individually plucked and molecular identification can begin. In this new procedure, genomic DNA is isolated directly from nematodes within the soil matrix using a series of buffers and magnetic nanoparticles. This increases the efficiency of nematode identification for this species while also increasing precision as it may be used in tandem with *M. enterolobii*-specific molecular assays. The DNA extraction procedure directly from soil was optimized for a 40 g soil sample and the sensitivity of the procedure was tested using samples inoculated with 0, 1, 5, 10, 50, 100, 500, 1,000, 5,000, and 10,000 second-stage juveniles. Initial results suggest that the lower limit of sensitivity may be 5 juveniles per 40 g soil when combined with standard PCR and species-specific PCR primers. Final results were obtained within 1.5 days of receiving the soil samples, nearly half the time required for the standard Whitehead tray extraction and downstream identification. The DNA extraction procedure was also performed on twelve soil samples from 5 different counties in North Carolina all with known *M. enterolobii* infestations. Eight out of the twelve sites were positively identified to contain *M. enterolobii* using this method, despite some the samples being approximately 3.5 months old. This work supports the efficiency and effectiveness of the procedure in detecting and diagnosing *M. enterolobii* populations in infested fields.

Evolution of soybean cyst nematode virulence and its implications for SCN management

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Abstract PI 88788 and Peking are two major sources of soybean cyst nematode (SCN) resistance in commercial soybean cultivars. Two long-term field experiments, conducted from 2003–2014 in Waseca and 2008–2022 in Lamberton, evaluated how monoculture and rotation of soybean cultivars with different resistance sources influence SCN population dynamics, virulence evolution, and crop yield. Experiments were established in fields initially infested with low-virulence (HG Type 0, Waseca) or PI 88788-virulent (HG Type 2.5.7, Lamberton) SCN populations. Continuous planting of PI 88788-derived cultivar (R1) increased the Female Index (FI, level of reproduction) on PI 88788, while Peking-derived cultivar (R2) imposed the strongest selection pressure, rapidly elevating FI on Peking. PI 437654-derived cultivar (R3) maintained the lowest SCN population densities and did not select for virulence against its own resistance source, though it did increase virulence on PI 88788 and Peking. No treatment increased virulence on PI 437654. Planting susceptible soybean reduced virulence on Peking, indicating a fitness cost associated with Peking-virulent genotypes, and decreased reproduction factor over time, suggesting the development of soil suppressiveness. All resistant cultivars lowered SCN population densities relative to the susceptible check, with R3 being the most effective. Although R1 produced the highest absolute yields, R3 suffered the least relative yield loss from SCN due to its strong SCN resistance. Overall, the results demonstrate that PI 88788 and Peking confer distinct resistance mechanisms and that rotating these resistance sources remains an effective strategy for slowing virulence adaptation, whereas prolonged use of a single resistance source accelerates resistance breakdown.

Potential for pennycress to select virulent soybean cyst nematode populations depends on host–pathogen genotype interactions

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Abstract Pennycress (*Thlaspi arvense*) is a winter-hardy species in the Brassica family and is being domesticated as a winter oilseed crop for inclusion in U.S. Midwest cropping systems. Pennycress has been reported to be a moderate host of the soybean cyst nematode (SCN; *Heterodera glycines*), a destructive pathogen of soybean, one of the major crops in the Midwest. The impact of introducing pennycress into soybean cropping systems on SCN population dynamics and subsequent soybean yield remains unclear, and management strategies are needed to minimize the potential effects of pennycress on SCN. To address this issue, understanding the reproductive potential of diverse SCN genotypes on different pennycress genotypes is critical. In this study, we evaluated 16 SCN inbred lines representing diverse genotypes and virulence phenotypes, along with four field populations representing four HG Types, on 14 pennycress genotypes to determine their interactive effects on SCN development. The 14 pennycress lines were selected based on previously observed high and low levels of female development by three field SCN populations in Minnesota and Missouri. A strong interaction between SCN lines and pennycress genotypes was observed, measured as Female Index (FI = number of SCN females on a plant $\times$ 100/average number of females on susceptible soybean cv. Williams 82). FI values for individual SCN–pennycress combinations ranged from 1.9 to 191.7, with an average of 40.4. Seventy of the 280 SCN–pennycress combinations had FI values $\geq$ 60, a susceptible range, raising concern about the potential development of highly virulent SCN populations on pennycress. Highly significant correlations were observed among pennycress lines for FI across different SCN lines, indicating that the genetic factors in pennycress controlling SCN reproduction are similar among pennycress genotypes. Interestingly, responses of pennycress lines did not correlate with those of most SCN-resistant soybean lines, including PI 88788, Pickett, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316, and PI 548489B. However, three pennycress lines were negatively correlated with soybean line Peking, and 10 pennycress lines were negatively correlated with soybean line PI 567516C. These soybean lines possess distinct sources of SCN resistance. The lack of positive correlation between pennycress and SCN-resistant soybean lines suggests that SCN resistance genes effective in soybean may not exist or function similarly in pennycress. The molecular mechanisms underlying the negative correlations between pennycress and Peking, and between pennycress and PI 567516C, warrant further investigation.

Evaluation of plant growth-promoting rhizobacteria for suppression of *Meloidogyne incognita* in resistant and susceptible cotton varieties

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Abstract *Meloidogyne incognita* (southern root knot nematode, sRKN) is one of the most destructive plant-parasitic nematodes affecting cotton production, causing significant yield losses by disrupting root function and reducing water and nutrient uptake. The objective of this study was to evaluate selected plant growth-promoting rhizobacteria (PGPR) for their ability to suppress sRKN reproduction and enhance cotton growth and to determine whether their effects differed between a resistant and a susceptible cotton variety. Previous greenhouse and field screening of PGPR strains using the susceptible cotton variety PhytoGen 340 identified several promising candidates for further testing. A greenhouse experiment was conducted using two cotton varieties, the resistant DP 2141NR B3XF and the susceptible DP 2333 B3XF. Ten PGPR strains ST-172 *Bacillus safensis*, ST-182 *Bacillus velezensis*, ST-181 *Paenibacillus physcomitrellae*, ST-128 *Priestia aryabhattai*, ST-97 *Psychrobacter nivimaris*, ST-156 *Pseudomonas koreensis*, ST-211 *Pseudomonas paraglyciniae*, ST-167 *Rosellomorea vietnamensis*, ST-20 *Salinicola lusitanus*, and ST-102 *Sphingobium xenophagum* were evaluated along with three controls: water (negative control), fluopyram (chemical control, 10 ppm), and Majestene (heat-killed *Burkholderia* spp. strain A396 cells plus fermentation media, 19 L ha⁻¹). Bacterial suspensions were prepared at 1×10^7 CFU ml⁻¹ and applied in-furrow at planting at 1 ml per cone-tainer. Plants were inoculated with 2,000 eggs per cone-tainer 1 day after PGPR treatment application in a factorial randomized complete block design with five replications. The variety $\times$ strain interaction for plant height ($P = 0.8841$), biomass ($P = 0.8913$), and egg densities (eggs g⁻¹ root) ($P = 0.504$) was not significant, indicating the PGPR affected both variety similarly. Compared with the susceptible variety DP 2333 B3XF, numerically egg densities in the resistant variety DP 2141 NR B3XF were reduced by 62%, 59%, 41%, and 18% for ST-128, ST-181, ST-172, and ST-211, respectively. Nematode reproduction was numerically 68% lower ($P = 0.143$) in DP 2141 NR B3XF compared to DP 2333 B3XF. Fluopyram supported the lowest egg density, representing a 98% reduction compared to the untreated control. Among the PGPR strains ($P = 0.3608$), ST-211 *P. paraglyciniae* showed the greatest numerical suppression of sRKN reproduction, reducing eggs densities by 89% relative to the untreated control and 40% relative to Majestene. ST-172 *B. safensis* was the strongest biological growth-promoting PGPR, increasing biomass by 72% relative to the untreated control while also reducing egg density by 87%. ST-128 *P. aryabhattai* and ST-156 *P. koreensis* also showed favorable numerical reductions in egg density and improvements in plant growth. Overall, ST-211 *P. paraglyciniae* and ST-172 *B. safensis* were the most promising strains, with ST-211 *P. paraglyciniae* showing stronger nematode suppression and ST-172 *B. safensis* showing greater plant growth promotion. These findings support the potential of selected PGPR strains as biological tools for sustainable integrated management of sRKN in cotton and underscore the importance of evaluating their performance in combination with host resistance.

Nematode-associated bacteria as novel biocontrol agents for beech leaf disease

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Abstract Beech leaf disease (BLD), caused by the foliar nematode *Litylenchus crenatae* (Lc), is an emerging threat to American beech (*Fagus grandifolia*) and other *Fagus* species in North America. Since its first detection in Ohio in 2012, BLD has spread rapidly, leading to interveinal banding, canopy decline, reduced mast production, and increased tree mortality. Current management options are limited, with chemical and cultural approaches providing costly, inconsistent or short-term suppression and facing logistical and environmental constraints in forest ecosystems. The complex transmission pathways of BLD, involving biotic and abiotic factors, further hinder effective disease control. Given these limitations, biologically based management strategies targeting Lc are of increasing interest. An influx of BLD-positive samples obtained by the Kantor Nematode Diagnostic Laboratory at Penn State University prompted an investigation of bacterial communities associated with infected Lc specimens, with the goal of identifying potential biological control agents. Nematode samples displaying bacterial infections were either collected from buds or obtained during Lc quantification for other research projects. Specimens showing symptoms of bacterial infections were surface sterilized, cultured on LB medium, and bacterial isolates were purified and preserved at -80°C . To date, 83 bacterial isolates have been recovered from Lc-infected specimens, representing the genera *Acinetobacter*, *Aquitalea*, *Bacillus*, *Curtobacterium*, *Erwinia*, *Flavobacterium*, *Niallia*, *Pseudomonas*, *Stenotrophomonas*, *Sphingobacterium*, *Xanthomonas*, as well as several unknown genera. To assess the potential effects of these bacterial isolates on Lc motility and survival, *in vitro* assays were conducted in 96-well plates using eight replicates per isolate. Nematode motility was evaluated at 24, 48, and 72 h after exposure. While some isolates had minimal effects on nematode activity, approximately 25% induced nematode mortality within 24 h of exposure, with symptoms including gut disruption and cuticle degradation. This work lays the foundation for developing novel biocontrol strategies to support integrated management of beech leaf disease.

Diagnosing emerging threats: From sample submission to actionable insights for managing plant parasitic nematodes

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Abstract Accurate and timely nematode diagnostics are essential for guiding management decisions; however, the path from sample submission to actionable recommendations is often complex, particularly when emerging or poorly documented species are involved. Detection of emerging nematode species requires not only accurate identification, but also active communication with the submitting grower and collaboration with extension specialists to contextualize results, assess risk, and inform management decisions in the absence of established thresholds or crop-specific management guidelines. Current diagnostic approaches for plant-parasitic nematodes integrate morphometric analyses with molecular tools, including sequencing (most commonly ribosomal DNA *loci* such as D2D3 regions of 28S rDNA). When needed, pathogenicity assays conducted under greenhouse conditions can be used to fulfil the Koch's postulates and confirm biological relevance. By linking diagnostic outputs with applied research and Extension engagement, this presentation highlights the role of diagnostic laboratories as a critical entry point for coordinated responses to emerging nematode issues. It also emphasizes the need for continued development of molecular diagnostic tools, expanded reference datasets, and strengthen feedback loops among laboratories, Extension programs, and growers. Together, these efforts improve our ability to respond to emerging threats, ensuring that diagnostic results are not only accurate but also meaningful and actionable for specialty crop producers.

A comprehensive survey of nematodes affecting potato production systems in Pennsylvania

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Abstract Pennsylvania (PA), often referred to as the “potato chip capital” of the United States, ranks first in the nation in potato chip production, despite not being a major potato-producing state. This unique dynamics with potato post-harvest value-addition in PA underscores the importance of monitoring quarantine pests threatening potato supply chains. Surveys targeting quarantine organisms are critical for early pathogen detection facilitating effective disease management by limiting the spread, and potential for successful eradication. The objective of this study was to conduct a field survey targeting potato cyst nematodes (PCN) (*Globodera pallida* and *Globodera rostochiensis*), a high-priority quarantine pest in the country. Surveys were conducted across six major potato-producing counties in Pennsylvania during the fall of 2025. In total, sixty-six samples were collected, processed, and analyzed using USDA approved PCN extraction protocols. Although no PCN were detected in the surveyed area, several other economically important plant-parasitic nematodes were recovered. Two root-knot nematode species, *Meloidogyne naasi* (barley root-knot nematode) and *M. hapla* (northern root-knot nematode), were identified from three PA counties. Additionally, two cyst nematode species: *Heterodera glycines* and *Heterodera trifolii* were detected and confirmed using morphological and molecular identification. Economically important dagger nematodes (*Xiphinema* spp.) were also detected from 12 soil samples across 3 PA counties. Notably, a new foliar nematode species from genus *Cotylenchus* was detected and confirmed from 2 counties, representing a potential novel and previously unreported nematode occurrence in PA potato growing regions. Continuous monitoring and collaborative surveys are critical for the identification and management of economically important pathogens.

Integrating DNA metabarcoding and morphological approaches to assess soil biodiversity and health

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Abstract Soils harbor a high diversity of microbial and microeukaryotic organisms that play critical ecological roles in nutrient cycling and overall soil health. Among soil microeukaryotes, nematodes are particularly abundant and diverse, span multiple trophic groups, and are widely used as bioindicators. Understanding how nematode communities respond to soil management practices is key to improving soil biodiversity and health, especially in restored systems. In this study, we combined DNA metabarcoding (soil eDNA and bulk nematode DNA) with morphological approaches to investigate changes in microeukaryotic and nematode communities across different soil habitats at Tumpinyeri Gardens, Victoria, Australia. These habitats included Fence Line, Abandoned Paddock, Cover Crop Paddock, Market Garden, Protected Market Garden, and Biodiversity Belt. Our analyses showed that community composition and alpha diversity (Shannon and Simpson indices) differed substantially between metabarcoding datasets (soil eDNA vs. bulk nematode DNA). For instance, soil eDNA samples exhibited higher diversity metrics than nematode bulk DNA, particularly in the Biodiversity Belt habitat. Soil eDNA samples were dominated by fungi, whereas bulk DNA was, as expected, dominated by nematodes. Despite these differences, beta diversity analyses revealed similar ecological patterns across both datasets, with samples clustering by management practice and reflecting comparable levels of disturbance. Morphological and metabarcoding approaches produced generally consistent results for nematode trophic composition, revealing a dominance of bacterivores (Cephalobids, Plectids, Rhabditids) and plant parasites (Tylenchids). Together, our findings support the conclusion that management practices affected soil and nematode communities, which are critical for improving soil health and productivity.

Declining effectiveness of PI 88788 and peking resistance to *Heterodera glycines* in OHIO: A Bayesian hierarchical analysis of virulence shifts over three decades

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Abstract The soybean cyst nematode (SCN; *Heterodera glycines*) remains the most devastating pathogen of soybeans in the United States and is managed primarily through host resistance and rotation with nonhost crops. In the North Central United States, the extensive deployment of PI 88788-derived resistance has selected for virulent nematode populations; however, contemporary quantitative assessments of this adaptation are lacking. In Ohio, the last comprehensive virulence survey was conducted over 30 years ago. We conducted a statewide survey across 67 counties from 2018 to 2025 to characterize SCN population densities and virulence phenotypes, and to quantify temporal shifts relative to data from the 1990s. Modified HG Type tests revealed that only 22% of contemporary populations were avirulent (HG Type 0–), compared to 73% in the 1990s, while HG Type 2– (virulent on PI 88788) now predominates, representing 41% of populations. To move beyond binary virulence classifications, we analyzed female index (FI) data using a Bayesian hierarchical model that treats virulence as a continuous trait. This approach provided high-resolution, probabilistic estimates of virulence intensity, showing that PI 88788 FI increased approximately 3.4-fold (6% to ~20%) and Peking FI increased approximately sevenfold (0.84% to ~6.1%) since the 1990s. The model identified substantial spatial heterogeneity in virulence, with county-level variation more pronounced for Peking than PI 88788. These results quantify a marked shift in the SCN virulence landscape in Ohio, demonstrating widespread loss of effectiveness of PI 88788. This study establishes a rigorous, quantitative baseline for SCN monitoring and provides a statistical framework for detecting emerging virulence, directly informing resistance stewardship strategies to support the long-term sustainability of soybean production.

Spatial and temporal variation of Tobrilid nematode microbiomes in the Western Nebraska Sandhills

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Abstract Nematodes can be found in all habitats, including the most extreme such as the soil of the Antarctic Dry Valleys and the arsenic-rich, hypersaline Mono Lake. Nematode association with microbiomes contribute to this success as microbiomes play a crucial role in nematode fitness, growth, and defense against pathogens. However, the mechanisms underlying microbiome established and their resilience to environmental change remain poorly understood. The lakes of the western Nebraska Sandhills offer a unique opportunity to explore these questions, as they form a natural experiment with alkalinity levels ranging from neutral (pH ~7) to highly alkaline (pH ~11). Importantly, climate-driven drought likely exacerbates these extreme conditions, pushing the lakes to even more extreme alkalinity. Previous studies have shown that nematode (dominated by Tobrilidae) and microbial diversity in these lakes were negatively affected by alkalinity increases over time. We hypothesized that sediment and nematode microbiome diversity and composition would be shaped by temporal variation of lake characteristics. To test this hypothesis, we collected four replicate sediment samples from lakes varying in alkalinity (pH 8–11) in October 2023 and 2024. Sediments and hand-picked individual Tobrilid specimens (three species) were characterized for bacterial and eukaryotic microbiomes using 16S and 18S rRNA metabarcoding. Nematodes were identified with microscopy and Sanger sequencing with 18S rRNA. Additionally, we characterized lake sediment biogeochemistry to examine its role in shaping bacterial and eukaryotic microbiomes. Our results show that bacterial and eukaryotic sediment microbiomes were significantly more diverse than Tobrilid gut microbiomes in both years. Although sediment microbiomes, both bacterial and eukaryotic, were consistently least diverse in the most alkaline lake, their diversity declined over time. Tobrilid bacterial microbiomes were less diverse in the most alkaline lake in 2023, but increased in diversity in 2024. In contrast, Tobrilid eukaryotic microbiomes were generally similar across lakes, but decreased in diversity in 2024. Random Forest models showed that sediment bacterial and eukaryotic microbiomes were most influenced by biotic variables, with pH-related variables, lake, and year also shaping bacteria but not eukaryotes. In contrast, the diversity of Tobrilid microbiomes were predominantly shaped by pH-related variables, although year played a role in eukaryotic microbiomes as well. PERMANOVA and dbRDA analyses indicated that the composition of both sediment and Tobrilid microbiomes, including bacterial and eukaryotic, was most strongly influenced by lake-specific characteristics and year. Our results indicate that alkalinity, lake-specific characteristics, and year are the primary drivers of Tobrilid microbiomes. In addition, the influence of year on microbiome composition suggests nematode-microbiome relationships might be vulnerable to the impacts of climate change driven drought.

The right stuff: What makes a good sample for advising growers?

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Abstract When many think of nematode diagnostics they think of simply extracting nematodes from soil, identifying them, and counting them. While that is an important part of it, there are many other factors involved in providing meaningful advice. A good sample for advising growers depends largely on four diagnostic factors: (i) what is sampled, (ii) how the sample is collected and handled, (iii) how the sample is processed in the lab, and (iv) how the findings are interpreted. Generally, the person collecting the sample is a crop consultant, distributor, grower, extension agent, or someone else besides the diagnostician. The “how”, “what”, and “when” will vary from commodity to commodity. So, for the first two diagnostic factors extension efforts focus on educating submitters on how and when to sample, and what to submit specific to the commodity being sampled. For extension educators, it is important to understand which kinds of nematodes are most likely to be a problem on specific commodities and the economic and sociological factors faced by the stakeholders, in order to develop and deliver appropriate educational programming for them. Similarly, different agriculture commodities and their key problem nematode genera often require specialized extraction procedures, sometimes multiple procedures are needed for a single sample to provide a good diagnosis. Therefore, diagnostic staff must be trained to recognize which protocols are appropriate when a sample arrives and to apply them correctly. Finally, interpretation requires the diagnostician to mentally (and sometimes physically) step outside of the lab and into the grower’s field to understand what nematode identifications and numbers mean in their world. This requires knowledge of nematology, as well as agronomy, economics, sociology, meteorology, agriculture engineering, and other disciplines. That knowledge must then be synthesized into advice that is both meaningful and practical.

Beyond the numbers: What do nematode thresholds really mean?

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Abstract When clients send nematode diagnostic samples to a lab, nematodes are typically extracted from soil and/or plant tissues, identified to genus, and counted. The diagnostician then compares the number of nematodes for the relevant genera detected to some kind of “threshold” for the commodity being sampled. But where do these thresholds come from, what do they mean, how accurate are they? Different threshold terms are commonly used, such as damage threshold, economic threshold, economic injury threshold, or action threshold. Some thresholds are derived from experiments while others are best estimates. Even when derived from experimental data the thresholds may be specific to the environment, nematode population, and crop cultivar used in the experiment but may not be relevant in other contexts. Our research in perennial turfgrass systems reveal that nematode counts are more useful for predicting future damage than estimating current damage and, therefore, are more valuable for economic thresholds than economic injury thresholds. Nematode thresholds often consider plant response, nematode counts, and often economic factors, but they rarely account for the sociological factors that influence growers’ decision making. Not considering these human factors can cause misunderstanding and poor communication between growers and diagnosticians, leading to frustration and lack of trust. At the University of Florida Nematode Assay Lab we utilize risk categories instead of thresholds for advising our clientele, assigning sample results into broad risk categories; low risk, moderate risk, or high risk of damage occurring if management strategies are not implemented. These determinations are made by our state extension nematologists that have specific crop expertise and knowledge of grower needs. The goal of our extension education effort is to assist our clientele in making educated decisions based on the level of risk they are willing to assume. In this model diagnosticians, extension specialists, and growers work hand in hand in decision making.

Genomic chaos enables global success in a clonal plant pathogen

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Abstract Clonal reproduction is often viewed as an evolutionary dead end, yet some clonal species achieve remarkable ecological success. Root-knot nematodes exemplify this paradox. Despite reproducing primarily through cloning, *Meloidogyne incognita* ranks among the world's most destructive agricultural pathogens, devastating crops across diverse environments. To understand the genomic basis underlying this pathogen's global success, we conducted a comprehensive genomic analysis of 307 globally collected *Meloidogyne* isolates, combining population-scale sequencing with chromosome-level genome assemblies of ten *M. incognita* isolates and representatives of *M. enterolobii* and *M. hapla*. Our analyses reveal that *M. incognita* exhibits extraordinarily low nucleotide diversity ($\pi = 2.15 \times 10^{-6}$), confirming its predominantly clonal lifestyle, yet pan-genome analysis uncovered extensive gene presence-absence variation across populations, suggesting that an open pan-genome provides a cryptic source of genetic diversity. Population structure analyses identified two deeply diverged lineages (L1 and L2) exhibiting extreme genetic differentiation ($F_{st} = 0.67\text{--}0.85$), which carry different mitochondrial SNPs alongside divergent nuclear signatures and lineage-specific patterns of chromosomal fusion, revealing that they originated through independent hybridization events rather than simple evolutionary divergence from a single ancestor. Loss-of-heterozygosity mapping shows that genome diploidization followed markedly different trajectories in each lineage, with most heterozygosity loss occurring between homoeologous A1 and A2 subgenomes prior to polyploidization, but via distinct mechanisms in L1 versus L2 populations. Comparative genomics further revealed extraordinary karyotypic instability, with chromosome numbers varying from 39 to 45 even within lineages due to frequent, recent chromosomal fusion events that drive gene expression divergence, particularly in subtelomeric regions enriched for effector genes. Phenotypic screening of over 150 populations against three widely used nematicides identified multiple highly resistant isolates, while genome-wide association mapping (GWAS) revealed candidate loci potentially underlying resistance evolution. Together, our findings demonstrate that independent hybridization events, structural genome remodeling, and gene content diversification have jointly shaped *M. incognita* evolution. These mechanisms enable clonal populations to maintain adaptive potential, reframing our understanding of clonal pathogen evolution while providing genomic resources for monitoring resistance evolution.

The master regulators of cyst nematode parasitism gene expression

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Abstract Plant-parasitic nematodes, like other pathogens, secrete overlapping and sequential waves of effectors to manipulate their host. What plant-derived information drives this pattern, and how that information is integrated in the nematode, is poorly understood. Here, we present recent advances in our understanding of transcriptional master regulators in the cyst nematode *Heterodera schachtii*. We show that a pair of nematode transcription factors (SUGR-1 and DGR-1) are stimulated by discrete plant signals termed effectostimulins. Effectostimulin-activated transcription factors work both antithetically, yet in concert, to define and fine tune effector gene expression during the early stages of host invasion. SUGR-1 activates early-stage effector expression required for plant invasion, while its homologue, DGR-1, acts as a dual-functional switch that “switches off” SUGR-activated early-stage effectors, and “switches on” the next wave of later-stage effectors associated with biotrophic establishment in the host. Together, the DGR-1/SUGR-1 transcription factor duet controls nearly one half of all *H. schachtii* effectors expressed before 48 hours post infection, and over one fifth of effectors of any kind. Importantly, given that knockdown of *sugr-1* reduces root penetration by 80%, while knockdown of *dgr-1* delays development of those infecting juveniles, blocking the regulators of effector deployment may open novel and generalisable control mechanisms to pathogens that secrete effectors.

Life monitor: An emerging method for soil monitoring and qualification

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Abstract Life Monitor is an advanced platform designed to automate nematode morphological analyses through the integration of artificial intelligence. The system is designed to rapidly image soil suspensions. Efforts are currently directed toward nematode populations due to their different roles in soil food webs and high economic importance in agriculture. This technological development can address, at the same time: (1) the quantification and identification of free-living soil nematodes to calculate *Nematode Based Indices (NBIs)*, as indicators of soil health; and (2) the detection, quantification, and viability assessment of plant-parasitic nematodes, including cyst contents. Artificial Intelligence modeling is ongoing at all taxonomic levels from order to species. For monitoring purposes, this technology would provide proxies that can function both as indicators of soil function or soil health and provide actionable insights. On a field scale, it would allow farmers/operators to profit from *NBIs* sensibility to land use intensity, to further their potential as an indicator of the impact of agricultural management practices on the soil biome. On a regional and national level, it could provide a standardized, scalable and affordable method to assess nematode abundance, for which thresholds could be established at Member State level.

Summer cover crops for nematode and nitrogen management in florida tomatoes

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Abstract Florida is the number one producer of fresh market tomatoes in the US. Root-knot nematodes (*Meloidogyne* spp.) are one of the major constraints to tomato production in Florida and one of the main reasons for the continued use of soil fumigants. Growing cover crops is an age-old practice that can help to reduce plant parasitic nematode populations in agricultural systems. Cover crops have many other benefits including erosion reduction and nutrient cycling and have the potential to reduce fertilizer inputs and nitrogen (N) leaching. Further research is needed to determine the effect of cover crops on these factors, especially in sandy soils in Florida that are prone to leaching. Field experiments were conducted at the University of Florida's Gulf Coast Research and Education Center between 2020 and 2025 to evaluate the most used summer cover crops in Florida. The cover crop treatments were sunn hemp (*Crotalaria juncea*), sorghum-sudangrass (*Sorghum x drummondii*) and cowpea (*Vigna unguiculata*), both in monoculture and biculture. Cover crops were grown for approximately 80 days and then mowed and incorporated into the soil. Shortly after cover crop incorporation, raised plastic-mulch vegetable beds with drip irrigation were formed, and tomatoes (*Solanum lycopersicum*) were planted and grown for approximately 14 weeks. No fumigants or nematicides were applied during the entire study period. Cover crop growth varied by year depending on environmental conditions, with bicultures generally offering greater resilience and reliability in terms of biomass production. Sorghum sudangrass and sunn hemp reduced root-knot nematodes but increased lesion nematodes (*Pratylenchus* spp.), while the opposite was noted for the weed fallow. All cover crop treatments improved tomato yields compared to the weed fallow control. Soil N data varied significantly with sampling time and depth. Few trends were noted, other than that higher total concentrations of extractable nitrate and ammonia were generally found in treatments containing sunn hemp. Overall, these findings support integrating cover crops in Florida tomato systems for root-knot nematode management and improved yield. Future research should quantify root biomass contributions to soil microbiomes and refine termination-to-transplant intervals that synchronize N release with tomato demand while minimizing early nitrate leaching.

Genome assembly supports new entomopathogenic nematode species in Wisconsin

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Abstract Wisconsin is the United States and the world's top cranberry producer, generating almost half the global supply. However, warmer weather and fluctuating winters are driving higher pest pressure, prompting more insect-related challenges for cranberry growers in the state. Because commercially available entomopathogenic nematodes (EPN) formulations have not been effective on cranberry pests, there is an urgent need for viable biocontrol agents, such as native EPN, for North American cranberry production. Surveys of nematodes conducted in Wisconsin cranberry marshes recovered a native EPN species similar to the entomopathogen *Osccheius onirici*, a species first characterized from karst cave soil in central Italy. Since the two habitats differ significantly in terms of their soil ecology spectrum, with the former being alkaline and mineral-rich, whereas the latter being acidic and organic-rich, the genomic differences between these two nematodes can inform successful biocontrol strategies. Here, we used Oxford Nanopore long-read sequencing to assemble the genome of a native EPN species from Wisconsin, which we propose to designate as *Osccheius wisconsinii*. The final assembly comprises 27 contigs, including 19 major contigs, with a total genome size of ~92 Mb, a largest contig of ~7.8 Mb, and an N50 of ~5.56 Mb. Gene prediction using BRAKER3 identified 25,629 genes, and genome completeness was high, with ~99.3% complete BUSCOs. Taxonomic profiling revealed no prominent bacterial taxa at either the species or family level from surface-sterilized nematodes, suggesting that putative symbionts, if present, may reside externally or in low abundance. This genome provides a valuable resource for future comparative phylogenomic analyses of *Osccheius* species and other EPN, enabling deeper insight into host range, ecological adaptation, and the development of improved biological control strategies.

Investigating the genetic basis of body size in soybean cyst nematode

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Abstract Despite potential implications for fitness and virulence, little is known about factors controlling body size in plant-parasitic nematodes. Environmental factors are expected to play important roles, but genetics are also likely to be relevant. Previous studies in *C. elegans* have identified genes associated with collagen, actin, and somatic polyploidy as contributing to body size. In this study we set out to determine the relative roles of genetics and environment on soybean cyst nematode (*Heterodera glycines*; SCN) body size and to identify specific genes contributing to body size. We used a set of 178 inbred lines of SCN which have been previously sequenced with Illumina short-read technology. We cultured each line on a susceptible soybean, extracted cysts and adult males from roots and soil, and hatched juveniles from young cysts. We then measured length and width of 40 juveniles (J2 stage), 30 cysts, and 20 adult males of each inbred line. We also used the cyst measurements to calculate cyst length/width ratio and cyst volume. There is substantial variation within lines, which can likely be attributed to environmental factors. Analysis of variance and broad-sense heritability calculations show that genetics also play a significant role. Despite this, genome-wide association analysis only identified loci significantly associated with male length. Candidate gene analysis of these loci shows they are linked to genes encoding proteins important for maintaining muscle structure, increasing fat storage, and protein-protein interactions. We speculate that our inability to identify loci associated with the other traits in this study, despite high broad-sense heritabilities, is because the traits are controlled by many genes with small effects.

Greenhouse evaluations of eleven common cover crops for management of *Trichodorus*/*Paratrichodorus* spp. in corn and *Heterodera glycines* in soybean production

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Abstract *Trichodorus/Paratrichodorus* spp. and *Heterodera glycines* both cause major yield losses in two of North Carolina's most valuable crops, corn and soybean, respectively. Management of these nematodes is best accomplished with an integrated approach, including crop rotations with poor- or non-host plants. The objective of these studies was to test the host status of common cover crops against both *Trichodorus/Paratrichodorus* spp. and *Heterodera glycines* to determine the best crop rotation options in soybean and corn production. Eleven different cover crops were grown in replicated greenhouse trials and inoculated with either *Heterodera glycines* race 2 as egg inoculum or *Trichodorus/Paratrichodorus* spp. as infested field soil. Soybean followed by soybean and corn followed by corn were used as susceptible controls. To simulate a field rotation in the greenhouse, each trial had two different data collection dates, namely Takedown 1 and Takedown 2. The cover crops grew for 60 days post inoculation, after which a 500 g subsample of soil from each pot was mixed with sterile soil and seeded with the susceptible crop; corn for the evaluation of *Trichodorus/Paratrichodorus* spp. and soybean for *Heterodera glycines*. A separate subsample of soil was also removed to quantify nematode populations (Takedown 1). The corn or soybean grew for 60-days post seeding, after which shoot weights and final nematode counts were evaluated (Takedown 2). *Heterodera glycines* cysts were extracted from the soil using the wet-decant sieving technique and *Trichodorus/Paratrichodorus* spp. were extracted from the soil with a modified Whitehead tray assay with 100 g soil and root fragments. Crimson clover, sunn hemp, white mustard, field pea, flax, and alfalfa all had significantly less *Heterodera glycines* cysts per sample than the susceptible soybean control at Takedown 1 and all eleven cover crops had significantly less cysts per sample than the soybean control at Takedown 2. There were no significant differences between the cover crops and the corn susceptible check in the *Trichodorus/Paratrichodorus* spp. experiments for either Takedown 1 or 2. However, numerically, cowpea, flax, oats, oilseed radish, and sunn hemp had less *Trichodorus/Paratrichodorus* spp. per 100 g soil than the corn control for Takedown 1 and all eleven cover crops had less *Trichodorus/Paratrichodorus* spp. per 100 g soil for Takedown 2. The use of these cover crops could be beneficial to use in rotation with corn and soybean production to mitigate populations of *Trichodorus/Paratrichodorus* spp. and *Heterodera glycines* in fields.

Rapid loop-mediated amplification (lamp)-based assay for field detection of the gall forming nematode *Anguina funesta*, a regulated seed-gall nematode in the United States

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Abstract Gall-forming nematodes of the family Anguinidae are obligate, highly specialized plant parasites with economic importance in several countries. They induce gall formation in multiple plant organs, including leaves and seeds. Several *Anguina* spp. (*A. agrostis*, *A. funesta*, *A. paludicola*, and *A. tritici*) are designated quarantine pests in numerous countries. Among them, *A. funesta* is of particular concern because it serves as the primary vector of *Rathayibacter toxicus*, a gram-positive bacterium that produces a tunicamycin-like corynetoxin within ryegrass seed galls. Consumption of contaminated material by grazing animals can lead to fatal poisoning known as Annual Ryegrass Toxicity, a major issue in Australia. *Rathayibacter toxicus* poses a significant threat to agriculture, livestock, and human health, and it is listed as a Plant Pathogen Select Agent by APHIS. *Anguina funesta* and *A. agrostis* are both present in the United States and difficult to distinguish morphologically, creating major challenges for current diagnostic methods, highlighting a persistent need for rapid, accurate detection tools for gall-forming nematodes. A real-time loop-mediated amplification (LAMP) based assay was developed for rapid detection of *A. funesta*. Ideally, primers would be designed to a unique gene target, but no whole-genome sequences are currently available. Instead, the assay was designed to target the internal transcribed spacer (ITS) sequence. The assay was evaluated in a portable real-time fluorescence monitoring device (Genie III). Additionally, a simple and rapid DNA extraction procedure was performed directly from a single gall containing *A. funesta*, without the use of a commercial kit. The resulting crude extract provided sufficient template DNA for immediate use in the LAMP assay. The LAMP assay was able to accurately detect *A. funesta* and distinguish it from other gall-forming nematodes, including *A. agrostis*, in less than 20 minutes. Validation parameters, including sensitivity, specificity, intermediate precision, and reproducibility were evaluated; the assay was shown fit-for-purpose. These results demonstrate the assay's reliability as a rapid, cost-effective, field-deployable diagnostic. Given this performance, the assay also has potential for integration into regulatory workflows, where it could support laboratories in evaluating suspect galls during routine surveillance and strengthen phytosanitary decision-making.

Reklemel™ active (fluazaindolizine), an effective tool to manage plant parasitic nematodes in ornamental crops

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Abstract Reklemel™ active (fluazaindolizine) is a novel, non-fumigant chemical nematicide recently commercialized by Corteva Agriscience for the management of economically important plantparasitic nematodes and represents a new mode of action as the first sulfonamide nematicide introduced for agricultural use. The active ingredient has demonstrated selective and effective control of key plant parasitic nematodes across a wide range of crops and production systems. This presentation summarizes results from multiyear, farm-based studies evaluating Reklemel for nematode management in ornamental crop production. From 2023 to 2026, trials conducted on *Caladium bicolor* in a horticultural production setting demonstrated high efficacy of Reklemel against *Meloidogyne arenaria*, consistently reducing nematode populations while improving plant growth and marketable yield.

Teaching nematology in the age of AI: Tools, opportunities, and pitfalls

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Abstract The development of effective educational resources is essential for training future nematologists, supporting extension programs, and communicating nematology to diverse audiences. However, producing high-quality teaching materials remains time- and resource-intensive. Recent advances in artificial intelligence (AI) provide new opportunities to streamline the creation of visual, interactive, and adaptive learning tools while enhancing teaching efficiency and student engagement. In this session, real-world examples from graduate instruction, extension programming, and outreach will demonstrate how AI can be used to develop instructional materials, including illustrations, videos, and schematic diagrams; generate flashcards and interactive learning modules; design quizzes and assessment tools; produce outreach materials for non-specialist audiences; and create 3D models for nematology education. Attendees will gain practical strategies for integrating AI into teaching and extension workflows. The session will also highlight common pitfalls, limitations, and unintended outcomes associated with AI-generated content.

Impact of planting date on Southern root-knot nematode galling and yield in soybean

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Abstract Southern root-knot nematode (*Meloidogyne incognita*) is a major yield-limiting pathogen of soybean (*Glycine max*) in the mid-southern United States. Management of *M. incognita* relies on an integrated approach that includes crop rotation, host-resistance, and nematicides; however, there is interest in cultural practices such as planting date. To assess this, three soybean cultivars marketed as susceptible (Dyna Grow 45ES10, Dyna Grow 49EN12, and Delta Grow DG46E30) and one cultivar marketed as resistant (Pioneer P46Z53E) to *M. incognita* were evaluated in 2025 at two different planting dates in a field experiment. The planting dates were three weeks apart, with the first planting on June 3 and the second on June 23, which is a late-planted soybean in Arkansas. The end-of-season *M. incognita* damage threshold was exceeded at both planting dates, with an average population density of 247 J2/100 cm³ of soil in planting date 1 and 478 J2/100 cm³ in planting date 2. Root galling was assessed twice during the season, once at the V5/V6 growth stage, and again at the R5/R6 growth stage, based on the percentage of root system galled of eight root systems per plot. There was a significant cultivar by planting date interaction for galling at both sampling times. These data suggest planting date has a greater effect on galling early in the season, while host-plant resistance has a greater effect later in the season. Across cultivars, planting date 2 had significantly less galling than planting date 1 by 95.5 and 40.0% at the early and late sampling times, respectively. Pioneer P46Z53E had significantly less galling than Dyna Gro 45ES10 at the V5/V6 growth stage, and all other cultivars at the R5/R6 growth stage. Regardless of planting date, Pioneer P46Z53E and Delta Grow DG46E30 had significantly greater yield than Dyna Grow 45ES10 and 49EN12. Overall, these data suggest that planting date affects galling early in the season, but that host-plant resistance and, in this study, tolerance are important factors in maximizing yield protection in a field with a high density of *M. incognita* in Arkansas soybean production.

ReklemelTM and cotton: Managing the *Meloidogyne incognita*-*Fusarium* wilt disease complex in Alabama

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Abstract The southern root-knot nematode, *Meloidogyne incognita*, causes significant damage to cotton production in the Southeastern United States, resulting in economic losses. The organism has a known relationship with the causal agent of Fusarium wilt, *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). This relationship entails a disease complex caused when FOV enters plant roots through wounds created by *M. incognita*. To reduce losses associated with this disease complex, management strategies must be implemented. The objective of this study was to determine the efficacy of ReklemelTM, an in-furrow nematicide, in conjunction with susceptible and resistant cotton varieties planted in a field known to have a history of the disease complex. Field trials were established wherein two cotton varieties DP 2333 B3XF (moderate Fusarium resistance) and DP 2414 B3TXF (moderate Fusarium susceptibility) were tested using three rates of ReklemelTM (476.4, 945.7, and 1891.4 g/ha), Velum Prime (420.3 g/ha), and an untreated control. Velum Prime (fluopyram) is a broad-spectrum nematicide and Group 7 fungicide while ReklemelTM (fluazindolizine) is marketed as a nematicide specific to plant-parasitic nematodes. Root samples were collected 35 days after planting, where nematode reproduction was quantified as root-knot nematode eggs per gram of fresh root weight (RK eggs/gram of root). Statistical analysis was performed using R Studio Version 4.5.1 and showed numerical reductions in nematode reproduction with chemical treatments compared to untreated controls. The untreated control averaged 7,087 eggs/gram of root, while ReklemelTM at 476.4 g/ha reduced reproduction to < 2,000 eggs/gram of root. ANOVA showed no overall difference among nematicides ($P = 0.1719$). These findings indicate that while nematicide treatments reduce root-knot nematode reproduction numerically, high variability limited statistical significance under the conditions of this study. Similarly, Fusarium wilt incidence was numerically decreased through the addition of chemical treatments. Velum Prime and the 476.4 g/ha treatment of ReklemelTM reduced the percent wilt incidence by > 3% ($P = 0.39681$). When looking at the effect of variety, DP 2414 B3TXF had higher biomass ($P = 0.008217$), higher seed cotton yield ($P = 0.02973$), and higher Fusarium wilt incidence ($P = 0.06985$) when compared to DP 2333 B3XF. Additionally, DP 2414 B3TXF supported numerically fewer RKN eggs/gram of root than DP 2333 B3XF ($P = 0.7401$). These findings indicate that while DP 2333 B3XF possesses innate Fusarium resistance and did have biological reductions in wilt ratings, the FOV disease pressure seen in this trial did not inhibit the performance of DP 2414 B3TXF.

Nematodes as bioindicators of ecosystem services

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Abstract Soil is a major reservoir for biodiversity. In healthy soils, this biodiversity plays a crucial role in regulating ecosystem functions, including primary production, biochemical cycling, and carbon sequestration. Nematodes are a key component of soil biodiversity. Through abundance, diversity, and positioning at most trophic levels within food webs, nematodes contribute to ecosystem functioning. As a result, nematodes have been well recognized as bioindicators of soil and ecosystem health. To monitor ecosystem functions and health, a wide range of nematode community metrics have been employed, including abundance, ecological alpha diversity indices, and ecological indices specifically developed for nematodes such as maturity and foodweb indices. Despite their widespread use in informing about ecosystem functions, surprisingly few studies have tested these relationships. The primary goal of this study was to assess the effectiveness of nematode community metrics in understanding ecosystem functions and health. To achieve this goal, we conducted a systematic literature search and assembled a dataset comprising over 2,000 pairwise comparisons of nematode communities and associated ecosystem functions. First, we performed a random effects and multilevel meta-analysis to evaluate the effect sizes of nematode measures and ecosystem functions in response to experimental treatments, such as disturbance, enrichment, and low-impact management, across ecosystems. We then performed a meta-regression analysis, in which ecosystem function effect sizes were modeled as a function of nematode measure effect sizes. Our results showed that effect sizes in response to treatments were specific to both nematode metrics and ecosystem functions, suggesting that different environmental settings impose distinct responses from nematode communities and ecosystem functions. The meta-regression analysis confirmed this result. Overall, changes in nematode abundance due to treatments were the strongest predictor of changes in ecosystem functions across studies, however this relationship was highly context dependent. On an individual nematode metric basis, abundance was a poor predictor of changes in ecosystem functions. In contrast, increases in most ecological nematode indices were associated with positive changes in ecosystem functions. Nematode metrics generally predicted shifts in carbon cycling and plant productivity, but not nitrogen cycling. Increases in total and omnivore/predator abundance generally predicted positive changes in carbon cycling, but this relationship was opposite for plant productivity. Alpha diversity was positively associated with increased nitrogen cycling, but negatively with carbon cycling and primary production. Increases in nematode metrics predicted increases in ecosystem function under low impact management only, but they were insensitive to disturbance or enrichment. Finally, changes in nematode metrics were most useful in predicting functions in agroecosystems compared to forests and grasslands.

Investigation of the molecular basis of *GMSHMT08*-mediated soybean cyst nematode resistance in soybean

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Abstract Soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pathogen of soybean (*Glycine max*), causing an estimated \$1.5 billion in annual yield losses in the United States. Planting SCN-resistant soybean cultivars is the most effective control strategy, with two major types of resistance widely deployed in commercial cultivars. These include PI 88788-type resistance requiring high copy *rhg1-b* (*GmSNAP18-b*) and Peking-type resistance requiring an epistatic interaction between *rhg1-a* (*GmSNAP18-a*) and *Rhg4*. *Rhg4* encodes a serine hydroxymethyltransferase (*GmSHMT08-a*) that differs by two amino acids from the susceptible version (*GmSHMT08-b*) and impacts the enzyme's ability to bind folate, leading to a gain-of-function in resistance to the SCN. SHMTs are critical for 1-C metabolism in all organisms, catalyzing the interconversion of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene-THF. The soybean genome harbors two cytosolic SHMT genes, *GmSHMT08* and *GmSHMT05*, that exhibit similar tissue-specific expression but are functionally distinct. *GmSHMT08* and *GmSHMT05* are believed to be the result of a recent duplication event in the *Glycine* lineage. The emergence of *GmSHMT08-a*, which is absent in wild soybean, is a result of artificial selection through the domestication process. Here, we show, using biochemical and gene-editing approaches, that *GmSHMT05* retains normal enzymatic activity and can complement the loss-of-function in *GmSHMT08-a*, whereas *GmSHMT08-a* cannot complement the loss-of-function in *GmSHMT05*, resulting in lethality. Furthermore, a phenotypic analysis of a unique set of soybean *Gmshmt08-a* mutants suggested that the neofunctionalization of *GmSHMT08-a* in SCN resistance may involve a trade-off with reduced pod numbers. To better understand the molecular basis of *GmSHMT08-a* mediated resistance, we leveraged the soybean *Gmshmt08-a* mutants to pinpoint *GmSHMT08-a* dependent gene expression changes in response to SCN via transcriptomic profiling. A specific and significant induction of the cysteine-methionine metabolism pathway suggests an increase in S-adenosylmethionine, thereby increasing methylation and ethylene biosynthesis. Elevated ethylene levels likely contribute to enhanced ROS (Reactive Oxygen Species) generation, leading to a robust hypersensitive response and ultimately cell death of the syncytium that restricts nematode development. Mechanistic insight into *GmSHMT08-a* function may provide new strategies for metabolic engineering of soybean cultivars with diverse resistance mechanisms to counter evolving virulent SCN populations. Current work focuses on targeted editing of selected differentially expressed genes within this pathway to confirm the role of cysteine-methionine biosynthesis in *GmSHMT08-a* mediated SCN resistance.

Evaluating integrated soybean cyst nematode management using spatially informed mixed models

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Abstract Evaluating management strategies for soybean cyst nematode (SCN) under field conditions remains challenging because the pathogen's inherently patchy spatial distribution inflates experimental error and can obscure treatment effects. This study combined spatially explicit linear mixed models with a split-plot field evaluation of two SCN-resistance sources (PI 88788 and Peking) and fluopyram seed treatment against nematode populations capable of reproducing on both resistance sources in Ohio. Six models spanning a gradient of spatial complexity were compared; tensor-product penalized spline models improved experimental efficiency by 33–39% relative to conventional non-spatial models. Peking-derived cultivars outperformed PI 88788 by 257 kg ha⁻¹ and suppressed nematode reproduction by 36%, demonstrating a clear advantage under these SCN population conditions. However, Peking exhibited slightly greater yield sensitivity to increasing initial SCN population density (P_i ; -5.1% per log-unit increase) than PI 88788 (-4%), revealing a trade-off between reproductive suppression and yield stability. Fluopyram seed treatment increased yield by 111 kg ha⁻¹ but did not reduce SCN reproduction factor, indicating a yield-protective effect rather than direct suppression of nematode population growth. These results demonstrate that effective management in fields with populations capable of reproducing on both resistance sources requires diversification of resistance, with Peking-derived cultivars representing an important complement to PI 88788 and seed treatments providing supplementary yield protection. The spatial modeling framework developed here provides a template for improving precision in soilborne pathogen research.

Applications and constraints of molecular and morphological approaches to nematode identification in ecological research

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Abstract Nematodes are key members of the soil food web and widely used as indicators of soil and sediment health in both natural and managed ecosystems. Therefore, robust identification is central to ecological analyses. Morphological identification has been traditionally used in nematode ecology, allowing for assignment of taxonomy, trophic group, and life stage. However, this approach is time intensive and dependent on taxonomic expertise. Molecular methodologies, particularly 18S rRNA metabarcoding, have become increasingly popular in ecological studies of nematode communities due to their high throughput and accessibility. However, molecular methodologies are limited by incomplete databases and primer coverage. While morphological and molecular methods differ in how community data are represented, both can be used to understand ecological community structure. Here we will use two distinct Midwest grasslands to demonstrate ecological analyses of nematode communities while identifying advantages and pitfalls of each due to the method of identification. In Michigan, nematode communities in early successional grasslands exposed to experimental warming were identified to genus using morphology. Warming increased total nematode abundance, with colonizer taxa responding directly and persister groups shifting indirectly in response to temperature-induced stress. In contrast, a study of the Nebraska Sandhills using 18S rRNA metabarcoding captured patterns of nematode diversity across a drought gradient. Nematode communities in the Sandhills were highly diverse and included rare taxa that can be difficult to resolve morphologically. Random forest analyses suggest potential biotic interactions within nematode communities and between nematodes and other soil microorganisms. These interactions may confer resilience under drought stress. Together, these comparisons demonstrate how methodological choice shapes ecological interpretation of nematode community response to environmental change. Integrating both morphological and molecular approaches may therefore provide a more complete understanding of soil food web dynamics across spatial and environmental gradients.

Unexpected association of *Pasteuria penetrans* with *Meloidogyne enterolobii*

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Abstract Root-knot nematodes (*Meloidogyne* spp.) are among the most damaging plant-parasitic nematodes worldwide. Their management typically relies on integrated strategies that include cultural, chemical, and biological approaches. Among biological control agents, the obligate bacterial parasite *Pasteuria penetrans* has been widely studied for its ability to suppress several *Meloidogyne* spp. However, attempts to evaluate its attachment and development on *Meloidogyne enterolobii* have not been successful. During routine nematode analysis, root galling and second-stage juveniles (J2) resembling those of *Meloidogyne* spp. were found in two soil and root samples, DPI LIST 01142026-00324-1-2 and 03262026-02538-1-9 collected from *Melaleuca viminalis* (= *Callistemon viminalis*) in Lake and Orange Counties, Florida respectively. These samples were submitted by our plant inspectors for the annual nematode certification renewal through the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. Microscopic examination revealed J2 of *Meloidogyne* heavily encumbered with *Pasteuria* sp. spores. DNA was extracted from single females (*Pasteuria* sp. infected female; non-infected female; nematode female cuticle only) and endospore-attached J2. DNA sequences of mitochondrial COX1 (mmT5/COI-R9), COX2 (COX2F/COX2R), and isozyme ($N = 52$; VS1-S1; N1a) data acquired from the females, and COX2 from J2 identified the nematode as *Meloidogyne enterolobii*, a species of significant regulatory concern in the USA and other countries. The bacterium was confirmed as *Pasteuria penetrans* using 16S rRNA, gyrase subunit B (*gyrB*), and sporulation sigma factor E (*sigE*) gene sequences. BLAST searches of *P. penetrans* 16S rRNA sequences matched 18 strains from various collections, including some previously reported from Florida (e.g., strain 30ssp, *M. arenaria*; KT923066). *SigE* and *gyrB* sequences matched *P. penetrans* strains PP1 (*M. javanica*; California, USA; HQ849348, HQ849297), P20 (*M. arenaria*, Florida, USA; AY570907), PPE (*Meloidogyne* spp., Queensland, Australia; HQ849350), RES147 (*M. javanica*/*M. incognita*, Papua New Guinea; HQ849352) and RES148 (*M. incognita*, USA; HQ849300). The detection of *P. penetrans* parasitizing *M. enterolobii* represents, to our knowledge, the first such report worldwide and may suggest the presence of a potentially novel or locally adapted *Pasteuria* isolate capable of infecting this nematode species. This unexpected association may introduce a promising new perspective for the biological management of *M. enterolobii*. Greenhouse culturing of *M. enterolobii* alongside *P. penetrans* is currently ongoing to facilitate studies on *P. penetrans* attachment and development, providing better understanding of this host-parasite interaction.

Does efficacy of cotton cultivars resistant to *Rotylenchus reniformis* vary by crop rotation?

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Abstract Resistant cotton cultivars are a relatively new tool for management of *Rotylenchulus reniformis* (reniform nematode, RN) that can be effective, especially under severe RN pressure. Crop rotation is another effective tool for RN management with greater efficacy the longer the period between host crops. In an integrated approach, a combination of these management practices are often used together, but a better understanding of their interaction is needed. The objective of this study was to evaluate resistant cotton cultivars for efficacy at managing RN abundances and protecting from yield suppression across different crop rotation and irrigation systems. A long-term cropping system site in Quincy, FL was used for this purpose in 2024 and 2025. Each year, this site has each crop phase of conventional and sod-based cropping systems under both irrigation and rain-fed systems. Conventional rotation is two years of cotton followed by a year of peanut. Sod-based rotation is two years of bahiagrass followed by a year each of peanut and cotton. During this study, each plot in a cotton phase (1st and 2nd year conventional cotton, or sod-based cotton) was split between a RN-susceptible (Deltapine 2038 B3XF in 2024 and Deltapine 2333 B3XF in 2025) and RN-resistant cultivar (Deltapine 2142NR B3XF). The site is known to have substantial RN infestation. Reniform nematode soil abundances were monitored regularly during the study, RN abundances from roots were assessed at 6 weeks after planting each year, and cotton yield was assessed at the end of each season. Based on the results of this study, strategies for integrating resistance and crop rotation for management of RN will be discussed.

Gradients in belowground herbivory may drive geographic clines in plant root defenses

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Abstract The longstanding biotic interactions hypothesis predicts that plants from lower latitudes experience stronger herbivore pressure and should thus invest in more potent defenses. However, the evidence is mixed and highly skewed towards aboveground tissues. To address knowledge gaps regarding belowground herbivory, we studied phenotypic variation in root defense against *Meloidogyne* root-knot nematodes (RKNs), which attack a wide variety of plants. RKN activity is influenced by temperature and typically higher at lower latitudes. Strikingly, a latitudinal cline in plant defense against RKNs was visible in all monocot and dicot plant species analyzed, with generally lower nematode fitness on low-latitude plants. Among the plant species in our dataset was *Arabidopsis thaliana*, which produces glucosinolates as defensive chemicals. Across European *Arabidopsis* accessions we identified heritable latitudinal clines in levels of certain glucosinolates. In particular, long-chain aliphatic glucosinolate levels were higher in low-latitude accessions and negatively correlated with RKN fitness. In addition, RKNs imposed fitness costs on plants that varied in magnitude depending on root long-chain aliphatic glucosinolate accumulation. To summarize, we find patterns consistent with the biotic interactions hypothesis: more intense nematode herbivory may have contributed to the evolution of more effective chemical defenses in roots of plants from lower latitudes.

Assessing metabolic resistance to fluopyram in resistant nematode populations using a synergist bioassay

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Abstract Fluopyram resistance has recently been confirmed in populations of the grass root-knot nematode, *Meloidogyne graminis*, and sting nematode, *Belonolaimus longicaudatus*, collected from a Florida golf course with repeated fluopyram exposure. While reduced sensitivity has been verified phenotypically and biochemically, the underlying resistance mechanism remains unknown. In animal parasitic nematodes and insects, well-vetted xenobiotic metabolism inhibitors are commonly used to implicate major detoxification enzyme classes. In this study, we are using piperonyl butoxide (PBO), a cytochrome P450 inhibitor; diethyl maleate (DEM), a glutathione S-transferase inhibitor; and Tribufos (DEF), a carboxylesterase inhibitor to investigate possible metabolic resistance to fluopyram in resistant plant-parasitic nematodes (PPN). We will conduct *in vitro* bioassays using resistant and susceptible populations of *M. graminis* and *B. longicaudatus*. Each well will contain 50 juveniles of *M. graminis* or 25 adults of *B. longicaudatus*. Nematodes will be pre-exposed to sublethal concentrations of PBO, DEM, and DEF, followed by exposure to fluopyram, with solvent controls included for comparison. Mortality will be recorded at 24 and 72 hours after treatment using sodium hydroxide as a stimulus. We will evaluate inhibitor effects by determining the synergism ratio (SR), calculated as LC50 of fluopyram alone divided by LC50 of fluopyram plus Inhibitor. An SR greater than 1 would suggest a likely metabolic contribution to nematicide resistance. This study provides a first step toward characterizing xenobiotic metabolism in resistant nematode populations and will be paired with future RNA-seq analyses to identify candidate genes and enzymes involved in resistance.

Distribution and prevalence of plant-parasitic nematodes in soybean fields of West Tennessee

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Abstract Plant-parasitic nematodes (PPN) cause billions of dollars in yield losses each year in the United States, substantially reducing farmers' economic profitability. Effective management strategies depend on accurate identification of PPN populations present in production fields. In Tennessee, the last formal statewide PPN survey was conducted in 1980, underscoring the need for updated information. The objective of this study was to detect and identify PPN that pose emerging threats to soybean production in the state. In 2025, a total of 122 soil samples were collected from nine major soybean-producing counties in west Tennessee. Soybean cyst nematode (SCN) cysts were extracted using a semi-automated elutriator, while other PPN were processed using sugar flotation and centrifugation technique. The PPN were morphologically identified at a genus level and counted. Nine PPN genera were detected: soybean cyst (*Heterodera glycines*), spiral (*Helicotylenchus* spp.), lance (*Hoplolaimus* spp.), lesion (*Pratylenchus* spp.), stunt (*Tylenchorhynchus* spp.), dagger (*Xiphinema* spp.), root-knot (*Meloidogyne* spp.), reniform (*Rotylenchulus* spp.), and pin (*Paratylenchus* spp.) nematodes. SCN was the most prevalent genus, detected in 94% of samples, followed by spiral nematode (92%). Overall, 78% of samples exhibited moderate to high risk of yield loss from at least one PPN genus. These findings highlight the need for ongoing monitoring of nematode population dynamics and implementation of appropriate management practices to mitigate yield losses. Further survey efforts are recommended, particularly in soybean-growing counties not included in this study.

Meloidogyne javanica MjMAP19 and MjMAP40, amphid-associated MAP-1 effectors coordinating host perception, immune suppression, and climate-responsive parasitism

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Abstract Root-knot nematodes (RKNs) are major constraints on global crop production, and identifying key molecular regulators of parasitism is essential for developing sustainable management strategies. Here, we functionally characterize two amphid-associated MAP-1 family effectors from *Meloidogyne javanica*, MjMAP19 and MjMAP40, identified through oxylipin-responsive transcriptomic profiling of pre-parasitic juveniles. Fluorescence *in situ* hybridization localized both transcripts to amphidial sensory organs, revealing a previously underexplored link between chemosensory perception and effector-mediated parasitism. Expression profiling showed strong induction at the pre-parasitic J2 stage, followed by rapid downregulation after host entry, with MjMap40 displaying a markedly sharper transcriptional response. Functional analyses demonstrate that both effectors act as central regulators of early infection. In planta, MjMAP19 and MjMAP40 localize to the nucleus, cytoplasm, and endomembrane system (ER/Golgi), and suppress Gpa2/RBP-1-triggered programmed cell death, indicating potent interference with effector-triggered immunity. Consistently, overexpression in tomato hairy roots significantly enhanced gall formation, female development, and nematode reproduction, whereas RNAi-mediated silencing impaired host recognition and reduced root penetration, establishing their essential role in infection success. Despite these shared functions, the two effectors exhibit striking functional divergence. MjMAP19 possesses an expanded architecture including an expansin-like domain and shows strong temperature responsiveness, maintaining activity under elevated temperatures where *Mi-1*-mediated resistance is compromised. In contrast, MjMAP40 represents a structurally reduced variant with a sharp, stage-specific transcriptional burst and a prominent role in feeding-site expansion, including increased giant-cell size and nematode development. Together, these findings reveal that MAP-1 family effectors integrate sensory perception, immune suppression, and developmental regulation to coordinate nematode parasitism. Their functional diversification and temperature-dependent behavior highlight adaptive strategies underlying virulence and resistance breakdown. Importantly, the essential roles of MjMAP19 and MjMAP40 in early infection identify them as promising molecular targets for innovative RNAi-based and integrated nematode management approaches.

Identification of soybean cyst nematode-specific neuronal network underlying effector secretion for targeted nematode management

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Abstract The soybean cyst nematode (*Heterodera glycines*) is among the most economically damaging plant-parasitic nematodes worldwide, causing annual yield losses exceeding \$1 billion in the United States. Over the past decade, to identify *H. glycines*-specific control targets, significant progress has been made in identifying esophageal gland effectors and elucidating their roles in parasitism. The timing and regulation of effector secretion likely depend on a parasitic-specialized nervous system involving serotonergic signaling and constraining muscles, which are absent in free-living nematodes. However, the underlying neuronal mechanisms remain largely unknown. Identifying the neuronal network governing this behavior could reveal parasitism-specific control targets that selectively disrupt *H. glycines* while preserving beneficial soil free-living nematodes. Reconstruction of the *H. glycines* esophageal map, including anatomical details and synaptic connectivity (connectome) enables systematic identification of parasitism-specific nervous system components by comparing with free-living nematode esophageal connectomes. We reconstructed the esophageal connectome from two *H. glycines* second-stage juveniles using serial-section electron microscopy. Our comparative connectomics found that the *H. glycines* esophagus shares the highly conserved neuroanatomical organization of their free-living relatives that allows us for direct neuron-by-neuron comparison of synaptic connectivity across multiple nematodes with different feeding habits and developmental stages. In contrast to structural conservation, the chemical synaptic connectivity is extensively rewired mapping directly onto effector secretion-associated structures, such as the subventral and dorsal glands, and the constraining muscles. We found that these structures have acquired *H. glycines*-specific presynaptic inputs, while connections shared among free-living species are lost. In particular, the I3 neuron-dorsal gland connection was identified as a consistent *H. glycines*-specific connection. These findings demonstrate that *H. glycines* plant parasitism is supported by selective rewiring of a conserved neuronal scaffold. The candidate neuronal targets exclusive to *H. glycines* provide a basis for sustainable management strategies that minimize impact on beneficial soil organisms.

Competition analysis of symphylans and plant-parasitic nematodes in pineapple in Hawaii

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Abstract Symphylans (Arthropoda: Symphyla) are cryptic, soil-dwelling arthropods that are root herbivores. In Hawaii, symphylans have long been recognized as pests of pineapple, where their feeding disrupts root system development and compromises plant productivity similar to that of plant-parasitic nematodes. The reniform nematode, *Rotylenchulus reniformis*, is one of the most economically important nematodes of pineapple in Hawaii. The extent to which these organisms interact, whether through competition, facilitation, or independent co-occurrence, remains poorly understood. This study investigated the spatial co-distribution and potential competitive interactions between symphylans and reniform nematodes in pineapple in Hawaii. Field sampling was conducted in pineapples 6-, 12-, 18-, 24- and 30-months after planting. Each plant age was represented by three transects across a block. Symphylans were collected from 500 cm³ carrot-baited traps, collected 1 week after placement, and extracted using a water flotation and soil disaggregation technique. Soil for nematode analysis was collected near each symphylian trap when it was harvested. The soil for nematode analysis was sieved through a 1-cm pore screen and a 250 cm³ subsample subjected to elutriation and centrifugation. Nematodes were counted using a microscope. Comparative statistical analyses were used to evaluate aggregation indices and co-occurrence relationships. Both symphylans and reniform nematodes exhibit aggregated spatial distributions, resulting in patchy field-level damage. Preliminary analyses indicate no significant correlation between symphylian and *Rotylenchulus reniformis* densities, suggesting independent co-occurrence rather than direct competition.

Identifying QTLs associated with soybean cyst nematode (*Heterodera glycines*) suppression in pennycress using GWAS

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Abstract The soybean cyst nematode (SCN; *Heterodera glycines*), is one of the most economically damaging pathogens of soybean (*Glycine max*) in the United States, with estimated annual yield losses ranging from \$1.24 to \$1.69 billion annually. Pennycress (*Thlaspi arvense*) is a winter annual herbaceous species in the Brassicaceae family and has been identified as an alternative host for SCN. Therefore, it is essential to investigate its susceptibility or resistance to SCN. Pennycress has garnered increasing attention as a versatile cover crop with potential for integration into soybean rotation systems. The most significant crop planted in spring just before or soon after pennycress harvest is soybean, though acreage of susceptible dry edible beans (common bean, *Phaseolus vulgaris*) is increasing. A potential challenge with this rotation is that pennycress may also act as a host for SCN. In our research, we challenged a collection of 387 diverse wild accessions of pennycress with SCN HG Type 0 (race 3) and found a continuum of SCN reproduction suppression, with cyst counts ranging from 15 to 129 per plant and female index (FI) ranging from 5.9 to 50.3. Using this phenotypic data and publicly available genotypic data, we were able to identify four distinct and consistent quantitative trait loci (QTL) and seven candidate genes that may be associated with the SCN suppression trait in a genome-wide association study (GWAS). These findings highlight the potential to develop pennycress breeding lines and cultivars with enhanced SCN suppression through marker-assisted selection and genomic prediction, supporting the sustainable integration of pennycress into existing crop rotations.

Seed treatment nematicides for protecting soybean cyst nematode (*Heterodera glycines*) and sudden death syndrome (*Fusarium virguliforme*): suppression of the disease complex

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Abstract Soybean cyst nematode (SCN; *Heterodera glycines*) and sudden death syndrome (SDS; *Fusarium virguliforme*) are among the most economically important constraints to soybean production in North America. The interaction between these two pathogens is complex and often synergistic, with SCN infection frequently exacerbating SDS severity. Seed treatments with both fungicidal and nematicidal activities represent a promising integrated approach for managing these pathogens. In this study, we evaluated the efficacy of the recently developed nematicide TYMIRIUM® (active ingredient: cyclobutirfluram) and ILEVO® (active ingredient: fluopyram) as seed treatments in managing SCN and SDS, under growth room and greenhouse conditions (27°C with a 16 h light/8 h dark). In the first experiment, a dose response of SDS was evaluated at six inoculum levels (from 1:10 to 1:60 sorghum seed: soil-sand mixture, w/w) with or without SCN (3,000 eggs per 100 cm³ soil). In the main experiments, cyclobutirfluram was evaluated at three rates (standard: 0.085 mg ai/seed; one-third: 0.028 mg ai/seed and threefold: 0.255 mg ai/seed) and ILEVO® was applied at its standard rate (0.15 mg ai/seed). Experiments were conducted in cone-tainers (4 cm diameter × 13.5 cm height) in a growth room and in 10-cm-diameter pots under greenhouse conditions, targeting the first and second SCN generations, respectively. Results indicated that the 1:30 inoculum level was optimal for the main seed treatment experiments, providing sufficient disease pressure while avoiding excessive SDS severity. All seed treatments significantly reduced SDS root rot index (1–2 vs. 4) and foliar symptoms (25–50% vs. 80%) compared to the untreated control ($P < 0.05$). SCN egg population density was reduced from approximately 26,000 in the control to 1,000–15,000 eggs/100 cm³ soil across seed treatments, representing a 40–96% reduction. ILEVO® resulted in the greatest suppression of SCN egg population density in the first generation (95–96% reduction), and differed significantly from cyclobutirfluram treatments (40–87% reduction; $P < 0.05$). Overall, nematicide seed treatments provided substantial protection against both SCN and SDS, individually and in combination, under controlled conditions. These findings support the use of integrated seed treatment strategies as an effective management tool for improving soybean health in SCN- and SDS-infested systems.

What are we missing? Comparative extraction techniques and potential hurdles to global standardization

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Abstract Recent global initiatives aim to standardize and unify methods to collect soil fauna at multiple levels, including soil mesofauna such as nematodes. The Soil BON (Soil Biodiversity Observation Network) is a global partnership dedicated to observing, understanding, and predicting soil biodiversity. Within Soil BON, the global research initiative, Soil BON Foodweb (SBF), shares sampling sites and joint data analyses, but with a specific focus on micro, meso, and macrofauna. One aim of SBF is to provide open, standardized, and globally representative methods for monitoring soil animal communities. For soil mesofauna like nematodes, SBF recommends a standardized Baermann funnel method, an active technique which requires nematodes to be able to crawl out of the soil into the water. However, globally standardizing nematode extraction is more easily said than done, since each lab has its own tried and true techniques and each extraction method has tradeoffs. Another method, sugar flotation, is a passive technique that extracts all nematodes, active and inactive, alive or dead, by density. The literature and a pilot study presented here indicate that sugar flotation is more efficient than Baermann funnel extraction, giving a better representation of the nematode community. While some have raised concerns about larger bodied nematodes being lost in the flotation method, the pilot study found more predators and omnivores with sugar flotation than with the Baermann funnel. The sugar flotation method also had a greater relative abundance of root-herbivore nematodes, as well as higher counts of nematodes overall. The funnel method, in contrast, had greater relative abundance of bacterial feeding nematodes. This indicates that sugar flotation may be a better choice when the goal is to characterize nematode pests and ecological structure indicators like predators.

Phenotypic analysis reveals multiple modes of action of the nematophagous fungus *Hyalorbilia oviparasitica*

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Abstract *Hyalorbilia oviparasitica* and closely related species are a group of nematophagous fungi with biological control potential against plant endo-parasitic nematodes. However, phenotypic diversity among strains and its relation to biocontrol efficacy remain poorly characterized. This study reports the phenotypic analysis of 14 nematophagous *H. oviparasitica* strains. They were isolated using baiting techniques with various California soils, where *H. schachtii* populations in sugar beet and Cole crop production areas had previously been reported at low levels. Sequence analysis of the ribosomal RNA gene internal transcribed spacer (ITS) region revealed 99% to 100% similarity of the 14 strains to the *H. oviparasitica* ImV27 strain. Despite high genetic similarity, considerable variation in growth patterns was observed across 3 culture media under multiple conditions. Nematophagous ability of the new strains was further examined through a series of *in vitro* and greenhouse assays. All 14 strains were found capable of parasitizing up to 33.3% of the eggs of *H. schachtii*, *Meloidogyne incognita* (race 1 and race 3), and *M. javanica*. In addition, all 14 strains significantly reduced the total female and cyst counts of the *H. schachtii* population on cabbage in a greenhouse trial, with suppression rates ranging from 72.1% to 97.4%. Further enzyme secretion analysis of the *H. oviparasitica* strains showed production of 12 enzymes involved in cell wall degradation, protein hydrolysis, and lipid/phosphate metabolism. Collectively, these results suggest *H. oviparasitica* suppresses plant-parasitic nematode populations through multiple modes of action and by targeting multiple life stages, supporting its potential as a versatile and effective biocontrol agent.

Identification and development of genomic resources of *Solanum brevicaule* (PI 473011) Y1-5, a wild potato clone with robust resistance to potato cyst nematodes

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Abstract Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are major quarantine pests that threaten global potato production. Host resistance represents the most effective, economical, and environmentally sustainable control strategy. Using the canister and pot assays, we screened 217 accessions from five wild potato species, including *Solanum brevicaule*, *S. boliviense*, *S. berthaultii*, *S. vernei*, and *S. microdontum*, from the U.S. Potato Genebank collection. Screening identified a *S. brevicaule* clone (PI 473011, designated Y1-5), that exhibited robust resistance to both PCN species. We subsequently sequenced the Y1-5 genome using PacBio-HiFi technology and generated a haplotype-resolved assembly. The two haplotypes were assembled to 763.49 Mb and 764.93 Mb, respectively, and together contain 80,021 predicted protein-coding genes. We identified a comprehensive set of genes encoding nucleotide-binding and leucine-rich repeat (NLR) proteins, which are intracellular receptors involved in disease resistance. These *NLR* genes were enriched on chromosomes 4, 6, and 11. Notably, the Y1-5 genome encodes a greater number of coiled-coil-type NLRs (CNLRs) than several previously sequenced wild *Solanum* clones. The identification of Y1-5 and the development of its genomic resources provide a foundation for investigating the mechanisms of PCN resistance and for supporting potato breeding efforts to develop potato cultivar with durable nematode resistance.

Managing emerging nematode pressures in cut flower crops: A collaborative journey to protect plant health and profitability

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Abstract What happens after a grower receives a nematode diagnosis, and who helps translate that information into action? This presentation shares the experience of a cut flower farmer producing dahlias who encountered unexplained plant decline and ultimately identified plant-parasitic nematodes as a key constraint to production. The story begins in the field, where early symptoms prompted uncertainty and iterative troubleshooting. As plant health declined, the grower engaged a plant diagnostics laboratory followed by a nematode diagnostic laboratory, where a standard nematode diagnostics result confirmed the presence of damaging nematode populations. However, like many diagnostic reports, the results provided limited, static information, leaving a critical gap between identification and management. This case study focuses on the post-diagnostic phase as a pivotal moment for extension engagement. Faced with complex and economically significant decisions, the grower worked together with the diagnostics laboratory to interpret the results and evaluate management options, including crop rotation, cultural adjustments, and changes in field allocation. Throughout this process, interactions with extension specialists and diagnostic personnel played a key role in contextualizing results, refining management strategies, and adapting recommendations to the realities of a diversified, high-value cut flower system. By tracing this collaborative process, the presentation highlights how effective communication and iterative feedback among growers, diagnostic laboratories, and extension programs can improve decision-making under uncertainty. It also identifies persistent challenges, including limited crop-specific guidelines, lack of actionable thresholds for specialty crops, and the need for more integrated recommendations following diagnosis. At a broader scale, this grower-centered narrative underscores the importance of strengthening the continuum from diagnostics to management through coordinated extension efforts. It points to opportunities for developing more responsive support systems, where diagnostics are paired with tailored, system-specific guidance and ongoing technical support. Ultimately, this presentation positions extension as a critical bridge between nematode diagnostics and on-farm implementation, demonstrating how collaborative approaches can enhance resilience, protect profitability, and better equip specialty crop growers to manage emerging nematode pressures.

A root awakening: Assessing plant parasitic nematode survivability in treated Delaware fields

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Abstract Southern root-knot nematodes (*Meloidogyne incognita*, RKN) and soybean cyst nematodes (*Heterodera glycines*, SCN) thrive in Delaware (DE) crop production systems, causing significant yield losses. SCN is the most widespread and damaging disease to soybean production in DE and across the US. RKN also impacts soybean production and its wide host range contributes to elevated populations across other crops grown in DE, such as lima beans and corn. With limited host resistance available, growers are interested in chemical management options. Soil applied fungicides, such as Velum (active ingredient Fluopyram), are labeled for broad spectrum control across several soilborne pathogens, including nematodes. To assess the efficacy of Velum for management of RKN and SCN, on-farm and small plot trials were conducted from 2024–2025. A field with history of RKN was selected for on-farm strip trial evaluation of Velum in corn, applied 2 × 2 at planting. An additional field was selected for small plot evaluation of SCN management comparing various rates of Velum applied in-furrow to a no in-furrow treatment. Composite soil samples were collected from each plot to quantify second-stage juvenile population densities (# J2 per 500 cc soil) at the beginning and end of the season and used to calculate reproductive factor ($[\text{final population}]/[\text{initial population}]$, RF) and percent population change ($[(\text{final population} - \text{initial population})/(\text{initial population}) \times 100]$). RKN soil populations were higher in 2024 and SCN populations were higher in 2025. RKN RF values were higher in 2025, but no differences were observed in RKN RF or percent population change by treatment in either year. Despite this, the average percent population changes suggest elevated rates of reproduction in corn fields, regardless of treatment application ($>5,000\%$ in Velum plots, $>13,000\%$ in non-treated plots). Numerically, Velum applied at 3 oz/A had the lowest percent change in SCN populations in both years, and Velum applied at 5 oz/A had significantly higher yield (43.9 bu/a) compared to the non-treated control (40.7 bu/a) ($p = 0.02$) in 2024. Results from these trials suggest that corn supports high rates of RKN reproduction despite at-plant treatment application and that in-furrow application rates of 3 or 5 oz/A of Velum provide best response of SCN populations and potential for yield increase in soybean. Broad spectrum fungicides applied at planting should be used as a tool in combination with other management approaches for RKN and SCN.

Engineering molecular immunity for root-knot nematode resistance in potato

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Abstract Root-knot nematodes (RKN, *Meloidogyne* spp.) are a major problem for the potato industry, where potato tuber damage by RKNs can significantly affect market value. Although chemical nematicides can be effective in controlling RKNs, producers and consumers are concerned about the toxicity to mammals, impacts on the environment, and rising costs. In addition, there is no commercially available potato cultivar that is genetically resistant to RKNs in the United States; therefore, three novel control strategies to combat RKNs are proposed. Applying beneficial microbes can boost plant innate immunity; however, inconsistent performance and deployment challenges have limited its application under field conditions. To address these limitations, PHYTOcapsules were created to encapsulate the *Bacillus subtilis* that secretes the phytoimmunostimulents to prime plant pattern-triggered immunity. *B. subtilis* was optimized to produce the highest amount of immune triggering peptide derived from potato (StPep1) and encapsulated into alginate capsules, which are a better solution for transport, storage, and application of the bacterium. In soil, the capsules slowly degrade over a prolonged time, releasing *B. subtilis* that secretes StPep1 near the potato roots. Treating potatoes with these probiotic capsules can result in up to a 70% reduction in the formation of root galls by RKNs. One promising strategy for developing RKN resistant varieties is to introgress resistance traits from wild potato relatives. *Solanum sisymbriifolium* (litchi tomato) displays strong resistance to RKNs and represents a valuable potential source of resistance for crop improvement. To identify and characterize the underlying RKN resistance genes in litchi tomato, root transcriptomes of *S. sisymbriifolium* with and without *M. hapla* infection were analyzed. This analysis revealed an autoactive coiled-coil nucleotide-binding leucine-rich repeat (NLR) receptor, designated SsiCNL-1. The expression of **SsiCNL-1** in potato hairy roots reduced RKN infections. These findings identify a potentially transferable NLR-mediated resistance mechanism against RKN and provide a genetic foundation for engineering durable nematode resistance in cultivated potato. Progress in understanding RKN–plant interactions has been limited by a fundamental technical barrier: nematode feeding sites occupy only a small fraction of the root, causing bulk transcriptomic approaches to obscure highly localized host and pathogen responses. To overcome this limitation, we applied an enhanced laser capture microdissection RNA-seq workflow to resolve cell-type-specific gene expression within intact, infected root tissues. By profiling nematode infection sites, we captured spatially resolved transcriptomes from nematodes, directly infected giant cells (PRIMER cells), and neighboring bystander cells. Our analyses reveal distinct immune cell states associated with nematode infection, characterized by suppression and manipulation of immune responses within PRIMER cells, alongside the emergence of immune “hot-spots” in surrounding bystander cells. In parallel, we identified candidate nematode effectors whose expression patterns suggest their potential roles in maintaining the feeding site and modulating host immunity. Disruption of these RKN manipulations could lead to developing cell-targeted strategy in sabotaging RKNs parasitism.

Characterization of a candidate virulence gene reveals SNP-associated adaptation to resistant soybean in soybean cyst nematode populations

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Abstract The soybean cyst nematode (SCN), *Heterodera glycines*, continues to be the most economically damaging pathogen affecting soybeans, primarily because of its ability to overcome host resistance. Recent analyses using pooled sequencing of SCN populations have pinpointed candidate genes that may be linked to virulence, though their specific roles are not yet fully understood. In this research, we focused on a prioritized candidate gene to assess its potential involvement in SCN virulence. Sequence analysis of various SCN populations with different virulence traits uncovered polymorphisms within a specific SNP region that are associated with adaptation to particular soybean resistance sources, with each population showing distinct signatures related to its host genotype. Developmental expression studies in virulent SCN revealed gene upregulation upon infection of resistant hosts with peak expression occurring during parasitic juvenile stages. *In situ* hybridization pinpointed transcripts in the dorsal gland esophageal gland, which is involved in stylet-secreted effector secretion during establishment of the feeding site (syncytium). These findings offer multiple lines of molecular evidence indicating that this candidate gene may play a role in SCN virulence. Ongoing functional validation through overexpression and RNA interference studies will directly assess the gene's role in virulence and adaptation, enhancing our understanding of SCN adaptation mechanisms and informing strategies for sustainable soybean resistance.

Subterranean biocontrol: Interactions between *Bacillus thuringiensis* and entomopathogenic nematodes modulate larval mortality and native soil biota recruitment

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Abstract Subterranean infected larvae can serve as ecological hotspots, mediating interactions between microbial biocontrol agents and native soil fauna. However, the extent to which infection by microbial agents influences these community-level interactions remains poorly understood. Here, we investigated how *Bacillus thuringiensis* subsp. *tenebrionis* (Btt), applied as a soil inoculant, affects larval mortality of *Diaprepes abbreviatus* and belowground recruitment of entomopathogenic nematodes (EPNs) and free-living nematodes (FLNs). A multi-tiered experimental approach was employed, including adult behavioral assays, larval infection rates in diet, and greenhouse soil conditions, and a field trial. Btt reduced neonate survival and disrupted larval development, with limited efficacy in older stages. *Steinernema riobrave*, applied 14 days after larval exposure to Btt, enhanced larval mortality and increased root and shoot biomass in the greenhouse. In field plots, Btt-treated larvae attracted significantly more EPNs and FLNs than controls. Soil samples were collected for metabarcoding to assess bacterial, fungal, nematode, and arthropod community shifts. The results suggest that Btt-infected larvae may function as biological signals that modify larvae cues and promote soil enemy recruitment. Our findings highlight how combining microbial agents may contribute to pest suppression and the engineering of soil food web responses, providing a foundation for future studies in functional soil ecology.

Nanopore sequencing to identify native entomopathogenic nematodes for corn rootworm biocontrol

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Abstract The use of entomopathogenic nematodes (EPNs) to manage agriculturally important insect pests is gaining popularity in the context of sustainable agriculture. EPNs of the genera *Heterorhabditis* and *Steinernema* are well-studied and proven effective in controlling various insect pests. Multiple strains of EPNs are commercially available, and endemic strains continue to be discovered. MtDNA COI barcoding and phylogenetic analysis has demonstrated the presence of endemic EPNs in Nebraska soils. Amplified DNA from individual nematodes provided a consistent diagnosis, however DNA collected from a bulk sample of nematodes emerging from infected waxworms often resulted in patterns suggesting sequence heterogeneity. A study was designed using Oxford Nanopore Technologies' MinION sequencing platform to assess these patterns. Soil samples from corn fields in west-central Nebraska were baited with *Galleria mellonella*, infected larvae were placed on white traps, and DNA was extracted from the emerging nematodes by Qiagen Blood and Tissue kits. A 720 bp fragment of COI DNA was amplified using the primer pair F1KF/R2KF, which amplifies both *Steinernema* and *Heterorhabditis* as well as associated scavenger nematode species. MinION sequencing often resulted in a collection of heterogeneous sequences occasionally dominated by *Pristionchus* or *Oscheius*. The amplified sequences also allowed discrimination of endemic and commercial EPN strains. Overall, this study reveals the dynamics and complexities of EPN recovery from field soils and the utility of nanopore sequencing in metabarcoding-based diagnostics.

Biological control of guava root-knot nematode (*Meloidogyne enterolobii*) using native bacterial isolates under laboratory and screenhouse conditions

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Abstract Guava (*Psidium guajava*) is threatened by the guava root-knot nematode (*Meloidogyne enterolobii*) in South Florida, necessitating the development of efficient management strategies. This study investigated local bacterial diversity as a potential biological control resource against *M. enterolobii*, offering an environmentally friendly alternative. Twenty-five *M. enterolobii*-infected root and rhizospheric soil samples were collected from four guava fields in Homestead, Florida, and used to isolate nematode-antagonistic bacteria using egg masses and second-stage juveniles (J2s) as baits. Molecular identification of isolates was performed using 16S rRNA gene sequencing. The nematocidal activity of bacterial isolates was evaluated through three *in vitro* bioassays assessing J2 mortality after 24 h exposure at 25°C with four replicates per isolate. In the first assay, 100% bacterial culture filtrates prepared in Luria broth (LB) were tested against 100 J2s. In the second assay, ethyl acetate-extracted secondary metabolites (SMs) from culture filtrates were evaluated against 100 J2s. In the third assay, 50 µL bacterial cell suspensions (10^7 CFU mL⁻¹, OD600 = 0.01–0.05) were applied to water agar 24-well plates containing 100 J2s per well. The most effective isolates were subsequently evaluated for their ability to suppress nematode penetration into tomato roots under controlled conditions. This involved application of 3 mL bacterial filtrate to 50 g soil infested with 100 J2s, followed by a 10-day incubation. LB-treated nematode-inoculated plants served as controls, with six replicates per treatment. Promising isolates were further assessed in a 2-month screenhouse pot experiment using 500 g of soil, 1,000 J2s, and 50 mL bacterial filtrate. Data were analyzed using ANOVA followed by Tukey's HSD test. A total of 39 bacterial strains representing different genera were recovered. Culture filtrates of *Enterobacter pseudoroggenkampii*, *Delftia tsuruhatensis*, *Pantoea agglomerans*, *Achromobacter xylosoxidans*, *Bacillus* sp., and *Staphylococcus gallinarum* induced > 80% J2 mortality compared with the control (3.02%). SMs from *P. agglomerans*, *D. tsuruhatensis*, *Stenotrophomonas* sp., *Serratia* sp., and *Pseudomonas putida* caused > 90% mortality ($P < 0.0001$) relative to the control (2.4%). In the direct contact assay, bacterial cell suspensions of *Stenotrophomonas* sp., *Bacillus* sp., *A. xylosoxidans*, and *Stenotrophomonas maltophilia* induced ~40% J2s mortality versus 1.2% in the control. Under laboratory conditions, *D. tsuruhatensis*, *Stenotrophomonas* sp., *Serratia* sp., *P. agglomerans*, and *Bacillus* sp. reduced J2 penetration by 96–98% ($P < 0.0001$). In the pot experiment, *P. putida* and *Bacillus* sp. significantly reduced J2 soil populations ($P < 0.05$) compared with both untreated and positive controls (*Bacillus amyloliquefaciens*), and decreased egg production per root system. Among plant growth parameters, a significant increase in root fresh weight was observed only in plants treated with *Bacillus* sp. compared with the healthy, non-inoculated control. These results demonstrate the strong potential of local bacterial isolates as biocontrol agents against *M. enterolobii*, warranting further field evaluation to assess their efficacy for sustainable nematode management in guava.

Biocontrol potential of *Oscheius* nematodes and their associated bacteria from florida fruit orchards against selected insect pests in laboratory conditions

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Abstract Some species of *Oscheius* (Rhabditidae) are increasingly recognized as entomopathogenic nematodes (EPNs) with significant potential for use in biological control programs due to their associations with insecticidal symbiotic bacteria. In the present study, three *Oscheius* species (*O. tipulae*, *O. carolinensis*, and *O. myriophilus*) were isolated from soil samples collected in Florida fruit tree orchards using *Galleria mellonella* as bait. This study represents the first report of these species in Florida soils, demonstrating their successful adaptation to humid subtropical environmental conditions. Species identification was achieved through an integrative taxonomic approach combining morphological characterization with molecular analyses based on 28S rRNA and internal transcribed spacer (ITS) markers. In addition, bacterial symbionts associated with these nematodes were isolated and identified using 16S rRNA gene sequencing. Six bacterial isolates were assigned to four species: *Providencia rettgeri*, *Proteus mirabilis*, *Clostridium senegalense*, and *Brucella anthropi*. The diversity of these bacterial genera supports previous findings that *Oscheius* spp. maintain flexible and diverse symbiotic associations beyond the classical Enterobacteriaceae-linked entomopathogenic nematodes. The entomopathogenic potential of both nematodes and their associated bacteria was evaluated under *in vitro* conditions against three economically important pests of fruit crops: the tea shot hole borer (*Euwallacea perbrevis*), the two-spotted spider mite (*Tetranychus urticae*), and the polyphagous mealybug (*Dysmicoccus grassii*). All *Oscheius* species demonstrated clear entomopathogenic activity, with *O. myriophilus* and *O. tipulae* exhibiting the highest virulence. Specifically, *O. myriophilus* caused 100% mortality in *T. urticae*, while *O. tipulae* achieved 93% mortality in *D. grassii* within 72 h of exposure. In contrast, moderate mortality (45–67%) was observed against *E. perbrevis*, indicating differences in host susceptibility, likely due to variations in cuticle structure and immune defenses. Bacterial isolates also exhibited strong, host-dependent pathogenicity. All isolates caused rapid and near-complete mortality in *T. urticae* (up to 100% within 48–72 h), while *D. grassii* showed high susceptibility (90–100% mortality after 72 h), particularly to *P. rettgeri* and *C. senegalense*. In contrast, *E. perbrevis* exhibited moderate susceptibility, with mortality ranging from 45% to 67%, where *P. rettgeri* and *B. anthropi* were the most effective. These findings indicate that the virulence of *Oscheius* spp. is strongly influenced by their associated bacterial communities, supporting the concept of a synergistic nematode–bacterium interaction driving host mortality. Importantly, this study provides the first evidence of pathogenicity of *Oscheius* spp. against an acarine host (*T. urticae*), significantly expanding the known host range of this genus beyond insects. Furthermore, the high reproductive capacity highlights their suitability for large-scale mass production. Further greenhouse studies are warranted to validate their efficacy under natural conditions and to optimize their application strategies.

Elucidating the mechanisms of resistance to *Meloidogyne incognita* in soybean PI 438489B

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Abstract Southern root-knot nematode (SRKN, *Meloidogyne incognita*) is an economically significant threat to soybean production in the southern United States, accounting for an estimated \$100 million yield loss annually in Arkansas. Previous studies identified a quantitative trait locus *Rmi1* (*Resistance to Meloidogyne incognita 1*) on chromosome 10 that is linked to *M. incognita* resistance in a few soybean genotypes, including PI438489B. However, unlike resistance in other crops, this region lacks conventional resistance (R) gene, and its defense mechanism remains poorly understood. To shed light on this non-canonical resistance mechanism, we evaluated *M. incognita* penetration and subsequent emergence rate, formation of feeding sites, nematode development inside the roots, and reproduction. Penetration and emergence assays were conducted in the susceptible Magellan, the resistant PI 438489B, and the resistant control Forrest. Number of nematodes inside the roots was assessed at 12, 24, and 48 hours post-inoculation (hpi) with second-stage juveniles (J2s) using Acid Fuchsin staining, while emergence was evaluated at 3–5 days post-inoculation (dpi). Although more nematodes penetrated resistant roots over first two days of infection, a greater proportion subsequently emerged, without setting permanent feeding sites, compared to susceptible Magellan. To evaluate later infection stages, nematode development and reproduction together with root galling were assessed in five soybean genotypes including Magellan, PI 438489B, Forrest and two nearly isogenic lines (NILs) from a Magellan × PI 438489B cross. Nematode development evaluated at 7, 14, 21, and 28 dpi showed that PI 438489B significantly delayed the progress of nematode life cycle, with markedly fewer individuals reaching the female stage by 28 dpi. This was further supported by reduced galling and lower egg counts, confirming that *Rmi1* resistant locus does not only limit number of nematodes inside the roots but also impairs *M. incognita* ability to properly develop. Furthermore, confocal microscopy revealed significantly smaller giant cells in PI 438489B compared to Magellan, suggesting that restricted feeding site development may contribute to host resistance. To gain insight into the molecular pathways underlying *Rmi1* resistance, we conducted comparative RNA-seq analysis between susceptible Magellan and resistant PI 438489B at 0, 2, 7, and 14 dpi. Large number of differentially expressed genes (DEGs) at the early infection phase aligns with observed phenotypic responses. Our data suggests that PI 438489B activates multiple defense pathways early during infection, which likely disrupts nematode ability to establish and properly develop permanent feeding sites, consequently constraining their life cycle progression and reproduction. These findings improve our understanding of non-canonical mechanisms of resistance to *M. incognita* and provide useful insights for developing durable and effective resistance strategies in soybean breeding programs.

Cotton development as influenced by *Rotylenchulus reniformis* of distinct geographic origin

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Abstract Studies were conducted to assess the impact of reniform nematode (*Rotylenchulus reniformis*) isolates originating from nine states in the US cotton belt (TN, AL, MS, LA, TX, AR, FL, SC, and GA) on development of cotton. Two cotton cultivars, Deltapine 2141NR B3XF, marketed as *R. reniformis* resistant, and Deltapine 2317 B3TXF, a susceptible control, were employed. Origin of *R. reniformis* significantly influenced plant height, number of leaves, boll weight, chlorophyll content and plant vigor, but not photosynthesis and transpiration. While cotton plants inoculated with any of the isolate sustained negative developmental impacts in comparison with the uninoculated plants, the level of these impacts differed by the origin of the isolate. The isolates originated from the delta region (AR, MS and TN) had the most pronounced negative impacts on cotton. Development of plant inoculated with the FL, SC, TX, and LA isolates were moderately impacted while those of the AL and the GA isolates inoculated ones experienced least impact. Across all isolates and in comparison with the susceptible control, the resistant cultivar was taller, produced more leaves and bolls, and had superior vigor, chlorophyll content, photosynthesis and transpiration. Results from this study implied a need for the development of niche-specific reniform nematode management method in cotton as the ability of *R. reniformis* to impact cotton development can differ based on geography. Provided this study was conducted in a greenhouse environment for two months, results may differ when assessed for a full crop growing season in field environments. As such, field studies across multiple cotton-producing regions with similar soil types and initial soil population density of *R. reniformis* are necessary to better understand the impact of reinform nematode on development of cotton.

Formulated prototype product with their active ingredients of single and blended essential oil phytonematicides on mortality viability and egg-hatch ability of root-knot nematode J2 larvae *in vitro* assays

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Abstract The effect of formulated prototype product with their active ingredients of single and blended essential oil phytonematicides on the mortality and egg-hatch ability of the J2 nematode larvae of *Meloidogyne incognita*, *M. enterolobii* or *M. javanica* was tested. A 100 µl suspension of distilled water containing 100 ± 20 freshly collected J2 or eggs was added to each well containing 100 µl of formulated prototype products (concentrations ranged from 2,000 ppm to 16 ppm). The treatments were assigned in triplicates to the wells in a completely randomized design with six wells representing replications per treatment. The mortality and egg-hatch assays were terminated after 24-, 48-, 72-h and 7-, 14- and 21-day exposure times, respectively. Both bioassays were repeated once. All formulated prototype products 1–14 treatments with their active ingredients of single, blended essential oil phytonematicides resulted in a significant ($P \leq 0.05$) increase in J2 mortality of *M. incognita*, *M. enterolobii* and *M. javanica* from 70–116% after 24-, 48- and 72-h exposure time compared with a solution of dH₂O (negative control) treatments. Egg hatching bioassays showed similar results. The observed efficacy of the EO extracts provides evidence of the potential usefulness of these EOs in the management of plant-parasitic nematodes.

Temporal trends in male and juvenile root-knot nematodes on bermudagrass golf and sports turf in Florida

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Abstract Root-knot nematodes (*Meloidogyne* spp.), particularly the grass root-knot nematode *Meloidogyne graminis*, remain among the most economically important plant-parasitic nematodes affecting bermudagrass used on golf courses and sports fields in Florida. These nematodes are associated with chlorotic blotching, thinning turf, reduced root function and uneven playing surfaces that negatively impact golf course quality. Florida's warm climate and sandy soils favor root-knot nematode development, making routine diagnosis essential for golf course and athletic field management. The University of Florida Nematode Assay Laboratory receives approximately 5,000 samples annually from across the US, with a largest proportion originating from bermudagrass in Florida where root-knot nematodes are common turfgrass pathogens. The objective of this study was to evaluate the temporal pattern in male and juvenile root-knot nematode counts in bermudagrass samples submitted between 2022 and 2025. Mobile life stages of root-knot nematodes were extracted from turf plugs using the mist chamber extraction method. These nematodes exhibited clear temporal patterns, with J2 consistently showing higher monthly mean counts than males and greater year-to-year variability. Monthly profiles revealed recurrent early-year peaks (January–May) followed by lower counts in late summer and fall, with sharp spring maxima in juveniles that contrasted with more moderate male peaks. This indicates peak reproduction during the early months of the year and is important information for treatment application timing. Despite these short-term oscillations, linear trend analysis of counts detected significant negative slopes for both stages, consistent with a gradual decline in nematode pressure across the study period. Average J2 counts decreased from approximately 331 individuals per sample in 2022 to 185 in 2025, a 44% reduction. During the same period, average male counts declined from about 96 to 34 individuals per sample, representing a 65% reduction. Total positive detections also declined annually, indicating a measurable downward trend in population pressure statewide. The observed reduction in root-knot nematodes detected is at least partially due to our extension efforts focusing on root-knot nematode diagnosis and management on turfgrasses over the past 10 years. These extension efforts include: *i*) developing and implementing sampling and extraction protocols that specifically target diagnosis of root-knot nematodes, *ii*) raising awareness of root-knot nematodes as these protocols have become standard, and *iii*) providing management recommendations specifically targeting root-knot nematodes. Continued submission of samples to the University of Florida Nematode Assay Lab remains critical for tracking statewide nematode dynamics and guiding integrated pest management programs on Florida golf courses.

Root-knot nematode resistance in flue-cured tobacco and implications for North Carolina production systems

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Abstract North Carolina is the leading producer of flue-cured tobacco in the United States, with over 114,000 acres harvested in 2024, making tobacco a critical component of the state's agricultural economy. Root-knot nematodes (*Meloidogyne* spp.) are a persistent pathogen of tobacco, and the continued spread of *Meloidogyne enterolobii*, a highly aggressive species capable of overcoming resistance genes in other crops, has raised concerns regarding the durability of resistance in commercial tobacco germplasm. Understanding cultivar-specific responses to multiple *Meloidogyne* species is essential for guiding nematode management and crop breeding efforts. This study evaluated commercial flue-cured tobacco cultivars and breeding lines for resistance to five economically important root-knot nematode species: *M. arenaria*, *M. enterolobii*, *M. hapla*, *M. incognita*, and *M. javanica*. Greenhouse trials were conducted using widely grown commercial cultivars and advanced breeding lines with reported resistance to soilborne pathogens. Plants were separately inoculated with 3,000 eggs from each nematode species. The host response was then assessed 60 days post-inoculation using root galling severity, reproductive factor, and eggs per gram of root tissue. Host resistance varied among cultivars and nematode species, and galling severity did not consistently correspond with nematode reproduction. This suggests post-infection resistance mechanisms in some genotypes. *Meloidogyne enterolobii* was the most aggressive species examined, inducing the highest galling severity and reproduction across cultivars. Meanwhile, *M. hapla* exhibited limited reproduction on tobacco. Several production lines, including NC960, NC196, NC1226, and PVH1600, consistently suppressed nematode reproduction across species and were identified as promising candidates for growers and breeding programs. Unexpectedly, *M. incognita* race 1 induced high galling and reproduction despite reported resistance in these cultivars, suggesting possible erosion of resistance in current commercial germplasm. These findings demonstrate that resistance in commercial tobacco is species-dependent and that *M. enterolobii* poses a substantial threat to existing resistant mechanisms. The identification of cultivars with reduced nematode reproduction provides immediate guidance for growers and valuable genetic resources for resistance breeding. This research highlights the importance of species-specific nematode diagnostics and resistance screening in forming tailored integrated nematode management strategies for flue-cured tobacco production systems.

Globodera pallida RHA1B metaeffector exploits the host proteasome to regulate post-translationally the cognate effector

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Abstract The potato cyst nematode, *Globodera pallida*, is a destructive pathogen that threatens potato yields worldwide. With genetic resistance representing the most practical and sustainable control strategy, advancing our knowledge of plant–nematode molecular interactions is key to developing long-term solutions. As a sedentary endoparasite that establishes long-lasting biotrophic relationships with its host, *G. pallida* secretes numerous effector proteins to manipulate host cell functions and suppress plant defenses. One such effector, RHA1B, functions as an E3 ubiquitin ligase, suppressing effector-triggered immunity by ubiquitinating plant proteins. Here, we hypothesize that RHA1B also acts as a metaeffector, regulating the stability of the cognate effectors *in planta*. To test this, we examined RHA1B's influence on effectors (ME1-5) clustered within the same putative effector island as the *RHA1B* gene. Using *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana*, we co-expressed tested effectors with RHA1B to assess protein stability via *Western blotting* and interactions via immunoprecipitation assays. Our data shows that RHA1B directly interacts with ME-4 and destabilizes it in an E3-dependent manner. We further confirmed that RHA1B is capable of ubiquitinating ME-4 *in vitro*. The roles of ME-4 in virulence were further characterized through Hypersensitive Response suppression and ROS scavenging assays, which both demonstrated that ME-4 interferes with host defenses. These findings suggest that, in addition to targeting host proteins, RHA1B also regulates the stability of other nematode effectors, adding a new layer of complexity to the nematode effectorome. This study identifies RHA1B as the first described meta-effector in nematodes. Although it remains unclear where, when, and why this regulation occurs, our findings strongly suggest that nematode effectors do not act in isolation but instead function as an interconnected network.

Microbial community structure of root-knot nematode suppressive soils across vegetable farms in Indiana and Kentucky

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Abstract Root-knot nematodes (RKN; *Meloidogyne* spp.) are a major constraint in organic vegetable production. Restrictions on synthetic pesticides increase the need for biologically based management. Naturally suppressive soils offer a promising resource for RKN management. In this study, we identified soils exhibiting suppressiveness against *M. incognita* and characterized the bacterial and fungal communities linked to suppressiveness across three compartments (bulk soil, rhizosphere soil, and egg mass). A total of 72 soils from high-tunnel and open-field vegetable farms in Indiana and Kentucky were collected and screened for indigenous RKN densities. Of these, 50 soils were further evaluated using growth chamber-based suppressive-soil assays. Suppressive potential varied widely among soils. Based on the assay results, six suppressive soils and four conducive soils were selected. Suppressive soils supported significantly lower nematode reproduction than conducive soils, and steam sterilization eliminated the suppressive effects. Representative suppressive and conducive soils were compared using 16S rRNA and ITS amplicon sequencing of bulk soil, rhizosphere soil, and egg mass compartments. PERMANOVA based on Bray-Curtis dissimilarities showed significant compartment-dependent differences in bacterial and fungal community composition. Across compartment comparisons, ANCOM-BC2 identified a greater number of differentially abundant bacterial genera than fungal genera. Differential bacterial genera included *Acidovorax*, *Mitsuaria*, *Allorhizobium*, and *Tahibacter*, and differential fungal genera included *Olpidiaster*, *Metacordyceps*, and *Colletotrichum*. In paired suppressive-versus-conducive comparisons, the strongest differences were detected in bulk and rhizosphere communities. Bacterial genera such as *Pseudomonas*, *Chitinophaga*, *Bradyrhizobium*, *Rhizobacter*, and fungal genera such as *Mortierella*, and *Chloridium* were enriched in suppressive soils. Microbial communities associated with egg masses from different soils showed no significant differential taxa in both bacteria and fungi, possibly because egg mass being specialized microhabitat with lower taxonomic diversity. These findings indicate that RKN suppressiveness is biologically mediated and associated with compartment-structured microbiomes and provides a basis for microbiome-informed RKN management in sustainable vegetable production.

Automated imaging and taxonomy-guided AI for accurate and scalable soil biodiversity diagnosis

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Abstract Soil biodiversity is crucial for our functional biosphere and 95% of our food relies on healthy soil. Yet over 70% of earth's soil is degraded, highlighting the urgency to restore soil health, a goal emphasized by the recent European Soil Monitoring directive. Soil-born nematodes exist within all trophic levels of the soil food web and represent a universal bioindicator of soil biodiversity, even in degraded soils. However, this indicator is not widely used and requires nematologist and soil ecology experts as well as significant labor-intensive manual analyses. With the support of EIC and EIT, we developed an automated end-to-end diagnosis tool, comprised of an automated soil sample imaging system (NEM-ASCOPE™) and a multi-level, taxonomy-guided computer vision AI for nematode species identification. Our technology provides quantitative soil biodiversity parameters based on the existing scientific framework of Nematode-based Indices (NBIs), assessing soil health, immunity, fertility, soil-based plant parasites, carbon cycling, pollution, and organic degradation pathway, among other NBIs for soil assessment. Validated by research phytopathogenic laboratories, the tool demonstrated to be in average more accurate (>90%) and over 20-times faster in end-to-end biodiversity analysis compared to manual analysis. The system's nematode identification performance was evaluated on Root-knot nematode (RKN) species level identification accuracy across *Meloidogyne* species that are among the most economically damaging plant-parasitic nematodes, using naturally infested field samples containing *M. chitwoodi*, which are challenging to distinguish from other *Meloidogyne* species due to their morphological similarities. Compared with manual identification, the AI-based approach achieved an accuracy of ~95% in identifying RKN genera with species-level prediction accuracy for *M. chitwoodi* with ~96%, essentially matching manual expert performance. Our platform demonstrates expert-level accuracy for nematode identification down to the species level particularly necessary for regulatory analysis and plant-parasite index (PPI) assessment. The technology allows scalable, industry-ready diagnostics addressing the global shortage of nematologist expertise with the potential to become a new standard in commercial and research sectors, aiding in the global efforts to manage and restore soil health.

Improved approaches to single-cell recovery and sequencing of plant-parasitic nematodes

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Abstract Single-cell sequencing technologies are powerful tools to unravel basic biology of model organisms. Complex symbioses, like that of plant-parasitic nematodes (PPN), necessitate single-cell resolution to unravel intricate interactions and characterize specific signaling events that dictate plant and animal development over time. One example is the decision of some PPN to initiate development from quiescent infective second-stage juveniles (J2), often compared to dauers, to that of an infective and developmentally active parasite. Here, we evaluated single-cell dissociation, flow cytometry, and transcriptome sequencing methods in the root-knot nematode *Meloidogyne hapla* naive dauer-like larvae compared with those of *Caenorhabditis elegans*, to elucidate as to why fewer viable singlet cells along with lower cDNA were consistently recovered from the former. Improved enzymatic digestion and mechanical disruption protocols followed by flow cytometry allowed for the comparison of viable singlet cells recovery from *M. hapla* and *C. elegans*. Normalization of cell recovery was based on the estimated total somatic cells per individual, reflecting the different innate biology between parasitic and free-living nematodes. To compare the percentage yield of viable singlet cells recovery, dissociated cell suspensions were sorted based on viability (Calcein-AM+ and DRAQ5+). In addition, individual singlet cells were assessed based on nucleic acid content (DRAQ5+ and Acridine Orange+), to calculate the percentage of cells with RNA and/or DNA, the ratio of RNA to DNA, and the relative RNA content (RNA/[RNA + DNA]). Improved methodologies for single-cell recovery allow for comparative transcriptional studies on the signals of *C. elegans* dauers and their PPN counterparts, providing new insights into the transcriptional control at the cellular level of these nematodes.

Effects of sheet Mulch on soil health in the establishment of an Avocado Orchard

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Abstract Sheet mulching is an agronomic practice of layering organic materials with a biodegradable weed barrier, such as cardboard, with the intention of enriching the soil without having to periodically replant a cover crop. The organic materials can be nitrogen- or carbon-rich, often gathered from fast-growing shelterbelt trees or shrubs nearby, chopped and dropped under the crop canopy. This is especially suited in an orchard system where crop rotation with cover crop is not feasible. While scientific studies of sheet mulching are limited, some common practices involve clipping from fast-growing nitrogen-fixing plants as the green layer. This project explored Mexican sunflower (*Tithonia diversifolia*) for use in sheet mulching due to its fast-growing characteristic, high carbon content, and known allelopathic compounds against plant-parasitic nematodes and weeds. Specific objectives were to evaluate the ability of Mexican sunflower to suppress weeds, retain soil moisture, and improve soil health in a newly planted avocado (*Persea americana*) orchard. In Hawai'i, avocado seedlings are typically transplanted and protected by a 1.2-meter-diameter metal cage wrap with shade cloth to protect the seedlings from wind and Chinese rose beetles. The field cage made it convenient to add green mulch on top of the sheet mulch periodically. In April 2025, a field trial was initiated to compare sheet mulching (SM) with (1) shredded Mexican sunflower (SF), (2) shredded pigeon pea (PP, *Cajanus cajan*) layered on top of cardboard, vs (3) cardboard only, or (4) standard grower practice with weed mat. Clippings of SF and PP were shredded using a wood chipper, and mulch was added on top of the cardboard at a 7.6-cm-thick layer every 2 months. Soil was monitored every 3 months. Both SM treatments improved the survival rate of avocado seedlings during a drought period in the summer of 2025. At 9 months after planting, SM with Mexican sunflower improved the structure of the soil food web as indicated by the highest structure index among the treatments based on nematode community analysis, whilst having the lowest abundance of plant-parasitic nematodes. To further understand if SF in the form of shredded mulch possesses allelopathic effect against plant-parasitic nematodes, two greenhouse trials were conducted to compare the antagonistic impacts of *T. diversifolia* mulch on root-knot nematode (*Meloidogyne incognita*) against pigeon pea (*Cajanus cajan*), and neem (*Azadirachta indica*) in autoclaved sand: soil mix. This study adds scientific knowledge to sheet mulching in the literature and quantifies the effects of this practice on soil health & plant parasitic nematode management.

Diversity and biological control potential of turfgrass-associated endophytic fungi against the root-knot nematode, *Meloidogyne incognita*

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Abstract Root-knot nematodes (RKNs) are among the most economically damaging groups of nematodes, causing substantial production and yield losses across a wide range of crops. Within the RKN species, *Meloidogyne incognita*, is of major concern due to its broad host range, including turfgrass, and widespread distribution. Management of *M. incognita* has traditionally relied on synthetic chemical nematicides to suppress populations below economically damaging thresholds. However, the increasing development of resistance to commonly used active ingredients in turfgrass systems and growing concerns about their ecological and human health impacts have intensified the need for sustainable management alternatives, such as biological control methods. Endophytic fungi are found within plant host tissues, often forming mutually beneficial relationships by priming resistance to pathogens, improving nutrient uptake, and stimulating growth. These fungi have previously demonstrated nematocidal efficacy in other crops and represent a promising biological control agent due to their minimal environmental disruption compared to synthetic chemicals. In this study, we evaluated the diversity and biological control potential of 131 turfgrass-associated endophytic fungal filtrates against *M. incognita* infective second-stage juveniles (J2s). Turfgrass core samples were collected from three locations: the Fort Lauderdale Research and Education Center and two golf courses in Bonita Springs, FL, and Davie, FL. These sites represented distinct bermudagrass (*Cynodon dactylon* (L.) Pers.) cultivars, including 'Latitude 36', 'Celebration', and 'TifDwarf'. Fungal endophytes were isolated and purified from the surface-sterilized roots and leaves of the collected samples and subsequently cultured in potato dextrose broth (PDB). After two weeks of incubation at 25°C on a shaker, fungal filtrates and mycelial growth were collected. Mycelial growth was processed for DNA extraction and molecular identification targeting the ITS rDNA gene region. Filtrates were used in a nematode mortality bioassay in which nematocidal activity was evaluated at a 50% concentration against *M. incognita* J2s in a 24-well plate. Mortality was assessed after 48 hours of exposure, with live and dead J2 numbers confirmed using sodium hydroxide (NaOH). Across the differing cultivars and locations, the 131 turfgrass-associated endophytes belonged to five classes, with the majority classified as Dothideomycetes (53.4%) and Sordariomycetes (38.9%). The remaining isolates belonged to Peronosporomycetes, Agaricomycetes, and Eurotiomycetes. The mortality bioassay results demonstrated a statistically significant increase in nematode mortality ($P < 0.0001$) from 14 endophytic isolates belonging to *Budhanggurabania*, *Codinaea*, *Curvularia*, *Fusarium*, *Gaeumannomyces*, *Trichoderma*, and *Tumenectria*, indicated by a 21–39% mortality rate against *M. incognita* J2s in comparison to the untreated PDB control (4.6%). Shared characteristics within taxonomic groups of fungal isolates could be further examined to identify the sources of the observed nematocidal activity. These findings suggest the potential for turfgrass-derived fungal endophytes to be utilized as a sustainable biocontrol alternative against *M. incognita*, with future research focusing on intra-genus interactions among closely related RKN species.

Food or Foe: Primary metabolic interactions between the root-knot nematode *Meloidogyne incognita* and plant host species

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Abstract Root-knot nematodes (RKN) are among the most destructive agricultural pests worldwide, inducing and maintaining metabolically active feeding sites in host plant roots as their sole source of nutrients throughout their life cycle. This long-term host–parasite relationship is driven by dynamic molecular and physiological interactions in which the parasite depends on the host for the nutrients and energy required for its survival, growth, and reproduction. Despite the central role of these metabolic exchanges in nematode proliferation and success, the dietary requirements of RKN remain largely unexplored. This study addresses the gap through metabolite profiling of *Meloidogyne incognita*-infected and healthy tissues across three host species, tomato, bean, and cucumber. Preliminary results reveal clear metabolomic differences between infected feeding sites and healthy roots. Notably, elevated accumulation of sugars and amino acids emerges as a consistent pattern across all hosts. To further resolve the origin and dynamics of these metabolites, we will extend profiling across RKN developmental stages, from egg to free-living juvenile to mature female, distinguishing nematode-derived metabolites from plant-derived metabolites and tracking how nematode metabolism shifts over time. Overall, this work will advance our understanding of RKN nutritional biology and inform the development of more targeted nematode management strategies.

Nematode community composition and nematicide efficacy in Alabama corn fields

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Abstract Despite increasing corn acreage and expanded nematicide use in Alabama, limited information is available on the prevalence, distribution, and economic importance of plant-parasitic nematodes in corn production systems. This study was conducted to characterize nematode community composition, quantify economically important taxa, and evaluate available nematicides for management of root-knot nematodes in Alabama corn production. In 2025, a preliminary survey was conducted using 52 composite soil samples collected from corn fields across central and southern Alabama. Nematodes were extracted using sieving followed by sugar flotation and centrifugation, identified to genus level, and quantified per 100 cm³ of soil. Spiral nematodes (*Helicotylenchus* spp.) were the most frequently detected (65.4%), followed by ring nematodes (Criconematidae) (46.2%), root-knot nematodes (*Meloidogyne* spp.) (30.8%), stubby-root nematodes (Trichodoridae) (28.9%), lesion nematodes (*Pratylenchus* spp.) (25.0%), and stunt nematodes (*Tylenchorhynchus* spp.) (5.8%). Spiral nematodes had the highest mean population density (213/100 cm³ soil), followed by root-knot (119/100 cm³ soil) and ring nematodes (103/100 cm³ soil). Root-knot nematodes were present at economically damaging levels in 50% of positive samples based on established threshold categories, confirming their importance as a major yield-limiting pest in Alabama corn. A field nematicide trial was conducted in 2025 in a root-knot nematode-infested field using nine treatments, including four seed treatments: Ileva (active ingredient [a.i.], fluopyram, 49.02%); BioST 2 G (a.i., inactivated *Burkholderia rinojensis* strain A396, 94.46%); Poncho/Votivo (a.i., clothianidin, 40.30% + *Bacillus firmus* I-1582, 8.10%); and Avicta 500FS (a.i., abamectin, 46.3%). Four in-furrow spray treatments included Averland FC (a.i., abamectin, 8.0%); OutReach SC (a.i., *Bacillus amyloliquefaciens* strain PTA-4838, 2.20%); Spectra (a.i., chitosan, 5.75%); and Spectra + Averland FC. One granular in-furrow treatment, Counter 20G (a.i., terbufos, 20%), was also included along with a non-treated control. Treatments were arranged in a randomized complete block design with five replications using corn hybrid DKC68-95. Early-season plant responses included stand count, plant height, plant biomass, root-knot nematode eggs per gram of root at 36 days after planting (DAP), and grain yield at 126 DAP. Stand counts differed significantly among treatments ($P < 0.0001$); however, differences were not consistently associated with nematicide application. Although plant height ($P = 0.3281$), biomass ($P = 0.6298$), nematode egg density ($P = 0.5429$), and yield ($P = 0.3063$) differences were not statistically significant, several nematicides numerically reduced root-knot nematode populations compared with the untreated control. Counter 20G showed the greatest reduction in nematode egg density (66%), followed by Averland FC (54%) and Spectra + Averland (42%). Poncho/Votivo, Counter 20G, and Avicta 500FS also produced numerically higher yields compared to the untreated control, corresponding to estimated additional returns of \$42, \$39, and \$35 acre⁻¹, respectively, based on a corn price of \$4.67 bu⁻¹. These findings support routine nematode diagnostics and targeted nematicide use as components of integrated corn nematode management in Alabama. Expanded sampling in 2026 will improve statewide representation and strengthen validation of nematicide performance across diverse production environments.

Pathogenicity differences between *Aphelenchoides pseudobesseyi* isolates and sequence variation in putative virulence-related aspartic protease genes

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Abstract *Aphelenchoides pseudobesseyi* is an economically significant plant-parasitic nematode that causes severe damage to various high-value crops worldwide. Understanding population-level variations in pathogenicity and their underlying molecular mechanisms is crucial for developing effective management strategies. In this study, three geographically distinct pure lines were established from populations collected from different ferns across Taiwan: HBL from *Diplazium esculentum* (Yuchi, Nantou), LSF from *Pteris fauriei* (South District, Taichung), and XJC from *Asplenium nidus* (Lüye, Taitung) through single female isolation followed by monoxenic culture on *Alternaria citri*. Comprehensive morphological analysis confirmed that all 3 isolates exhibited morphological *A. pseudobesseyi* characteristics, including rounded and slightly offset lip regions, post-uterine sacs posterior to vulva with length exceeding one-third of vulva-anus distance, and conoid tails terminating in cuspidate or brush-like mucrons bearing 2–3 pointed processes. Molecular confirmation through Bayesian phylogenetic analysis using cytochrome c oxidase subunit I (*COI*) gene sequences under the GTR + I + G evolutionary model clustered all three isolates within the *A. pseudobesseyi* clade. Pathogenicity assays using 250 nematodes inoculated onto *Asplenium nidus* leaves revealed significant pathogenicity differences among them after 30 days. The HBL isolate caused smaller lesions on the fern leaves compared to LSF and XJC isolates. The aspartic protease (ASP) genes, which are hypothesized to facilitate plant parasitism through host tissue degradation, were investigated. Substantial sequence variations were found between isolates, with single nucleotide polymorphisms ranging from 5–73 across four different ASP loci. Amino acid translation demonstrated that these variations significantly affect encoded proteins, particularly within signal peptide regions and around peptidase A1 domain functional sites. These findings demonstrate substantial genetic variation in ASP genes might be related to the virulence of *A. pseudobesseyi*.

Elucidating the role of MigPSY peptides in plant–root-knot nematode (RKN) interaction

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Abstract Plant-parasitic nematodes pose a significant threat to global food production by invading roots and establishing specialized feeding sites to extract nutrients from their hosts. To facilitate parasitism, these nematodes secrete effectors that manipulate host cellular processes, such as plant peptide mimics that resemble endogenous signaling molecules. Normally, plants produce a family of sulfated peptides called plant peptide containing sulfated tyrosine (PSY), which regulate root growth through cell expansion and proliferation. Interestingly, similar PSY-like peptides have evolved in pathogens; for example, in bacteria, *Xanthomonas* spp. secrete the effector RaxX, indicating a conserved strategy of host manipulation. Our previous work identified a group of PSY-like peptides in root-knot nematodes, called MigPSYs, that promote root growth and contribute to nematode virulence. Building on these findings, my research aims to understand how plants perceive and respond to MigPSYs. Additionally, we examined MigPSY's subcellular localization and tested its recognition via candidate receptors in *Arabidopsis* (PSYRs) and rice (XA21). Our results suggest that PSYR3 and XA21 may be involved in MigPSY-mediated signaling, indicating a conserved host perception mechanism across plant–microbe and plant–nematode interactions. By revealing how nematodes exploit peptide signaling pathways, this work provides new insights into host–parasite communication and offers strategies for controlling plant-parasitic nematodes.

Microscopic marvels: Multimedia content to communicate nematode diversity to the public audiences

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Abstract Nematodes are the most abundant and possibly the most diverse multicellular animals on the planet. In addition to their abundance and diversity, their functional and ecological significance is also remarkable. Despite their diversity and important roles in ecosystem functioning, nematodes remain largely unknown to the public, being hidden marvels appreciated mainly by the scientists who study them. To address this gap and promote public engagement, we developed *Microscopic Marvels*, a three-year interdisciplinary collaboration among scientists, science communicators, and ranchers through *The Streaming Science Project*, in partnership with *Platte Basin Timelapse* and supported by the National Science Foundation. The project is grounded in place-based and dialogic science communication frameworks, emphasizing the power of storytelling to connect audiences with complex, unseen ecological processes. Field-based research was conducted across the Nebraska Sandhills, including ranching systems, to document nematode diversity in aquatic habitats such as lakes, rivers, and wetlands. Scientists, land managers, and communicators co-developed narrative-driven multimedia content designed to translate complex, invisible ecological interactions into accessible, place-centered stories for public audiences. Specific communication outputs include a short documentary video, case study interviews, photo essays, posters, and podcast episodes. Additionally, teaching resources aligned with national science standards have been developed for middle/high school classes, as well as after school programs, to inform and engage students about nematodes and their roles in the environment. These resources aim to increase awareness of nematode diversity, illustrate their ecological roles, and connect learners to real-world scientific research and agricultural and natural ecosystems. Furthermore, social science methods were applied to examine how integrating raw Nebraska Sandhills nematode photos and field footage into University of Florida science communication courses influenced college students' learning about place and nematode diversity. Students engaged with authentic research media within project-based learning activities, using the materials to develop their own science communication products. Qualitative reflections were used to assess changes in students' knowledge, perceptions, and attitudes toward nematodes, ecosystem health, and the role of place in shaping ecological understanding. Findings suggest that incorporating place-based, real-world scientific media enhances learners' conceptual understanding of nematodes and strengthens their ability to connect invisible organisms to broader environmental systems. By making the invisible visible through interdisciplinary collaboration and course-based implementation, *Microscopic Marvels* contributes to both nematology outreach and science communication pedagogy. This work highlights the value of integrating research, storytelling, and experiential learning to expand awareness of nematode diversity and to promote more inclusive, place-based understandings of ecosystem health and sustainability.

Atroforce™: New biological nematicides for the control of plant parasitic nematodes in cotton

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Abstract AtroForce™ is a new biological nematicides containing *Trichoderma atroviride* K5 (NRRL B-50520) being developed by UPL for the control of plant parasitic nematodes. This new strain colonizes roots providing excellent endophytic and rhizospheric growth. Metabolites produced inhibits egg hatch and paralyzes juvenile and adult life stages. In addition to providing a unique mode of action for management of plant parasitic nematodes, root biomass is also increased via below ground bio-stimulation. *Trichoderma atroviride* K5 is able to grow at soil temperatures between 4–32°C with the optimum growth around 25°C. AtroForce provides a unique mode of action for management of plant parasitic nematodes.

Mechanisms that differentiate highly *Nematophagous hyalorbilia* spp. strains from those with lower effectiveness

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Abstract Cyst nematodes cause significant damage to economically important crops worldwide. Our group and others have shown that a group of naturally occurring fungi, *Hyalorbilia* spp., parasitize fourth-stage juveniles, females, and eggs of the sugarbeet cyst nematode (*Heterodera schachtii*). These fungi can reduce egg densities below the economic damage threshold and they have been effective in different agricultural soils. Their presence predicts which soils are likely to suppress cyst nematodes. The goal of this study was to determine the mechanisms that differentiate highly effective *Hyalorbilia* strains from those with lower efficacies. A series of studies was conducted on a collection of *Hyalorbilia* strains with varying nematode-suppression efficacies. The strains that are most effective at reducing *Heterodera schachtii* densities in soil were primarily female parasites rather than egg parasites. A comparative genome analysis identified genes unique to the most effective strains. A transcriptomic analysis revealed distinct sets of upregulated genes in *Hyalorbilia* strains parasitizing female sugarbeet cyst nematodes compared to those during saprotrophic growth on peat.

The silent invader: Uncovering the local dispersal of *Litylenchus crenatae* ssp. *mccannii* in U.S. Forest

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Abstract Beech leaf disease (BLD), caused by the *Litylenchus crenatae* ssp. *mccannii* (Lcm) nematode, is an emerging threat to beech trees. This disease is characterized by distinct leaf symptoms, including leaf interveinal banding and thickened leaf texture, which leads to eventual tree mortality. Understanding Lcm dispersal mechanism(s) is crucial for managing BLD, yet these remain largely unknown, posing a major barrier to its effective management. This study represents a pioneering investigation into the abiotic and biotic vectors that potentially contribute to the local dispersal of Lcm in natural American beech (*Fagus grandifolia*) forest systems in the Northeastern United States. An experiment was set up in Stone Valley Forest, Pennsylvania (PA), using four funnel stands placed at variable distances from naturally BLD-infected beech trees. This approach enabled the recovery of active Lcm nematodes from each funnel, demonstrating their ability to naturally disperse at least 11.74 m from the nearest BLD-infected tree. In addition, birds were investigated as potential dispersal vectors of Lcm via external and internal mechanisms. The objectives were to determine if Lcm can be detected from ectoparasites, feathers or feces collected from wild birds caught in BLD areas. The findings highlight the role of abiotic factors involved in the dispersal dynamics of Lcm, especially wind and humidity, as indicated by a generalized linear model. The current study also uncovered the incidental association of Lcm with other organisms beneath the canopy of BLD trees, including spiderwebs and caterpillars. The detection of Lcm DNA in seven ectozoochory and fourteen endozoochory samples from six different bird species further supports the idea that birds may play a role in transmitting the nematode. To our knowledge, this is the first study to document the potential vectors involved in the local dispersal of Lcm, offering valuable information for the biology of this nematode. The findings contribute to broader efforts in advancing the understanding of the local spread of BLD, highlighting the complex interplay of abiotic and biotic factors in this disease dispersal.

The national plant diagnostic network: Expanding nematology collaboration for national plant health efforts

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Abstract The National Plant Diagnostic Network (NPDN), established in 2002, is a coordinated system of diagnostic laboratories dedicated to protecting U.S. agriculture and natural ecosystems from plant pests and pathogens. Through integrated communication, quality diagnostics, and a nationwide laboratory network, the NPDN enhances early detection, rapid response, and overall plant biosecurity. More than 120 laboratories across all 50 states and four U.S. territories are affiliated with NPDN and provide expertise in plant pathology, entomology, nematology, plant and seed identification, and nutrient analysis. These laboratories function as sentinel sites for surveillance of emerging and regulated threats and offer distributed surge capacity during agricultural emergencies. The NPDN manages the only accreditation program specialized in plant diagnostic labs in the U.S. This program allows labs to demonstrate accuracy, reliability, and consistency in their diagnostic results while strengthening laboratory readiness for the early detection of exotic and emerging pests and pathogens. Accreditation builds confidence in laboratory results and promotes continuous improvement of diagnostic services. NPDN committees provide support to the diagnostic community through professional development, management of the national data repository, newsletter publications, development of outreach materials, connection with regulatory partners, protocols and other resources. This essential work creates a national framework for sharing information and supporting the early diagnosis and identification of plant health threats. Nematodes remain an underrecognized but economically significant component of plant health diagnostics, and expanded collaboration with the nematology community is essential to strengthening national detection and response capabilities. This presentation will highlight current diagnostic infrastructure, resources, and opportunities for engagement with the NPDN. Participation in NPDN can enhance visibility, collaboration, and impact of nematology expertise while contributing to a coordinated national system for plant health protection.

Combining *Purpureocillium lilacinum*, *Bacillus amyloliquefaciens* and sunn hemp to suppress *meloidogyne javanica* in florida vegetables

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Abstract *Purpureocillium lilacinum* (Pl), is a soil fungus widely used as a biological nematode control agent (BCA). The efficacy of a BCA often depends on its persistence, and higher survival rates usually correlate with better pest suppression. However, Pl spore viability declines rapidly in Florida sandy soils, due to high temperature and low organic matter. Practices that improve soil conditions, such as cover cropping, may help mitigate these limitations by increasing organic matter. In addition, other BCAs such as *Bacillus amyloliquefaciens* (Ba) may complement Pl through the production of nematocidal metabolites and plant growth promotion. Greenhouse and field studies were conducted in 2024 (organic field) and 2025 (conventional field) to evaluate the combination of *P. lilacinum*, *B. amyloliquefaciens* and sunn hemp (*Crotalaria juncea*) to suppress root-knot nematodes in vegetables. Sunn hemp was grown in the field during summer for 15 weeks prior to mowing and incorporation, after which plastic-mulch beds were made and planted with zucchini (2024) and tomato (2025). The trial was a split-plot design with four replicates, with cover crops as main plot and biocontrol treatments Pl, Ba, and Pl + Ba and a control as subplots. Biocontrol treatments were applied at labeled rates at 0, 2, 4 and 6 weeks after planting. Root gall ratings, and nematode soil populations were evaluated at mid-season, and at the end of the season. Fruit yield was evaluated by harvesting zucchini (4 times) and tomato (2 times). For the greenhouse trial, 2-liter pots were filled with soil that was collected from each field trial shortly after the cover crop incorporation. Zucchini and tomato were planted into pots and inoculated with 8,000 *M. javanica* eggs. The experiment was set up as a randomized complete block with five replicates, evaluating the same biocontrol treatments, a nematicide (fluopyram), and inoculated and non-inoculated controls across cover-cropped and fallow soil. All treatments were applied as a drench at the same rates and timings as the field trial. Plant growth, root gall ratings and RKN eggs per plant were recorded after eight weeks. In the greenhouse trial, none of the treatments improved tomato growth, but sunn hemp improved zucchini growth. Sunn hemp also significantly reduced root galls on both crops by 33–54% and egg production by 40–52%, regardless of the biological treatment. The biological agents provided some additional nematode suppression, especially in the cover crop soil, and mostly with zucchini. In the zucchini field trial, nematode infection was very low and none of the treatments affected yield. In the tomato field trial, sunn hemp gave a 30% reduction in tomato root gall ratings. In addition, sunn hemp improved tomato yield, resulting in an overall increase of 19% compared to fallow. Overall, sunn hemp cover crop suppressed root-knot nematodes in both trials, and improved tomato yield, whereas the biological agents provided some nematode control in zucchini but less in tomato.

Knotty little nematodes: Bioprospecting for green-alternative nematicides to control root knot nematode in the pacific northwest

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Abstract Plant-parasitic nematodes (PPN) are among the most destructive agricultural pests, causing substantial economic losses across a wide range of cropping systems. The primary control, soil fumigation, is costly and increasingly restricted due to environmental concerns and potential toxicity. Therefore, identifying alternative and sustainable PPN management strategies is essential. *Solanum sisymbriifolium*, (litchi tomato) is known for its resistance to several species of PPN and is a promising source of natural nematocidal compounds with potential applications in biological control. Research has demonstrated that litchi tomato shoot material can be a potent nematicide but the mechanisms of its activity on PPN remain elusive. Aqueous litchi tomato shoot extracts stimulated egg hatch by *Meloidogyne chitwoodi* but no significant effect on second-stage juveniles (J2) was observed. However, adding an emulsifier rendered the same aqueous extract highly toxic to J2 of *M. chitwoodi*, *M. hapla*, and *M. javanica*, suggesting that emulsification enhanced the solubility and/or bioavailability of nematocidal compounds. To further investigate this activity, concentrated litchi tomato shoot extracts were generated using a liquid-liquid extraction technique. Crude methanolic extracts of freeze-dried and milled litchi tomato shoot material were fractionated with solvents of varying polarity, listed here from most to least polar: 1-butanol, ethyl acetate, dichloromethane, and hexane. These were then evaluated for their effects on nematode hatch and viability. The 1-butanol fraction consistently produced the strongest suppression of egg hatching and reduction in J2 viability; however, all four fractions demonstrated nematocidal activity in at least one assay. These findings not only demonstrate the potential of litchi tomato as a source of new nematocidal compounds for use as biopesticides but also provide a clear opportunity for comparative chemical analyses identifying the mechanisms underlying its toxicity.

Separating integrated efficiency of regenerative agricultural practices on beneficial nematode trophic groups with the fertilizer use efficiency model

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Abstract The value of regenerative agricultural practices (RAPs) such as tillage, cover crops (CC) and nutrient amendments for improving soil health and generating desirable ecosystem services (DESSs), including beneficial nematodes (BNs), soil organic matter (SOM), and available nitrogen (NO_3 and NH_4), the role of BNs in nutrient cycling within the soil food web (SFW), and the Ferris *et al.* SFW model as a soil health diagnostic tool are well established. Depending on the targeted DESSs, soil health outcomes could be suitable, unsuitable or variable. For example, we reported that four years of corn production under tillage, winter rye CC, and 0, 112 or 224 kg N/ha from inorganic- and compost-sources resulted in primarily resource-limited and structured (data in Quadrant C) soil health conditions. Thus, suggesting the presence of process-limiting factors affecting nutrient cycling and raising research and management decision-making questions. First, how does the presence of process-limiting factors relate to bacterivore (Bv), fungivore (Fv), Predator (Pr) and omnivore (Ov) trophic groups? Second, are the generated DESSs and/or soil health outcomes sustainable, unsustainable or require additional measures to be sustainable? A sustainable outcome is one that generates the DESSs while meeting ecological and economic expectations simultaneously. This study answers the questions using the same data describing the presence of process-limiting factors and applying the fertilizer use efficiency (FUE) model to analyze integrated efficiency of the RAPs, DESSs and changes in total BNs, Bv, Fv, Pr and Ov trophic groups. The FUE model compares the effect of RAPs on changes in DESSs (y-axis) and BNs (x-axis) relative to the control and separates where DESSs and BNs increase (sustainable), decrease (unsustainable) or go in opposite directions (needing corrective factor(s) to be sustainable). The x- and y-axes start at zero, controls represent 100%, and changes on either axis have no upper limit. BNs decreased in CC treatments and increased in non-CC treatments in till or no-till plots, while SOM, NO_3 and NH_4 increased in both tillage and CC treatments in all N treatments. This suggests that the outcomes of DESSs and BNs in non-CC treatments are sustainable. When BNs were separated by trophic groups, differences in nutrient treatments emerged. The trends of Bvs, Fvs, Prs and Ovs in CC treatments were similar to that of BNs in CC treatments. The data points for the trophic groups fell within the sustainable zone for most nutrient treatments. However, the magnitude of changes for Prs and Ovs was significantly greater than that of Bvs and Fvs. This suggests that factors that boost Bvs and Fvs maybe need to address the process-limiting factors and improve efficiency of the RAPs.

Plant-parasitic nematode distribution in soybean fields of Mississippi: Implications for management

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Abstract Mississippi ranks 14th in soybean production in the United States in 2025 with a total production value of \$1.05 billion, and with an average yield of 56 bushels per acre across 1.81 million acres planted. However, plant-parasitic nematodes pose a significant threat to soybean production, resulting in substantial yield losses and reducing the soybean supply for both local and global markets. In Mississippi, soybean fields are commonly rotated with cotton, corn, and sweet potato. The determination of population levels of plant-parasitic nematodes present in the field is pivotal for soybean growers in the implementation of effective nematode management strategies. This survey study analyzed seven years (2019–2025) of data from 2,807 soybean field soil samples across counties in Mississippi. Samples were obtained from two primary sources: submission to the Mississippi State University Extension Service Plant Diagnostic Laboratory (MPDL) and collection from soybean growers' fields by project personnel. This study reported the 44 counties from soybean-growing Northeastern and Northwestern regions in Mississippi, and a few from Southern regions that do not widely cultivate soybean. Twelve genera of plant-parasitic nematodes were identified, including *Heterodera glycines*, *Meloidogyne* spp., *Rotylenchulus reniformis*, *Helicotylenchus* spp., *Hoplolaimus* spp., *Tylenchorynchus* spp., *Pratylenchus* spp., *Paratrichodorus* spp., *Xiphinema* spp., *Mesocriconema* spp., *Hemicyclophora* spp., and *Belonolaimus* spp. The distribution of major plant-parasitic nematodes of soybean was also mapped in Mississippi. Also, this study reported the percentage of samples containing nematodes and their populations above damage threshold per 100 cm³ of soil for soybean. Among these nematodes, spiral nematode was the most recovered, followed by reniform and soybean cyst (SCN). However, an analysis of samples exceeding the damage threshold revealed that reniform nematode was the most frequently observed, exhibiting the highest number of samples above the damage threshold, followed by the soybean cyst and root-knot nematode. This survey remains ongoing across soybean-growing fields throughout Mississippi to further understand the plant-parasitic nematode population profile and its impact on soybean production. Some of the growers historically and currently rotate soybean with cotton, in which reniform is a serious problem and very prevalent in the cotton belt. The findings of this survey are expected to facilitate the Mississippi soybean growers for the development of effective nematode management strategies.

A new era in soybean cyst nematode screening

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Abstract Soybean Cyst Nematode (SCN) enumeration is a critical first step in determining field management strategies, such as seed treatments or the planting of resistant variants. Currently, the industry-standard method requires large soil samples, laborious sieving, taxonomic expertise, and microscopic enumeration. These requirements result in expensive testing protocols and long turnaround times—hurdles that often lead to insufficient or infrequent sampling. Consequently, growers frequently receive only a partial picture of true nematode populations due to the non-homogeneous distribution of SCN in soil. The objective of this study was to evaluate NemaGENE, a new rapid qPCR assay, for the detection and enumeration of nematodes in 20cc fertility soil subsamples in under 2.5 hours. By integrating pathogen testing with routine chemical and physical soil analysis, this method aims to resolve the bottlenecks that currently limit widespread testing. Bulk soil samples were collected across Midwestern soybean production regions, representing a diverse range of soil types and SCN infestation levels, and subjected to standard fertility drying and grinding procedures. Samples then underwent a paired analysis comparing microscopy-based enumeration (conducted at Minnesota Valley Testing Laboratories and Iowa State University) and the NemaGENE qPCR assay. No significant statistical difference was found between the two methods. These findings support the use of the NemaGENE assay for SCN detection using small-volume subsamples derived from routine fertility sampling. Integrating pathogen detection into existing soil sampling workflows has the potential to increase sampling density and improve management decisions without increasing the sampling burden on growers.

Assessment of host response in sweetpotato cultivars and diverse germplasm to *Rotylenchulus reniformis*

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Abstract The reniform nematode (*Rotylenchulus reniformis*) has become a major pest in sweetpotato production. Multi-year field trials in Louisiana have shown that severe reniform nematode infestations result in a 30–40% yield loss. Currently, commercial sweetpotato production relies heavily on three cultivars: ‘Covington’, ‘Beauregard’, and ‘Orleans’, yet only ‘Beauregard’ has been characterized as a host to the reniform nematode. This study (i) evaluated the host status of broadly used sweetpotato cultivars to the reniform nematode, (ii) identified potential sources of resistance among sweetpotato germplasm from the USDA Genetic Resource Information Network (GRIN), and (iii) quantified the impact of reniform nematode parasitism on sweetpotato root mass. Reniform nematode reproduction and sweetpotato root weight were measured in nine commercial cultivars and 28 plant introductions (PIs) in greenhouse trials. All nine commercial cultivars supported similar numbers of eggs per gram of root as ‘Beauregard’, signaling widespread susceptibility. Four PIs (‘Saing-Mi’, ‘Regal’, ‘Morada Sombica’, and ‘Tainung 63’) supported significantly less reniform nematode reproduction compared to ‘Beauregard’, suggesting potential utility in breeding programs. When sweetpotatoes were inoculated with the reniform nematode, they had a 28.4% increase in fibrous root biomass and a 30.4% decrease in storage root weight. This study is the first to identify ‘Covington’ and ‘Orleans’ as excellent hosts to the reniform nematode and confirms previous findings that the reniform nematode is a major pest of sweetpotato through inhibition of storage root production.

Stage-specific plant responses to *Meloidogyne incognita* infection through integrative transcriptomic analysis

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Abstract Advances in omics technologies and bioinformatic tools have made large-scale gene expression studies more accessible. However, RNA-seq datasets are typically analyzed within the narrow scope of their original experiment. This approach provides only constrained insights into gene-trait associations and results in gene expression phenotypes that are limited to an individual host, nematode species, life stage, or experimental condition. This constraint is especially important and in contrast to plant-parasitic nematodes with large and diverse host ranges and with many genes lacking clear homology in protein databases making biological interpretation from individual omics datasets challenging. Therefore, we integrated several publicly available *Meloidogyne incognita* transcriptomics-datasets from multiple hosts to provide a broader view of gene expression patterns and identify conserved or condition-specific gene expressions associated with nematode-host interactions. Raw transcriptomic datasets were first assessed for quality, and only high-quality datasets were retained for downstream analysis. We then grouped curated datasets into early infection, representing 2 to 7 days post inoculation, and late infection, representing 2 to 3 weeks post-infection. Integrated cross-host transcriptomes from multiple host species for early and late infection were generated. We identified 1,461 and 2,979 differentially expressed genes at early and late infection stage respectively. These results suggest that host responses to *M. incognita* infection shift strongly over time, with more extensive transcriptional reprogramming happening during later infection. We also developed a co-expression gene network among host genes to identify novel gene pathway associations. Commonly induced pathways across hosts may provide broad targets for nematode management, while distinct host responses may help explain differences in host range and infection outcomes. This methodology adds novel value to historic transcriptomic datasets and can help drive database management for sustained utility while uncovering new biological insights to plant-nematode interactions.

New frontiers in root-knot nematode feeding tube composition and function: Novel targets for broad crop resistance to root-knot nematodes

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Abstract Root-knot nematodes (RKNs; *Meloidogyne* spp.) represent the world's most successful group of plant-parasitic nematodes, capable of parasitizing thousands of plant species and causing billions of dollars in crop losses worldwide each year. Armed with a protrusible mouth stylet used to secrete an arsenal of effectors, these sedentary endoparasites exploit the host's own physiology to form giant feeding cells within roots that serve as the sole source of nutrition required to complete their life cycle. Feeding tubes (FTs), unique structures formed by adult females, extend into the giant-cell cytoplasm, where they are immediately enveloped by a fine network of tubular endoplasmic reticulum. These structures serve as highly specialized conduits for nutrient uptake—the key to sustained parasitism. Although well described microscopically, the molecular composition of FTs has remained a mystery. Using a combination of approaches, we demonstrate that FTs are formed from stylet-secreted effector proteins derived from the secretory dorsal esophageal gland cell of actively feeding adult females. These effector proteins are unique to RKNs and conserved across the sequenced genomes of eight major species, opening the door for novel strategies targeted at disrupting FT formation to engineer crops with broad RKN resistance.

Comparative evaluation of commercial biocontrol agents for the management of *Meloidogyne incognita* in tomato

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Abstract Root-knot nematodes (*Meloidogyne* spp.) are among the most economically damaging soilborne pathogens worldwide, causing significant yield losses in a wide range of crops. Biological control has emerged as a promising and sustainable alternative to chemical nematicides. A growing number of commercial biocontrol products based on microbial agents are available now. However, the comparative efficacy of commercially available products remains insufficiently characterized. This study evaluated the nematocidal efficacy of three commercial biocontrol products, NemaClean® 10% WP, DoubleNickel® LC, and SoilGard®, against *M. incognita* in tomato under controlled growth chamber conditions. Over a 60-days experimental period, all three products significantly reduced gall index, total egg number, and egg number per gram of root compared to the untreated inoculated control. NemaClean® demonstrated the strongest overall nematocidal efficacy and modestly promoted root biomass. DoubleNickel® LC exhibited comparable suppression to NemaClean® and additionally produced significant increases in both root and shoot fresh weight. SoilGard® provided intermediate nematode suppression with no significant effect on plant biomass. Our findings highlighted the promising potential of biocontrol products as components of sustainable strategies. Future studies should investigate the synergistic effects of combining these products, their compatibility with organic or mineral fertilizers, and their performance across soils varying in organic matters, which may influence microbial activity and, consequently biocontrol efficacy.

Diversification drives subsurface stability: Aspirational cropping systems enhance soil nematode diversity and food web stability

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Abstract Soil nematode communities serve as sensitive biological indicators of soil food web health, yet the pathways through which aspirational cropping system (ASP) - comprising diversified crop rotations, cover cropping, and no-till improve food web stability compared to business-as-usual (BAU) management remain poorly understood. This study investigated nematode community composition, functional diversity, and soil food web stability across four long-term agricultural trials (Illinois, Michigan, Nebraska, and North Dakota) in the United States, with 260 soil samples collected across two seasons (pre-plant and harvest) and 104 nematode genera identified. ASP cropping system significantly increased genera richness, Shannon diversity, and the relative abundance of omnivorous and predatory nematodes compared to BAU, while generating compositionally more heterogeneous and spatially distinct communities. Geography (state) was the dominant driver of community composition (26.9%), though treatment (1.2%) and season (5.7%) effects were ecologically relevant. Redundancy analysis and variation partitioning revealed that soil physicochemical properties, particularly aggregate stability, soil moisture, and labile carbon fractions were significant mediators of treatment-associated community shifts, with site age amplifying the explanatory power of soil variables, emphasizing legacy effect of management practices. Nematode-based indices, including the maturity index, structure index, and enrichment index, alongside a novel quantitative rhombus-based soil food web assessment, consistently indicated higher food web maturity and structural complexity under ASP. These findings demonstrate that ASP management progressively restructures soil nematode communities toward more mature, stable, and functionally complex food webs, with important implications for biological monitoring of regenerative agricultural systems.

Characterization of *Ditylenchus* species of alfalfa in Virginia

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Abstract Alfalfa (*Medicago sativa*), a critical forage crop for the dairy industry, is ranked among the top three field crops in 26 states and grown in all 50 states. In 2025, U.S. alfalfa production reached around 50 million tons, with Virginia producing 112,000 tons valued at \$24.5 million. However, plant-parasitic nematodes, particularly, *Ditylenchus* species poses a threat to alfalfa production in the Western U.S., but its role in the Eastern states is unknown. These nematodes reduce plant biomass and shorten the longevity of this perennial crop posing an economic risk to production. Despite the significance of this threat, a comprehensive survey of *Ditylenchus* species has not been conducted in over 70 years making its current impact and distribution unknown. To address this gap in our knowledge, we conducted a survey to identify the *Ditylenchus* species present in alfalfa in Virginia. This study evaluated the morphology and morphometric characteristics of *Ditylenchus* spp. detected in Pulaski County. The population was characterized by morphological features using a light microscope and measuring them with Adobe® Photoshop. They were characterized by a moderately long female body (1124.6 ± 147.7 (922.9–1302.5)), a short stylet (10.5 ± 0.5 (10.0–11.3)), a sharply pointed tail (85.9 ± 11.5 (69.6–99.4)), and male with a leptoderan bursa and curved spicules (21.2 ± 0.7 (20.0–21.7)). To confirm the identification, molecular characterization was conducted using the ITS region. The molecular results also confirmed that the population belongs to *Ditylenchus dipsaci*. The combined morphological, morphometric, and molecular evidence provides confirmation of *D. dipsaci* associated with alfalfa in Virginia. These findings contribute to improving our understanding of the distribution and identity of *Ditylenchus* species in alfalfa production systems in the eastern United States. This information is important for strengthening nematode diagnostics and supporting future studies on the biology, distribution, and management of *D. dipsaci* in Virginia forage systems.

Dose–response profiles of fluopyram and garlic-derived diallyl polysulfides against *Belonolaimus longicaudatus*

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Abstract Sting nematodes (*Belonolaimus longicaudatus*) are one of the most damaging plant-parasitic nematodes in Florida sandy soils, particularly in high-value crops, like strawberries, where management continues to rely on soil fumigation. In the past decade, the introduction of several new non-fumigant and biological nematicides has provided alternative options for growers. Fluopyram (Velum®), a succinate dehydrogenase inhibitor, is now commonly used by Florida strawberry growers as a post-plant nematicide for management of sting nematode. However, to reduce the risk of resistance development, it is important to identify other nematicides that can be rotated with fluopyram. This study evaluated the *in vitro* effects of fluopyram, and garlic-derived diallyl polysulfides (DAPS; NEMguard™) on sting nematodes, using motility inhibition and mortality as endpoints. Nematodes were exposed to a range of concentrations across four time points (6, 24, 48, and 96 h) in 48-well plate assays. Fluopyram was evaluated at 1–1,000 ppm, and DAPS at 100–10,000 ppm. Responses were analyzed using a binomial generalized linear model (GLM) with log₁₀-transformed concentration, and EC₅₀ (motility) and LC₅₀ (mortality) values were estimated when supported by the data. Fluopyram caused rapid motility inhibition, with EC₅₀ values below 1 ppm at 6 and 24 h. DAPS also produced strong motility inhibition; however, the concentration range did not extend low enough to estimate an EC₅₀. Both products showed significant mortality at the tested concentrations. Fluopyram showed limited mortality at 6 h (LC₅₀ > 1,000 ppm), with lethal effects increasing over time and reaching an LC₅₀ of 110 ppm at 24 h and of 5.8 ppm at 96 h. In contrast, DAPS exhibited rapid mortality, with substantial effects observed as early as 6 h (LC₅₀ = 463 ppm), and LC₅₀ values remaining stable over time (~386–501 ppm). In summary, fluopyram exhibited early effects on motility at low concentrations, followed by increasing mortality over time, while DAPS showed strong motility inhibition and rapid lethal activity at higher concentrations, with effects occurring as early as 6 h and remaining consistent over time. DAPS may provide a useful rotational or complementary tool for sting nematode management in Florida if biologically effective concentrations can be achieved under field conditions. This is currently being evaluated in field trials.

Reproduction of *Pratylenchus neglectus* on corn cultivars and development of a QPCR assay for its detection and quantification in corn roots

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Abstract root-lesion nematode, *Pratylenchus neglectus*, is a migratory endoparasitic nematode that infects roots and causes significant yield losses in corn. The nematode penetrates, feeds and reproduces within root tissues, and reproduction may vary among corn cultivars. Higher nematode abundance in roots is associated with greater root damage and potential yield loss. Traditional identification and quantification methods are time-consuming and labor-intensive. Therefore, the objective of this study was to evaluate reproduction of *P. neglectus* on corn cultivars and to develop a real-time quantitative PCR (qPCR) assay for rapid and direct detection and quantification of *P. neglectus* in corn roots. Nematode reproduction was evaluated on 11 corn cultivars in two greenhouse experiments arranged in a randomized complete block design with five replications in 1.5 kg of infested soil per pot containing 1,350 *P. neglectus*. Reproductive factor (Rf = final population/initial population) was used to quantify nematode reproduction. The qPCR assay was developed using primers targeting the ITS region, previously designed to detect and quantify *P. neglectus* in soil, and was re-evaluated for specificity to account for differences in sample preparation and the presence of other nematode genera and closely related species. Assay sensitivity was evaluated using two-fold serial dilutions of DNA from 0.2 g of root inoculated with four *P. neglectus*. The assay was validated by comparing qPCR estimates with manual counts across eleven corn cultivars. Reproduction of *P. neglectus* varied significantly among corn cultivars ($P < 0.0001$), with Rf values ranging from 2.0 to 7.3 in trial 1 and 2.4 to 6.7 in trial 2. The qPCR assay was specific to *P. neglectus*, with no amplification observed for other control nematode species. The detection limit of the assay was 0.125 nematode equivalent in 0.2 g of corn roots. Standard curve generated by plotting quantification cycle (Cq) values from qPCR against log-transformed nematode numbers inoculated into non-infected corn roots showed a strong linear relationship ($R^2 = 0.99$) with an amplification efficiency of 90.5%. Validation of standard curve using additional inoculation levels showed a high correlation ($R^2 = 0.97$) between qPCR estimates and actual numbers of *P. neglectus*. Validation across the 11 corn cultivars showed strong positive correlations between qPCR estimates and microscopic counts in the two experiments ($R^2 = 0.73$ and 0.77). In a separate time-course validation across five sampling time points using one susceptible and one resistant cultivar, qPCR estimates were positively correlated ($R^2 = 0.82$) with microscopic counts, and *P. neglectus* was detected in corn roots at 21 days after planting, the first sampling time. The findings of this study demonstrate that reproductive ability of *P. neglectus* varies among corn cultivars. The qPCR assay developed enables rapid and sensitive detection and quantification of *P. neglectus* directly from corn roots without prior nematode extraction and supports early detection of nematode populations and improved monitoring and management decisions in corn production.

Nematode diversity across an alkalinity gradient in the Nebraska Sandhills lakes

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Abstract Nematodes are the most abundant and diverse metazoans on Earth, offering strong potential as indicators in ecological and environmental studies. However, their diversity remains unevenly explored across habitat types, with freshwater systems receiving comparatively little attention. The Nebraska Sandhills is the largest intact temperate grassland, characterized by unique hydrology and more than 1,600 lakes spanning a wide range of alkalinity from neutral to extremely alkaline (pH 7–11). To contribute to the understanding of nematodes diversity in freshwater habitats, we evaluated nematode diversity in the Nebraska Sandhills lakes and hypothesized that nematode communities would become less diverse with increasing alkalinity. By using a dredge, four replicate sediment samples were collected from nine lakes spanning an alkalinity gradient. Nematode were extracted using sugar centrifugation, and their communities were characterized using 18S rRNA metabarcoding. Microbial diversity was also analyzed using 16S rRNA (bacteria) and 18S rRNA (fungi and protists) metabarcoding, alongside measurements of key biogeochemical parameters. To assess the potential role of microbial and biogeochemical drivers in shaping nematode communities, Generalized Linear Model was used to test for differences in alpha diversity (Richness and Shannon index), while Random Forest models were applied to identify the important predictors of alpha diversity. Compositional differences among communities were assessed using PERMANOVA, followed by distance-based redundancy analysis (dbRDA) using the same as above predictors. Nematodes were detected from eight of the nine lakes and 26 out of 36 samples, with no nematodes detected in Benjamin's Floyd Lake, the most alkaline (pH = 10.2) lake. In total, 22 taxa belonging to six trophic groups (animal parasites, bacterivores, fungivores, omnivores, herbivores and predators) were identified, with declining taxonomic and trophic diversity as alkalinity increased. Random Forest analysis identified microbial diversity as the strongest predictor of nematode diversity (bacterial diversity was the most important, followed by fungal diversity), while most biochemical variables showed low predictive importance, except for pH, which had a moderate but significant influence. The dbRDA model showed that combined biogeochemical factors significantly explained nematode composition variation. Permutation tests revealed that pH and Iron were the most important factors in shaping community structure, while other variables showed no significant individual effects. Our findings align with previous studies reporting declining nematode diversity with increasing pH and the relatively important role of abiotic variables, especially pH along with potential biotic interactions in nematode community diversity in the Sandhills lakes. Overall, biotic and abiotic factors contribute to community patterns, but fully understanding nematode ecological dynamics and specific interaction with microbial communities will require deeper investigation into the biological networks and species interactions that drive community assembly across freshwater systems. We will also explore the potential of Geographical Information System and Remote Sensing to understand the effect of lakes trophic status and the land-use on the nematode diversity.

Management of *Rotylenchulus reniformis* in sweetpotato using non-fumigant nematicides integrated with cover crops

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Abstract Reniform nematode (*Rotylenchulus reniformis*) is one of the most significant plant-parasitic nematodes that restricts the production of sweetpotato (*Ipomoea batatas* [L.]). Sweetpotato is an economically important crop in the US, especially in Mississippi, whereby soil borne pests often reduce the yield and root quality. The reniform nematodes can cause losses of yield up to 70 percent, but there are few management options available due to the lack of resistant cultivars and the increasing restrictions on fumigant nematicides. Therefore, to ensure sustainable management of reniform nematodes, there is a need to have integrated management strategies that involve the integration of cultural practices and non-fumigant nematicides. A field experiment was conducted at the Pontotoc Ridge- Flatwoods Branch Experiment Station in Mississippi in a field that was naturally infested field with *R. reniformis*. This study evaluated the combined effects of winter cover crops and non-fumigant nematicides on suppressing nematodes and the yield of sweetpotatoes. This experiment followed a split-plot randomized complete block design with four replications. Cover crop treatments included fallow, wheat (*Triticum aestivum*), winter pea (*Pisum sativum*), canola (*Brassica napus*), and a mixture of wheat, winter pea, and canola. The treatments of nematicides were of fluopyram (Velum Prime), oxamyl (Vydate), and an untreated control. Soil samples were collected before planting, at mid-season, and at harvest to quantify reniform nematode populations using the sucrose-centrifugal flotation technique. Agronomic measurements, including storage root grading and total and marketable yields, were also recorded during harvesting. Cover crop selection significantly influenced reniform nematode populations. Treatments with winter pea, canola, and mixed cover crops significantly decreased the density of reniform nematodes compared to fallow and wheat. Mid-season observations indicated a strong interaction between cover crops and nematicide treatments, with significant nematode suppression by fluopyram and oxamyl primarily occurring in plots previously planted with mixed cover crops. The effects of nematicides on nematode population were reduced by harvest, while cover crop-mediated suppression remained evident. Mixed cover crops maintained the lowest population of nematodes, whereas the fallow plots had the greatest population. Yield trends reflected nematode pressure across treatments. Mixed cover crops and winter pea numerically produced higher sweetpotato yields compared to the fallow and wheat. Nematicide applications improved yield primarily in higher nematode pressure systems. These results demonstrate that cover crop selection strongly influences *R. reniformis* population dynamics and can affect the performance of non-fumigant nematicides. Integrating biologically suppressive cover crops with non-fumigant nematicide applications may provide a sustainable strategy for managing reniform nematodes and improving sweetpotato productivity.

Cotton variety performance under reniform nematode pressure: Shifts in soil microbial communities and implications for yield prediction

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Abstract Plant parasitic nematodes pose a substantial threat to cotton production in the Southeastern United States. Second, to *Meloidogyne incognita*, *Rotylenchulus reniformis* is a significant cotton pest, causing close to 40 thousand bale losses in 2024. Evaluating variety performance under nematode pressure and characterizing associated changes in soil microbial communities is critical for integrated management strategies that optimize cotton production. During the 2025 growing season, 18 cotton varieties were planted in reniform nematode (RN) infested and non-infested fields, with and without in-furrow granular nematicide (aldicarb), to assess nematode resistance and yield potential. Nematicide treated varieties produced a 75% reduction in RN egg density (471 eggs/g root) compared to untreated varieties (1,924 eggs/g root; $P < 0.001$). Thirteen out of the 14 varieties showed no statistical difference in RN nematode egg density compared to the resistant cotton variety with the lowest egg density, DP 2141NR B3XF. Lint yields were significantly affected by both nematicide and variety, with nematicide treated plants producing an additional 213 lint kg/ha ($P < 0.0001$), and the top five producing varieties yielding between 1,327 and 1,797 lint kg/ha ($P < 0.0001$). Soil samples were collected throughout the season to characterize phospholipid fatty acid (PLFA) composition to 1) assess how treatments (untreated, nematicide treated, resistant, susceptible, and ThryvOn cotton), sampling time, and presence of RN influence soil microbial community structure and 2) if soil microbial composition could predict yield outcomes. The presence of *R. reniformis* nematodes significantly decreased total living microbial biomass ($-2,468.7$ ng/g; $P < 0.001$), actinomycetes (-363.82 ng/g; $P < 0.001$) and arbuscular mycorrhizae (AM; -92.5 ng/g; $P < 0.001$) PLFAs, as well as fatty acid saturation ratios (-2.83 ; $P < 0.05$). Functional group diversity index significantly decreased as the growing season progressed ($P < 0.001$), fatty acid saturation ratios significantly increased ($P < 0.01$) and cyclopropyl fatty acids saw a large decrease in week 5 followed by recovery at harvest ($P < 0.0001$). Linear discriminant analysis was used to evaluate whether yield outcomes could be predicted by functional groups, fatty acid composition, and total community metrics. Fatty acid composition ratios at planting and total community metrics from week 5 independently achieved 60% accuracy at predicting lint production outcomes, demonstrating a potential early, but practical, diagnostic tool for assessing yield potential in the presence of nematode pressure. This integrated approach combines variety evaluation, microbial community profiling, and predictive modeling provides insights into the importance of nematode management strategies and the role of soil microbial communities in cotton production systems. Our findings revealed *R. reniformis* nematode pressure significantly alters soil microbial communities and these changes are dynamic throughout the growing season, likely due to the life cycle and fluctuations in nematode populations as well as the developmental stage of the cotton variety. This could provide another tool, alongside traditional management methods, to maintain consistent lint production outcomes in the presence of nematode pressure.

Differential infection and developemnt of soybean cyst nematode on nonhost legumes

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Abstract *Heterodera glycines* (SCN) is the most economically important pathogen affecting soybean yield in the United States. The overuse of the popular SCN resistance genes (R-genes) in soybean, *Rhg1* and *Rhg4*, has resulted in the emergence of virulent SCN pathotypes that will require new sources of resistance to manage. Nonhost resistance is an understudied phenomenon, and R-genes involved in nonhost resistance could help develop new resistance traits in soybean. We hypothesized that nonhost legume species closely related to soybean may exhibit unique mechanisms of resistance against SCN. To test this, we inoculated two nonhost legumes, *Medicago truncatula* and *Vicia villosa*, alongside a susceptible soybean cultivar (Williams 82) with stage 2 juveniles (J2) of SCN and monitored their infection and development over time via acid fuchsin staining. At 3 days post inoculation (DPI), we observed a high rate of SCN penetration on both nonhost species, with no differences observed in nematode growth or development across the three species. At 8DPI, we began to see significant differences in SCN growth and development across the three host plant species with no nematode growth or development observed on *M. truncatula*, and no adult females observed at 21 or 41DPI. In contrast, SCN infecting *V. villosa* showed some growth and development at both 8DPI and 13DPI but did not produce adult females at 21 or 41 DPI. Interestingly, we observed several adult males develop on *V. villosa*, indicating SCN can establish a feeding site in this species. This investigation confirms the presence of several distinct mechanisms of resistance to SCN in nonhost legumes and justifies further research into the mechanisms of this resistance and the genes that underly this phenomenon.

Assessing peanut breeding lines for resistance to peanut root-knot nematode

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Abstract *Meloidogyne arenaria* (peanut root-knot nematode, PRKN) is a major pest in peanut production. Although highly resistant peanut cultivars have been developed and produced substantially greater yields than susceptible cultivars in heavily infested fields, limitation in yield potential and other agronomic characteristics have hindered widespread adoption. The objective of this study was to screen peanut breeding lines for resistance to PRKN and agronomic performance. This objective was investigated in greenhouse and field conditions using ‘FloRun 52N’ as the susceptible check in both trials, ‘TifNV-HighO/L’ as the resistant check in the greenhouse and ‘TifNV-HG’ as the resistant check in the field. The small plot field trial was conducted in 2025 in a North Florida field with a high PRKN load, averaging 1,080 PRKN/100 cm³ soil at planting. Yield and PRKN abundance in soil, roots and damage ratings were the primary assessment. The susceptible check had greater PRKN abundance than many of the breeding lines, while most lines tested were resistant, maintaining low PRKN levels in roots and soil at midseason and harvest. Several breeding lines reduced midseason PRKN abundances from roots by 92–99% and final PRKN soil abundances 94–98% relative to the susceptible check. In contrast, one line was susceptible to PRKN, supporting 89% higher PRKN root abundance at midseason and 71% higher PRKN soil abundance at harvest relative to the susceptible check. Only ‘TifNV-HG’ and one breeding line produced significantly greater yields than ‘FloRun 52N’, with an increase of approximately 1,682 kg/ha. All other lines yielded similarly to the susceptible check. The greenhouse trial was conducted in 2025 and plants were grown for 90 days after inoculation with 5,000 PRKN eggs per pot. PRKN infection on roots and growth parameters were measured at the end of the trial. The susceptible check had greater PRKN abundances in roots than many of the breeding lines and plant growth was similar among lines. In summary, several lines demonstrated high levels of resistance to PRKN. Among these, one line appears particularly promising as a potential commercial resistant cultivar, both greatly suppressing PRKN infection and increasing yield.

Papaya ground seed extract triggers induced systemic resistance against *Meloidogyne incognita* in tomato

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Abstract Southern root-knot nematode (*Meloidogyne incognita*) is an important plant-parasitic nematode affecting vegetable crops in Hawaii. Organic producers have limited management options and urgently need an alternative strategy to control this damaging pest. Papaya seeds are a locally available waste product from the cut fruit industry in Hawaii. It contains benzyl isothiocyanate, a compound similar to the active ingredient of the commercial fumigant Vapam, making it a potential biofumigant. While papaya ground seed and papaya seed extract (PE) are highly effective in suppressing *M. incognita* (Mi) based on several lab and greenhouse pot studies by various researchers, the mechanism of control has not been fully understood other than attributed to its biofumigation effect. We hypothesize that drenching PE could also suppress *M. incognita* by mediating soil microbes that trigger induced systemic resistance (ISR). Two greenhouse trials were conducted using ‘Orange Pixie’ tomato grew in sterile sand soil mix (Trial 1) or field soil (Trial 2). Each tomato was planted in two 10-cm-diameter pots by splitting its roots between them. Treatments included (1) drenching with 0.5% or (2) 1% PE, (3) untreated control with Mi inoculation, and (4) autoclaved soil (without Mi inoculation). One week after transplanting, PE was drenched on one side of the split-pots in PE treatments. PE drenching was repeated every 2 weeks until 1.5 months after planting. On the other side of the split-root system, 200 second-stage juveniles of *M. incognita* were inoculated 24 h after PE drenching for treatment 1 to 3. Root tissues were subsampled from the nematode-inoculated side at 24 and 48 h after the initial PE drench to extract RNA (using Zymo Quick-RNA Miniprep kit) and synthesize cDNA (using the ThermoFisher Verso cDNA synthesis kit). ISR-associated *ethylene response factor 1* (*ERF1*) and *MYC2 transcription factor* (*MYC2*) genes, and the *actin* housekeeping gene were targeted for gene expression using qPCR. At termination of the experiment (2 months after planting), plant growth were recorded, and Mi root infection was quantified using acid fuchsin-staining. Expression of *ERF1* and *MYC2* were only induced in Trial 2, confirming the hypothesis that ISR genes are mediated by soil microbes. At 24 hours, both ISR genes were overexpressed in the 0.5% (15-fold) and 1% (30- to 100-fold) PE treatments compared to the control ($P < 0.001$), triggered by PE. However, 1% PE drench reduced the number of mature females compared to the untreated controls in both trials (with and without field soils) but 1% PE only reduced egg masses in Trial 2 ($P \leq 0.05$). This study confirmed that PE drenching can trigger the expression of ISR-mediated defense against *M. incognita*, but there might be other genes being induced by PE that are not mediated by soil microbes that warrant further investigation. Future work will evaluate the efficacy of PE under in field conditions.

Integrated weed and nematode management in Hawai'i pastures

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Abstract Himalayan raspberry (*Rubus ellipticus*) is an ever-growing invasive threat to the pasturelands of the Island of Hawaii. It was introduced to the island in 1960 as an ornamental and for its edible fruit but quickly became a noxious weed by 1961. Their high seed production, easy spread by animals, and thorny stems and leaves make these weeds difficult to manage and nonpalatable for high-value livestock. Gastro-intestinal nematodes (GINs) are among the most serious problems affecting sheep and goats in the U.S. Widespread anthelmintic resistance in small ruminants is a global problem, and so it is in Hawaii. The objective of this study was to examine an integrated weed management strategy to mitigate *R. ellipticus* and GIN pressure, improve soil health, enhance the indigenous nematode-trapping fungi (NTF). In collaboration with a small-scale cattle rancher in Volcano, a field trial is installed to examine mechanical mowing with a skid steer mulcher, followed by the use of anaerobic soil disinfestation (ASD) technique, where a silage tarp was mulched over the mowed *Rubus* residues for 4 weeks. Anaerobic soil disinfestation has been proven to degrade weed seeds and reduce the weed seedbank in other ecosystems. It is hypothesized that returning organic mulch into the pasture will increase carbon content, microbial activity, and nutrient cycling. New growth of *Rubus* was monitored monthly over 9 months. Initial *Rubus* viability was reduced by ASD 1-month after tarping. Nematode-trapping fungi (NTF) are cosmopolitan soil microbes and play a role in reducing GINs in pastures. Soil collected from the treatment plots will be quantified for NTF colony forming units using three-series soil dilution (0.05, 0.005, and 0.0005) using ¼-strength cornmeal agar. Once nematode-trapping structures are detected, they will be transferred to full-strength cornmeal agar for culture prior to molecular identification. DNA extraction and molecular identification will be performed to confirm NTF identification. Subsequent NTF abundance will be monitored using real-time PCR targeting on variable regions of ITS1 and ITS2 rDNA in particular for *Duddingtonia flagrans* and *Arthrobotrys oligospora*. Nematode communities will be monitored at 3-month intervals. This research will potentially identify alternatives to intense chemical control against Himalayan raspberry while enhancing biological management against gastrointestinal nematodes in pasture systems.

Host-induced gene silencing of a stylet-secreted effector protein family involved in root-knot nematode feeding tube formation

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Abstract Root-knot nematodes (RKN, *Meloidogyne* spp.) are sedentary endoparasites with a wide host range and worldwide distribution that cause significant agricultural losses. All RKN species parasitize host plants by releasing stylet-secreted effector proteins originating from esophageal gland cells, two subventral and one dorsal gland, which contribute to the formation of specialized feeding cells called giant-cells. Once giant-cells are established, the adult RKN female produces a specialized tube-like structure called a feeding tube. Recently, we identified a novel *M. incognita* (Minc) stylet-secreted effector protein family (MincAF-SSE-F7) as a core component of RKN feeding tubes. To investigate the role of MincAF-SSE-F7 in feeding tube formation, RNAi constructs were designed to target different regions of *MincAF-SSE-F7* using a host-induced gene silencing approach. Sequences targeting these regions were cloned into a vector backbone as inverted repeats flanking an intron to form a hairpin structure for production of double-stranded RNA (dsRNA), under the control of either a constitutive promoter or RKN-inducible promoter active in giant-cells. These constructs were transformed into *Rhizobium rhizogenes* and used to generate composite plants in two host species, soybean and tomato. Preliminary bioassay results showed that silencing of *MincAF-SSE-F7* results in reduced fecundity of adult females, indicated by a decrease in the number of total eggs per plant. Additional biological replicates are underway, and silencing efficiency of *MincAF-SSE-F7* is being determined by quantification of transcript levels in adult females feeding on RNAi versus control roots. The ability to target a RKN feeding tube gene that is essential for adult female reproduction offers a novel target for developing crops with broad resistance to RKN species.

Evaluation of factors that impact emergence of *Anguina funesta* on Oregon annual ryegrass seed

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Abstract *Anguina funesta*, the seed gall nematode (SGN), infects annual ryegrass (ARG, *Lolium multiflorum*) grown for seed in Oregon. Zero tolerance for SGN detections in internationally shipped seed lots leads to significant rejections and a lack of market access for Oregon ARG seed producers. Understanding the environmental conditions that contribute to this nematode's successful infection of ARG in Oregon is critical to evaluating further management strategies. We conducted three studies to evaluate nematode emergence from galls under various temperatures and substrates. In the first experiment, galls both with (intact) and without (disassembled) the lemma and palea intact were evaluated. Individual galls were placed singly into a well of a 12-well cell culture plate containing 2 ml of 0.22 µm filter sterilized tap water, and incubated at 4, 10, and 15°C in growth chambers in complete darkness. Daily emergence was recorded and galls were moved to new wells containing sterile tap water until galls were dissected to determine total initial population density and final emergence percentage. Emergence was significantly influenced by the interaction of temperature and the physical state of the seed gall (intact or disassembled). Removal of the lemma and palea significantly increased emergence across all temperatures. Intact galls – the state in which galls occur in nature – exhibited a distinct thermal optimum for emergence at 10°C, reaching 50% emergence in 29.6 days. Incubation at 4°C and 15°C delayed time to 50% emergence to 50 to 70 days, respectively. The second experiment evaluated emergence in greenhouse soil wetted to field capacity for the life of the experiment at 10°C. Only intact galls were evaluated. Emergence data was collected every 3 to 4 day for 100 days following an initial 14-day incubation. The soil substrate significantly altered emergence at the thermal optimum of 10°C, requiring an additional 30 days to achieve 50% emergence. A final experiment investigated the influence of host and non-host root exudates on emergence. As in the first experiment, intact galls were submerged in 2 mL of filter-sterilized root exudate or water. Experiments were conducted using exudates collected at one, two, and three weeks of seedling age, and incubated at 10°C for the life of the study. Emergence data was recorded every 3–4 days for 52 days following an initial 14-day incubation period. Comparisons in emergence will be made across host and non-host exudates to see what influences, if any, are caused by root exudates. If there are differences, gas chromatography-mass spectrometry will be used to identify potential chemistries of interest for future study. Together, these studies provide critical biological information to help ARG growers time SGN management programs.

Validating pacbio long reads to characterize nematode biodiversity

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Abstract Third-generation sequencing is becoming increasingly popular in metabarcoding studies and is contributing to the characterization of previously understudied taxa. Nematodes are among the most abundant and diverse metazoans on Earth. However, their true diversity remains largely unknown, with only about 3% of species formally described. Here, we used PacBio long reads to partially amplify the nematode ribosomal RNA (rRNA) repeat and assess its potential for metabarcoding studies. Nematodes were extracted from sediment and soil samples and identified by light microscopy (i.e., to the family and/or genus level). Single specimens and pooled nematode worms were subjected to DNA extraction and PCR using a modified PacBio long-read protocol. Our primer set targeted an amplicon of approximately 4,000 bp, including the 18S rRNA, ITS-1, 5.8S, ITS-2, and D1–D3 domains of the 28S rRNA. A total of 39 samples were sequenced on a PacBio Sequel II system. PacBio circular consensus sequences (CCS) were analyzed with DADA2 to generate amplicon sequence variants (ASVs). In addition, we Sanger sequenced the 18S rRNA for a subset of samples and compared these sequences with the PacBio data. A total of 3,946,570 PacBio HiFi reads were generated in this study. Variation in read count (30–210,323 reads) and sequence length (1,486–4,009 bp) did not appear to be related to the number of specimens used for DNA extraction or to nematode taxonomy, unlike the number of ASVs recovered by DADA2 (1–500 ASVs). Taxonomic assignments based on the 18S rRNA showed that the most abundant ASV in a sample often matched the nematode taxon of interest. Furthermore, comparisons between 18S rRNA sequences generated by Sanger and PacBio sequencing showed little variation: 18, 9, 3, and 1 nematode samples had 0, 1, 2, and 3 base-pair differences, respectively, most of which were transitions. The use of PacBio long reads for studying nematode biodiversity is highly promising and builds on current molecular databases. We successfully amplified about 4,000 bp of the nematode rRNA repeat from single specimens collected from terrestrial and marine systems. By enabling amplification of a much larger fragment, PacBio long-read sequencing is likely to improve our understanding of nematode biodiversity and phylogeny. Future studies should test this method using mock communities and raw soil and sediment samples with increasing nematode diversity complexity.

Sweetpotato × *Meloidogyne enterolobii*: Validation of methodological parameters for phenotyping approaches

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Abstract Precise and reproducible phenotyping protocols are essential for advancing studies on sweetpotato resistance to *Meloidogyne enterolobii* and for supporting breeding programs. Previous studies conducted in Brazil identified optimized methodological parameters for this pathosystem. Using the susceptibility and resistance standards ‘Beauregard’ and ‘Blesbok’, respectively, an initial inoculum level of 2,000 eggs was found to be ideal for evaluating the reproduction factor (RF) at 70–90 days after inoculation (DAI), whereas 16,000 eggs allowed more robust differentiation in gall severity scores, based on the diagrammatic scale of Bridge & Page (1980) at 90 DAI. Additionally, a new scoring scale (ranging from 0 to 5) was proposed specifically for gall symptoms in sweetpotato roots, based on illustrative patterns. The objective of this study was to validate these parameters under experimental conditions at North Carolina State University (USA), using clones from its germplasm collection, including the globally recognized susceptible standard ‘Beauregard’ and the resistant standard ‘Tanzania’. Two inoculum levels (2,000 and 16,000 eggs + J2 per plant) were evaluated. Experiments were conducted in a greenhouse using a completely randomized design in a 23 × 2 factorial scheme (clones × inoculum levels), with four replications, with 600 mL pots and one plant per pot. Evaluations were performed at 90 DAI focusing on the RF, and gall severity, which were assessed using both the Bridge & Page scale and the newly proposed scale. Resistance classification was based on the RF according to the Oostenbrink (1966) criterion, while quantitative variation in severity among clones was also assessed. A Tweedie distribution model was used to estimate differences among genotypes, inoculum levels (2,000 and 16,000), and their interaction, with direct implications for selection in breeding programs. The clone ‘New Kawogo’ showed RF values of 135.25 at 2,000 and 15.72 at 16,000 inoculum levels, whereas ‘Tanzania’ showed RF values of 0.08 (2,000) and 0.01 (16,000). The high inoculum density (16,000) induced a saturation effect in the host–nematode system, reducing the phenotypic amplitude of RF and compressing differences among susceptible genotypes. In contrast, the 2,000-level provided greater resolution of quantitative variation, better revealing differential susceptibility among genotypes. A multivariate model integrating symptom scores, RF, and the number of nematodes per gram of root was used to generate a global ranking of host suitability among genotypes. The results confirm that the use of a 2,000-egg initial inoculum provides a more robust and reliable classification of sweetpotato host suitability to *M. enterolobii* across different environmental conditions and genotypes, representing an important contribution to nematological experimentation and sweetpotato breeding.

Enhancing cellulose sponge formulations of entomopathogenic nematodes for improved survival and efficacy

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Abstract Two critical factors influencing the success of entomopathogenic nematodes (EPNs) as biological control agents are their capacity for mass production and effective formulation for long-term efficacy. The infective juvenile (IJ) stage of EPNs is highly susceptible to environmental stressors, including low oxygen, elevated temperatures, desiccation, and ultraviolet (UV) radiation, which can significantly reduce IJ viability and infectivity. Two studies were conducted to evaluate formulation strategies to enhance EPN performance. In the first study, *Steinernema feltiae* IJs were formulated on two sponge types (cellulose and microfibre) with a filter paper control and stored for 14, 30, and 60 days. Sponge type significantly affected waxworm larval mortality ($p < 0.001$), with cellulose sponge resulting in high mortality (62%), followed by microfibre (46%) and filter paper control (26%). Storage duration also had a significant effect ($p < 0.05$) on waxworm larval mortality, although no interaction between sponge type and storage time was observed. In the second study, cellulose sponge was used to evaluate the effect of Barricade gel at 0%, 1%, and 2% as a protective coating under UV exposure at different timepoints. Barricade-treated EPNs maintained high levels of infectivity, with no significant differences among gel concentrations, while the untreated control exhibited significantly reduced mortality. These findings demonstrate that cellulose sponge is an effective formulation substrate and that gel-based protective coatings can enhance EPN survival and efficacy under UV stress. Together, these strategies offer practical approaches to improving the field performance of EPNs in sustainable pest management programs. Further optimization of formulation methods is necessary to address desiccation, ultraviolet radiation, and temperature protection to enhance the long-term EPN storage for biocontrol applications.

Real-time RT-qPCR detection of *Tobacco rattle virus* in *Paratrichodorus allius* and its association with corky ringspot disease in POTATO

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Abstract Corky ringspot disease (CRS), caused by *Tobacco rattle virus* (TRV) and vectored by the stubby-root nematode *Paratrichodorus allius*, is an important disease of potato. Even low numbers of viruliferous nematodes can induce tuber symptoms and reduce marketability. Currently available molecular assays for detecting TRV in the nematode vector lack sensitivity and take longer to produce results. Therefore, this study aimed to improve TRV detection in *P. allius* and to assess the accuracy of the molecular method by comparing its results with field CRS disease incidence. Soil samples were collected from a 2025 field trial on potato cv. Milva, which included seven nematicide-based treatment protocols and a non-treated control arranged in a randomized complete block design with four replications. A SYBR Green-based real-time RT-qPCR assay was developed using RNA from viruliferous *P. allius*, recovered from the control field plots exhibiting CRS symptoms. The assay was specific to TRV, with no amplification for non-target potato viruses, including *Potato mop-top virus*, *Potato leafroll virus*, *Potato virus Y*, and *Tomato spotted wilt virus*. Specificity was further supported by a single melt-curve peak at 81°C and amplicon sequence confirmation. The sensitivity of the assay was evaluated using RNA extracted from two viruliferous *P. allius* individuals as the starting quantity, followed by two-fold serial dilutions. It was able to detect TRV at levels equivalent to 1/16th of a single nematode. The standard curve, generated by plotting quantification cycle (Cq) values against the log-transformed dilution series, showed strong linearity ($R^2 = 0.985$) and an amplification efficiency of 102.9%. The assay was further applied to 14 *P. allius*-positive field samples from Minnesota and Alabama, of which two tested positive for TRV (Cq < 34). To evaluate the assay's accuracy, soil samples from 32 plots in the field trial were processed for nematode extraction. CRS incidence in potato tubers from the corresponding plots was assessed at harvest and 90 days after harvest (DAH). Eighteen samples contained *P. allius*, and single nematodes recovered from these samples were used for assay validation. Of these, three samples were obtained from plots where tubers showed no CRS symptoms. RT-qPCR Cq values were negatively correlated with CRS incidence at harvest ($r = -0.858$) and at 90 DAH ($r = -0.773$). RNA extracts from *P. allius* obtained from CRS-negative plots showed no amplification, whereas TRV was detected in nematodes from symptomatic plots. These results demonstrate that the developed assay is a rapid, sensitive, and specific method for detecting TRV in single *P. allius* and a useful tool for determining vector viruliferous status and supporting assessment of CRS risk in potato.

Aquatic nematodes from the Alkaline Lakes, Rivers, and streams of the nebraska sandhills

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Abstract The Nebraska Sandhills are the largest grass stabilized dune system in the Western Hemisphere. The Ogallala Aquifer, one of the world's largest aquifers underlies the Nebraska Sandhills. We have sampled the sediment from five rivers that originate in the sandhills and nine uniquely high-potassium alkaline lakes. Measurements of pH in most of the lakes range from 9.0–10.5. The lakes are fed by atmospheric precipitation and are not connected to the river systems. Nematodes in the family Tobrilidae are the predominant taxa in both river and lake systems. Overall, we have examined 445 specimens in the Nebraska Sandhills and another 147 in ponds and streams east of the sandhills region. Individual nematodes in the genera *Tobrilus*, *Neotobrilus*, *Semitobrilus*, and *Brevitobrilus* were characterized by morphology, 18S, and COI DNA barcodes. A COI maximum likelihood phylogenetic tree representing 309 tobrilid specimens produced clades structured by sediment pH and geographic location. *Neotobrilus* was the only genus found in the Alkaline Lakes when pH was above 9.5. The high pH tolerant *Neotobrilus* was not found in any of the sandhills rivers. Rivers and relatively low pH lakes (less than 9.0) were populated by clades representing *Tobrilus*, *Semitobrilus* and *Brevitobrilus*. *Tobrilus* was collected from Sandhills lakes of lower pH and were common in streams and ponds east of the Sandhills. These eastern *Tobrilus* were genetically distinct from their western counterparts. Similarly, *Semitobrilus*, a conspicuously morphologically larger genus than the other tobrilid genera found in Nebraska, was genetically distinct from its western counterpart. The COI phylogenetic tree of Tobrilidae included 14 clades with moderate or well-supported bootstrap values. Only a single clade of 21 *Semitobrilus* specimens included members from a Sandhills lake and a Sandhills river.

Development of a lamp assay for detection of *Pratylenchus penetrans*

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Abstract Root-lesion nematodes (RLN) species in the *Pratylenchus* genus are considered polyphagous and have a wide host range. However, each species has host preferences in which they cause considerably high yield reductions in cereal and vegetable crops. *Pratylenchus penetrans* is of major concern in potato-producing regions in the US, especially when accompanied by the soilborne fungus *Verticillium dahliae*, causing Potato Early Dying (PED). PED is a disease complex characterized by the early senescence of the plant and decreased tuber yield and quality. Soilborne pathogenic fungi in the *Verticillium* genus that cause Verticillium Wilt are known to be the main pathogens of PED, specifically *V. dahliae*. However, disease severity is exacerbated when *P. penetrans* and *V. dahliae* are simultaneously present in the field. Although reports demonstrate this seemingly synergistic interaction between both pathogens, the same symptom exacerbation does not occur when *V. dahliae* is present alongside other species of the *Pratylenchus* genus. Methods used for early detection of PED from field samples often rely on non-species-specific RLN counts and presence of *Verticillium* DNA per grams of soil or by spore counts. However, as this disease complex is species specific, identifying the nematode to the species level is essential to predict PED occurrence in the field. Lack of specificity in detection methods could result in growers adopting unnecessary management practices, such as fumigation, even when the correct combination of pathogen species is not present. To provide accurate and timely PED prediction tools, a Loop-mediated isothermal Amplification (LAMP) assay to speciate *P. penetrans* was developed. LAMP happens at a single temperature, requires only LAMP reagents and primers, and results can be seen between 15–60 minutes, making it an affordable and quick assay. This detection method is easily adoptable with minimal investment and effort to enhance the accuracy of PED diagnoses in support of management recommendations.

Meloidogyne hapla strains resulting in differential host gene expression

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Abstract Genetic differences, such as resistance, in plant hosts are widely utilized to defend against plant pathogens, including parasitic nematodes. However, the impact of nematode genotype, its adaptability to host defense mechanisms, and its potential for plant developmental reprogramming is still unclear. Root-knot nematodes demonstrate considerable genetic diversity between and within species. Among them, *Meloidogyne hapla* presents a unique opportunity to study intraspecific genetic variation and its phenotypic responses from its hosts due to its small genome size and facultative meiotic parthenogeny mode of reproduction which allowed to generate recombinant inbred lines in F2 generation. Two strains of *M. hapla* VW9 and LM elicit markedly different phenotypic responses in their hosts. VW9 infected hosts characteristically develop smaller, less prominent galls, whereas LM infected hosts produce larger, prominent galls. Moreover, the transcriptomic profile induced by these two strains differ significantly with differential gene expression patterns observed consistently across multiple plant species. This phenomenon has been strongly correlated to a single locus in their genome known as host expression modulator (HEM1). In addition, plant host MADS-box transcription factors, which are normally expressed in flowers and play critical roles in reproduction, are ectopically expressed in RKN galls and had strikingly different expression levels dependent on the *M. hapla* genotype. Here, we identify genes in HEM1 locus responsible for modulating ectopic host gene expression and functionally characterize them with spatio-temporal expression profiles and reverse genetic approaches. This work addresses how genetic diversity within single nematode species generates differential host response advancing our understanding of the basis of nematode-host interactions.

Bacterial colonization of steinernema hermaphroditum nematodes

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Abstract Steinernema entomopathogenic nematodes and their *Xenorhabdus* bacterial symbionts infect and kill insects, defending and utilizing the cadaver as a food source. When nutrients in the cadaver run low and nematode densities are high, the bacteria re-colonize the nematodes in a tissue called the pharyngeal intestinal valve and the nematodes vector the bacteria to a new insect host. However, how nematode hosts recognize and select for their cognate symbionts, while excluding other bacteria is not well understood outside of the most well studied *S. carpocapsae*-*X. nematophila* host symbiont pair. We have characterized multiple aspects of this transmission process in *S. hermaphroditum* nematodes. Like other Steinernema nematodes, *S. hermaphroditum* are specifically colonized by *Xenorhabdus* strains of their cognate species *X. griffinae*, and not by other *Xenorhabdus* species. In contrast to *S. carpocapsae*-*X. nematophila*, the *S. hermaphroditum* pharyngeal intestinal valve is often colonized by multiple *X. griffinae* bacteria, ultimately resulting in *S. hermaphroditum* infective juveniles colonized by a non-clonal population of bacterial symbionts. Finally, we tested whether bacterial factors known to be important for *X. nematophila* colonization of *S. carpocapsae* are also necessary in *X. griffinae* and *S. hermaphroditum*. Loss of the feast or famine transcription factor *lrp* impacted the growth and emergence of *S. hermaphroditum* infective juveniles. Deletion of the sigma factor *rpoS* resulted in normal development and emergence, but a complete loss of the ability of the *X. griffinae* bacteria to colonize *S. hermaphroditum* nematodes. This suggests that *rpoS* may play a conserved role in regulating genes that promote colonization in *Xenorhabdus*. These findings serve as a foundation for our ongoing exploration of the conserved and novel molecular and cellular mechanisms that underpin the transmission process in *S. hermaphroditum*-*X. griffinae*.

Linking roots and shoots: Mechanisms of multitrophic interactions above and below ground

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Abstract Successful *Glycine max* (soybean) farming plays an integral part in today's economy with the soybean industry generates billions of dollars annually. However, crop pests substantially reduce yield and profitability. *Meloidogyne incognita* (southern root knot nematode; SRKN) is a major belowground pest of soybean, causing an estimated \$140 million in losses in 2024, with particularly severe impacts in southern soybean producing states. Soybean plants are frequently exposed to multiple stressors under field conditions, including below- and aboveground pests. *Chrysodeixis includens* (soybean loopers; SBL) is another major soybean pest—this defoliating insect cause approximately \$45 million in soybean yield losses in 2024. Notably, SBL infestations often temporally overlap with periods of active SRKN infection and reproduction, creating the potential for plant-mediated interactions between above- and belowground pests. Despite this overlap, the mechanisms underlying these interactions remain poorly understood. This study investigated above- and belowground multitrophic interactions in soybean using physiological, morphological and pest performance assays. Using the soybean cultivar, Magellan, plants were subjected to one of four treatments: SRKN challenge, SBL challenge, combined SRKN and SBL challenge, or an untreated control. To assess the importance of pest attack sequence, two experiments were conducted: one in which plants were first challenged with SBL prior to SRKN and a second in which plants were challenged with SRKN prior to SBL. Prior exposure to SBL herbivory was associated with a trend toward reduced SRKN infection. Pest stress altered plant morphological and physiological traits across treatments. The effects of SRKN on SBL performance were also evaluated. Early SBL growth was not significantly affected by plant treatment; however, trends emerged at later developmental stages. In the reverse sequence experiment, plant fresh weight was not significantly affected by treatment; however, volatile analysis conducted at vegetative stages revealed that plants exposed to any pest challenge exhibited changes in volatile profiles and concentrations. Additionally, SRKN produced fewer eggs on plants that experienced extended SBL pressure. Collectively, these results suggest that above- and belowground soybean pests can indirectly influence one another through plant-mediated mechanisms and that pest attack sequence plays an important role in shaping plant responses and pest performance. While further data analysis is required, this study highlights the importance of considering multispecies interactions and attack sequence when examining pest impacts on crop systems.

Leveraging resistance breaking behavior in root-knot nematodes to enhance the durability of resistance in pepper

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Abstract Root-knot nematodes (RKN), particularly *Meloidogyne incognita*, represent a major agricultural challenge for pepper (*Capsicum spp.*) cultivation worldwide, causing substantial yield losses due to root damage. The resistance genes *N*, *Me1* and *Me3* have historically provided protection against RKN in many pepper varieties. However, the emergence of virulent *M. incognita* populations capable of overcoming (a.k.a. “breaking”) these resistance genes pose a critical threat to sustainable pepper production. To better understand how *M. incognita* can break these resistance genes we used artificial selection in a laboratory environment to select break strains against all three pepper resistance genes. Consistent with previous reports, we selected multiple independent resistance breaking strains of *M. incognita* against the *N* and *Me3* genes, but could not develop the one that broke the *Me1* gene. We then conducted bioassays to understand how the virulence profiles of these different break strains had changed compared to the unselected populations. In an unexpected twist, virulent isolates of *M. incognita* that break resistance conferred by *N* genes also break *Me3* resistance and vice versa but none of these two break strains could break the *Me1* gene. In an effort to increase the durability of RKN resistance in pepper we crossed two pepper lines with complementary resistance gene HDA149 (*Me3* gene) and HDA330 (*Me1* gene) with the goal of stacking these two genes into a single pepper line. To design genetic markers in this highly repetitive region of the pepper genome, we sequenced and assembled two phased genome assemblies from both lines and discovered large changes within this region of the pepper genome that have implications into how these RKN resistance genes have evolved and how they can be utilized in breeding programs. We will present new data on how RKN is able to overcome these major resistance genes in pepper, and how we can use this behavior to inform future resistance breeding efforts.

Screening of the USDA sweetpotato germplasm collection for resistance to two root-knot nematode species *Meloidogyne enterolobii* and *M. incognita*

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Abstract Sweetpotato, a vital staple crop, faces major threats from root-knot nematodes (RKNs), particularly the southern root-knot nematode (*Meloidogyne incognita*) and the invasive guava root-knot nematode (*Meloidogyne enterolobii*) in the southeastern U.S. These pests cause substantial yield reduction and render storage roots unmarketable. While host resistance is the most economical and effective management strategy, sweetpotato cultivars combining consumer-desirable characteristics with RKN resistance are scarce, especially given the aggressive nature of *M. enterolobii*. To address this, we conducted a screening assay to identify sources of resistance to both *M. incognita* and *M. enterolobii* for future breeding efforts. We screened 89 sweetpotato accessions for *M. enterolobii* resistance and 83 accessions for *M. incognita* resistance from the U.S. Department of Agriculture germplasm repository using replicated greenhouse pathogenicity assays. ‘Beauregard’ served as the susceptible control, and ‘Regal’ as the resistant control. Rooted vine cuttings were arranged in a randomized complete block design and inoculated with 10,000 *M. enterolobii* eggs. After eight weeks, plants were harvested and assessed for root galling, and nematode eggs per gram of dried root. This comprehensive evaluation successfully identified around 20% and 54% of the screened accessions that exhibited resistance to *M. enterolobii* and *M. incognita* respectively, offering crucial genetic material to develop more resilient sweetpotato varieties.

Addressing the emerging threat of *Meloidogyne partityla* in georgia pecan orchards: Diagnostic assay development and nematicide evaluation

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Abstract Pecan, *Carya illinoensis*, is one of the most economically important tree nut crops in the United States, and Georgia contributes about one-third of total U.S. pecan production. In recent years, the pecan root-knot nematode (RKN), *Meloidogyne partityla*, has become an emerging threat to pecan production and is now considered the dominant RKN in pecan orchards in Georgia. Effective management of this nematode depends on both accurate detection and reliable management strategies. Therefore, this study was conducted to develop a recombinase polymerase amplification (RPA) assay for rapid detection of *M. partityla* and to evaluate the effectiveness of chemical nematicides for its management in commercial pecan orchards. For the RPA assay, primers and probes were developed targeting the internal transcribed spacer region (ITS) and tested for both sensitivity and specificity. The developed assay was able to detect DNA from a single adult female at 39°C in 20 min using the AmplifyRP XRT + Kit. It successfully amplified the target species and detected DNA at levels as low as 1/100 of a single female nematode. In addition, it showed high specificity, with no amplification observed for other non-target *Meloidogyne* species, including *M. incognita*, *M. arenaria*, *M. enterolobii*, and *M. floridensis*. Following accurate detection of *M. partityla*, chemical nematicides, including fumigants and non-fumigant products, are one of the most practical strategies for managing RKN in commercial pecan orchards. To evaluate the effectiveness of these nematicides against RKN, a field experiment was established in a commercial grower's orchard in Houston County, Georgia. A total of eleven treatments, consisting of fumigants Telone (1,3-dichloropropene) and Telone C-35 (65% 1,3-dichloropropene + 35% chloropicrin), the non-fumigant nematicides Velum (fluopyram), Nimitz (flusulfone), and Movento (spirotetramat), and their combinations, were arranged in a randomized complete block design. Fumigants were applied only as preplant treatments, whereas non-fumigant nematicides were applied annually over a three-year period from 2019 through 2022. Plant vigor and RKN population densities were recorded from 2022 to 2025. Based on pooled data across years, preplant application of the fumigant Telone followed by the non-fumigant nematicide Velum applied at planting and later drenched through microjets, was the most effective treatment. Relative to the untreated control, trees treated with Telone followed by Velum showed up to 95% decrease in *M. partityla* densities. Tree vigor ratings on a 0–10 scale further showed that all nematicide treatments improved tree growth compared with the untreated control, with the exception of Nimitz. These findings show that the RPA assay can reliably detect *M. partityla* and highlight the potential of integrating fumigant and non-fumigant nematicides for effective nematode management in commercial pecan orchards.

A rapid screening pipeline for identifying and predicting microbes that affect egg hatching in root-knot nematodes

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Abstract Root-knot nematodes (RKN; *Meloidogyne* spp.) cause billions of dollars in annual economic losses to US agriculture. Chemical control options remain extremely limited, and bio-based alternatives are in high demand. However, traditional screening for root- or soil-associated bacteria that suppress nematode eggs or juveniles has yielded few promising candidates, typically limited to *Bacillus* and *Pseudomonas* species. Additionally, nematode extraction from plant material for suppression studies is labor-intensive and time-consuming, thus becoming a major bottleneck. We hypothesized that the mechanisms underlying bacterial suppression of RKN are linked to their defenses against microbial predation. To investigate this, we selected 20 rhizosphere bacterial isolates that thrive under high predation pressure in co-culture with 10 protist species, then tested their effects on RKN egg hatching. Fourteen (70%) of these predator-resistant isolates significantly altered nematode hatching rates: eight reduced or abolished hatching, while six increased it, a rarely studied but potentially important phenomenon. We analyzed whole genomes of these bacteria using KEGG pathway prediction and AntiSMASH to determine if there were predictive patterns of genes previously used to screen for nematode suppression. Chitinase gene homologs were predicted in four of the six bacteria that inhibited hatching, while two other inhibitory isolates lacked a chitinase gene. At least two periplasmic protease genes were predicted in each genome, regardless of suppressive or induction activity, and no clear non-ribosomal peptide synthetase gene clusters were found. No combination of chitinase or protease genes reliably separated hatch-suppressing strains from nonsuppressive strains. To further probe the bacterial mechanisms affecting egg hatching, we evaluated the impact of nine selected hatch-active bacteria on *Colpoda inflata* (maize root isolate UC22), a ciliate abundant in the rhizosphere whose cysts share structural similarities with RKN eggs. One hundred freshly formed *Colpoda* cysts were incubated with nine bacterial isolates. Four of the five isolates that suppressed RKN hatching also significantly suppressed protist growth compared to controls, suggesting delayed or inhibited excystment. All three isolates that enhanced RKN hatching also increased protist growth. These findings indicate that similar bacterial mechanisms may regulate both protist excystment and nematode hatching. Our results suggest that protist predation yields a higher frequency of both RKN hatch suppressors and enhancers than is typically found in soil. This work lays the foundation for a rapid, effective screening pipeline to discover bacteria that modulate nematode hatching, offering new mechanistic and ecological insights into both inhibition and enhancement of RKN emergence.

Evaluation of cover crops on population dynamics of soybean cyst nematode (*Heterodera glycines*)

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Abstract Microplot experiments were conducted in 2024 and 2025 to evaluate the effects of cover crops on the population dynamics of the soybean cyst nematode (SCN, *Heterodera glycines*), a major soybean pest. Four cover crop species, oilseed radish (*Raphanus sativus*), cereal rye (*Secale cereale*), white mustard (*Sinapis alba*), and red clover (*Trifolium pratense*), along with their combinations with SCN trap soybean (*Glycine max*) were evaluated in soil naturally infested with SCN populations from Michigan fields. Crops were grown in outdoor microplots located at Michigan State University Entomology farm (38.1-cm diameter) beginning in early November. Soil samples were collected from each microplot prior to the new season before soybean planting in early June, and SCN cysts and eggs were quantified to determine the population reproduction factor (RF). Across the study, SCN population densities were not significantly reduced by any cover crop treatment or their combinations with trap soybean compared with the non-planted (fallow) control. However, numerically lower final SCN population densities were observed in treatments with white mustard and its combination with trap soybean for both cysts (RF = 0.16–0.86) and eggs (RF = 0.002–0.55). In contrast, oilseed radish (RF cyst = 0.85–0.95; RF egg = 0.02–0.52) and cereal rye (RF cyst = 0.95–1.03; RF egg = 0.20–0.53) generally resulted in equal or higher SCN population densities compared with the non-planted control (RF cyst = 0.38–0.73; RF egg = 0.01–0.68). Following cover crop termination and soybean planting, white mustard treatments resulted in significantly fewer SCN females on soybean compared with other treatments, indicating reduced nematode reproduction. Soybean following red clover also showed significantly higher shoot weight (53.17 ± 15.16 g/plant) and pod weight (31.97 ± 9.81 g/plant) compared with the control, likely due to improved nitrogen availability associated with biological nitrogen fixation. Overall, although none of the cover crop treatments consistently suppressed SCN populations across all measurements, white mustard showed a consistent trend of reduced nematode reproduction, suggesting potential value as a component in integrated SCN management strategies rather than as a stand-alone control method.

Determining threshold levels for root lesion nematodes (*Pratylenchus penetrans* and *P. crenatus*) in carrot production

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Abstract The root lesion nematodes (*Pratylenchus penetrans* and *P. crenatus*) are among the most damaging plant-parasitic nematodes in carrot production, causing yield loss, root necrosis, and deformation. Predicting damage associated with nematode population density is critical for establishing economic thresholds and guiding management decisions; however, threshold levels for these species in carrot production are not well defined. Microplot experiments were conducted at Michigan State University during the 2023–2025 growing seasons to determine population thresholds under controlled inoculation levels. Field soils were collected from two distinct sites naturally infested with *P. penetrans* and *P. crenatus*, respectively, and were used as inoculum sources. To maintain and increase nematode population densities, soils were cultured under greenhouse conditions using ‘Cupar’ carrot (*Daucus carota*) as a host prior to use in experiments. Five inoculation levels (0, 10, 50, 100, and 250 nematodes per 100 cm³ soil) were evaluated in a randomized complete block design with six replicates for each species. Total carrot biomass was not significantly affected by nematode density; however, marketable yield decreased with increasing inoculum levels. For *P. penetrans*, significant reductions were observed at 50 nematodes per 100 cm³ soil and higher, with average marketable yield reductions of 36.4%, 44.9%, and 65.3% at 50, 100, and 250 nematodes per 100 cm³ soil, respectively. A similar pattern was observed for *P. crenatus*, with reductions of 38.7%, 80.1%, and 83.7% at the same inoculum levels. These results indicate that 50 nematodes per 100 cm³ soil represents a preliminary economic threshold for both species, providing a practical guideline for nematode management in carrot production.

Evaluation of compost amendments for plant-parasitic nematode suppression and soil health in potato fields

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Abstract Michigan ranks eighth in the nation with more than 923,100 potato acres and an estimated \$4.6 billion farm-gate value. Plant-parasitic nematodes contribute to economic losses annually within the state's \$104.7 billion food and agriculture industry. In potato production systems, the root-lesion nematode, *Pratylenchus penetrans*, can interact with the wilt-inducing fungus, *Verticillium dahliae*, resulting in Potato Early Die, which damages root systems, reduces photosynthetic capacity, and decreases yield by 30–50%. Intensive management practices, including soil fumigation and nematicide applications, are commonly used but may negatively affect soil health, microbial diversity, and long-term soil productivity. Compost-based amendments have been increasingly explored as sustainable alternatives with potential to suppress soil-borne pathogens while improving soil biological activity. Therefore, the objective of this study was to evaluate the effects of compost amendments on plant-parasitic and free-living nematode populations and their implications for soil health in potato production systems. A field trial was conducted during the 2024 and 2025 growing seasons in a commercial potato field in southwestern Michigan, USA (41.76047, -85.51287) under a history of Potato Early Die. A randomized complete block design was used with six treatments and four replicates per treatment, including compost applied in spring, fall, and both seasons, Vapam fumigation, a chemical standard (oxamyl; Vydate), and an untreated control. Certified disease-free potato seed cv. Russet Norkotah was planted under standard agronomic practices. Soil samples were collected at early, mid-season, and harvest. Nematodes were extracted using a modified centrifugal flotation method, identified to genus or species level, and quantified. Population dynamics were expressed as reproduction factors (RF = final/initial population). Results showed that compost-treated soils supported higher free-living nematode reproduction (RF = 2.02 ± 0.40 – 3.46 ± 1.07), with the highest values in compost-fall (RF = 3.46 ± 1.07), compared with the untreated control (RF = 2.19 ± 1.08). Vapam fumigation showed intermediate reproduction (RF = 2.51 ± 0.60), while the chemical standard (Vydate) had the lowest RF (1.59 ± 0.37). However, differences among treatments were not statistically significant. Among treatments, only Vapam fumigation significantly reduced plant-parasitic nematode reproduction (RF = 0.24 ± 0.04), representing approximately 63% reduction compared with the untreated control (RF = 0.64 ± 0.12). Compost amendments resulted in moderate reductions (RF = 0.48 ± 0.11 – 0.59 ± 0.09 ; 7–26%). Specifically, root-lesion nematode *P. penetrans* reproduction was significantly reduced only by Vapam fumigation (RF = 0.08 ± 0.03), corresponding to > 90% reduction compared with the untreated control (RF = 0.80 ± 0.34). Compost treatments showed intermediate suppression (RF = 0.28 ± 0.07 – 0.38 ± 0.14) but were not statistically different from the control. Overall, compost amendments moderately suppressed plant-parasitic nematodes while supporting free-living nematode communities, indicating potential benefits for soil health enhancement, whereas chemical fumigation remained the most effective treatment for rapid nematode control.

Sheet mulching for nematode community, weed management and soil health improvement in a taro agroecosystem

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Abstract Resilient agriculture aims to reduce reliance on external input by sourcing resources within the farm. Sheet mulching layers natural materials to suppress weeds, retain soil moisture, and add organic matter, while improving soil health. The objective of this project was to evaluate the effects of sheet mulching using the fast-growing legume pigeon pea (*Cajanus cajan*) in a dryland taro cultivation system in Waianae, Hawaii. A field trial was conducted in 2024–2025 (Trial I) by comparing taro planted with sheet mulch (SM) vs air-impermeable synthetic mulch (no sheet mulch, NM). To prepare the SM plot, pigeon pea was clipped from the border rows and shredded into green mulch using a wood-chipper and layered on the soil surface between taro plants. On the side of the taro rows, cardboard was used to cover mushroom compost waste. Each treatment was consisted of 6 replications. Taro was grown for 7 months and harvested. The experiment was repeated in the same area in 2025–2026 (Trial II). In Trial I, SM significantly suppressed the abundance of root-knot nematodes (*Meloidogyne* spp.), increased the nematode enrichment index (EI) and abundance of predatory nematodes ($P \leq 0.05$), indicating a healthy bottom-up support soil food web. Taro corm weight was significantly increased by SM compared to NM ($P \leq 0.05$). In addition, SM reduced soil salinity and increased microbial soil respiration numerically and soil labile ammonia-nitrogen (SLAN, $P \leq 0.05$) over the entire 7-month period. SM also significantly reduced weeding time up to 4 months after taro planting, which was during the critical weed-free period. Surprisingly, volumetric soil moisture was reduced in the SM plots ($P \leq 0.05$). In Trial II, data were collected up to 3 months after taro planting. At this time point, SM numerically reduced the abundance of reniform nematodes (*Rotylenchulus reniformis*) and spiral nematodes (*Helicotylenchus* sp.), but it also reduced EI significantly ($P \leq 0.05$). On the other hand, SM increased the abundance of predatory nematodes by 70%. In terms of soil physical properties, SM continued to decrease soil salinity ($P > 0.05$), increased soil microbial respiration ($P \leq 0.05$) and SLAN ($P > 0.05$). Unfortunately, due to unexpected weather events, reapplication of sheet mulch was not timely executed, and weed pressure was slightly increased in SM plots. Nonetheless, sheet mulching combined with pigeon pea enhanced soil microbiome activity and increased the abundance of predatory nematodes, both of which could contribute to reducing plant-parasitic nematodes in both trials. Ability of sheet mulching to increase SLAN, which is an organic plant-available form of nitrogen resulted in higher taro yield. Benefits of pigeon pea sheet mulching in reducing soil salinity are encouraging for agriculture on the Waianae Coast, as this provides a low-input approach to managing the salinity stress that is increasingly threatening this key farming area in Hawaii.

Nematocidal efficacy testing of Aveo EZ[®] Nematicide against *Meloidogyne incognita*

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Abstract Aveo EZ[®] Nematicide, containing the active ingredient *Bacillus amyloliquefaciens* strain PTA-4838, is registered as a seed treatment for control of root knot, soybean cyst, and reniform nematodes. A series of *in vitro* experiments were performed to explore efficacy of this biocontrol microbe against multiple nematode life stages. Egg hatch inhibition and contact mortality assays using second-stage juveniles (J2) were conducted with *Meloidogyne incognita*. For each assay, approximately 100 eggs or juveniles (J2) were suspended in 1 mL of actively growing PTA-4838 cells, and this mixture was stored for the duration of the assay at 25 °C in the dark with shaking at 200 rpm. Egg hatching was assessed at four-day intervals for a total of 14 days, while juvenile mortality was evaluated daily for four days. PTA-4838 applied at concentrations ranging from 1×10^5 to 1×10^7 CFU/mL significantly reduced *M. incognita* egg hatch for up to 14 days post-exposure and caused significant *M. incognita* J2 mortality between 1 and 4 days post-exposure. These results illustrate the varied impacts of PTA-4838 across several nematode life stages and provide rationale for the benefits observed from Aveo EZ[®] Nematicide in field environments.

Functional characterization of a syncytium-localized putative effector (*HgSPE2*) in the late phase of soybean cyst nematode parasitism

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Abstract The soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging agricultural pest of soybean in the United States, with yield losses exceeding \$1 billion annually. As a sedentary endoparasite that creates a prolonged biotrophic relationship with its host plant, SCN deploys secreted effector proteins to facilitate parasitism and development. These effectors play critical roles in suppressing plant immune responses and/or manipulating host cells to generate a permanent feeding site, called a syncytium. Given that cyst nematode genomes contain hundreds of predicted effectors, identification of functionally relevant virulent proteins delivered into plant cells during parasitism remains challenging. Proteomic analysis of the beet cyst nematode (*Heterodera schachtii*) nematode feeding sites in *Arabidopsis* 10 days post-infection (dpi) led to the identification of a short list of syncytium-localized putative nematode effectors (SPEs). SPEs were predicted based on the presence of an N-terminal signal peptide, absence of a transmembrane domain, conservation among cyst nematodes, and lack of functional annotation or known roles in plant parasitism. Using these criteria, we selected SPE2 for further characterization. Although SPE2 was identified in the *H. schachtii*–*Arabidopsis* system, the *H. glycines* ortholog shares > 96% amino acid identity, and we hypothesize that it likely plays a similar role in cyst nematode parasitism. *In-silico* functional domain analyses of *HgSPE2* revealed a conserved calycin motif found in fatty acid-binding proteins (FABPs), indicating a potential role in lipid metabolism. Although fatty acid- and retinoid-binding effectors (FARs) are well-characterized in nematode parasitism, *HgSPE2* shares less than 20% sequence identity with *HgFAR*, and its β -barrels structure differs markedly from the α -helical architecture of FARs, suggesting a novel function. Gene expression profiling across developmental stages revealed uniquely elevated *HgSPE2* expression in females, supporting a role beyond early host infection. Subcellular localization analysis in *Nicotiana benthamiana* leaves placed the GFP-fused effector in both the nucleus and cytoplasm. Consistent with predicted function in nematode metabolism, *HgSPE2* did not suppress *Gpa2/RBP1*-mediated hypersensitive cell death, a hallmark of activated plant defenses, indicating that *HgSPE2* is unlikely to inhibit plant immunity. For further functional characterization, transgenic soybean hairy roots expressing GFP-fused *HgSPE2* were generated, and transgene expression was confirmed by Western blot analysis using an anti-GFP antibody. GFP-*HgSPE2*-transformed roots were then used for SCN bioassays to confirm contribution to nematode virulence and co-immunoprecipitation coupled with mass spectrometry (Co-IP/MS) to identify host protein targets of *HgSPE2*.

High-resolution genome assembly and linkage mapping in *Meloidogyne hapla* reveal non-canonical telomere repeats and recombination hotspots associated with effector proteins

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Abstract Root-knot nematodes cause significant yield losses across diverse crops. *Meloidogyne hapla* is a valuable model for studying root-knot nematodes due to its small diploid genome and reproductive strategy that facilitates genetic analysis. We report the most contiguous genome assembly to date for any plant-parasitic nematode, built using PacBio HiFi, Oxford Nanopore, Illumina, and Hi-C sequencing. Genetic linkage analysis of F2 populations derived from crosses between *M. hapla* strains validated the assembly but revealed anomalies indicating chromosome structure differences between parental isolates such as fissions, fusions, and rearrangements. Strikingly, we identified sharply delimited zones with extraordinarily high recombination on most chromosomes. Several of these high recombination zones were significantly enriched for genes encoding secreted proteins that contribute to parasitism. These findings suggest that meiotic recombination facilitates effector diversification and offer insight into how these parasites diversify their effector repertoire to expand their extraordinary host range. We further report the discovery of a novel 16-nucleotide tandem repeat and lack of canonical telomere repeats at chromosome ends. The localization of this 16-nt repeat at chromosome ends highlights a potentially divergent mechanism of chromosome-end maintenance. Overall, our study integrates high-resolution structural genomics, genetic mapping, and functional inference to uncover links between genome architecture, recombination landscapes, and host–parasite interactions.

Advances in production methods of entomopathogenic nematodes for use as biopesticides

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Abstract Entomopathogenic nematodes are mass produced for commercial purposes using *in vivo*, *in vitro* solid and liquid culture methods. Each method has its advantages and disadvantages. *In vivo* culture uses insects as host and lacks economy of scale due to the costs of labor and insects. *In vitro* culture requires a sterile monoxenic system. *In vitro* liquid culture uses the largest capital inputs but provides the greatest economic efficiency. *In vitro* solid culture is intermediate between the other two methods, requiring less capital investment but more employees. Recently, improvements in all approaches have been made. *In vivo* systems can be enhanced through mechanization whereas *in vitro* systems can be improved through optimization of media and system parameters. A sustainable system for farmer-based production has been developed for *in vivo* production. Additionally, a novel *in vitro* solid production was developed that uses the production media as a formulation and carrier, thereby substantially saving costs. Exposure to ascarioside pheromones may be used as a final step to enhance biocontrol efficacy. The improved production approaches indicate potential for wider application of entomopathogenic nematodes in biocontrol systems.

Artificial intelligence (AI)-based detection and quantification of soybean cyst nematode

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Abstract Soybean cyst nematode (SCN; *Heterodera glycines*) is a major pest of soybean and a persistent threat to soybean production in the United States. Accurate detection and quantification of SCN eggs and cysts are essential for estimating infestation levels, screening host resistance, and informing management decisions. However, routine microscopy-based diagnosis is labor-intensive, time-consuming, and confounded by debris and root fragments in extraction samples. To address this bottleneck, we are developing a deep learning framework for microscopy-based SCN diagnosis built from expert-annotated images collected under practical laboratory conditions. The current dataset contains 1,082 high-resolution microscopy images from egg suspensions and cyst extracts obtained from SCN-infested field and greenhouse samples. We developed an expert-verified dataset of 93,618 annotated objects that reflects the realistic complexity of field-collected soil samples. By including visually similar non-target objects, the dataset provides a robust foundation for training generalized models capable of detecting nematodes, including 17,289 eggs, 17,325 cysts, 36,837 debris objects, 21,951 root fragments, and 216 vermiform nematodes. Beyond primary targets, the dataset's inclusion of debris and root fragments offers broader utility to the research community, to enhance both diagnostic accuracy and model robustness. Furthermore, we developed a preliminary web-based platform that facilitates the export of YOLO-specific annotations into raw, model-agnostic annotation formats. This enables integration with various convolutional neural networks, object detectors, and transformer-based architectures for scalable training, benchmarking, and cross-model performance comparison. Initial models trained on this dataset, including YOLO-based detectors and RF-DETR, achieved mean average precision values approaching 99% for SCN detection on our curated image sets. Current work is focused on testing performance and deployment efficiency under more complex diagnostic conditions relevant to practical nematology workflows. This effort could lead to establishing a reproducible infrastructure for automated SCN diagnosis and creating a foundation for external validation and future decision-support tools. Collectively, these advances will support faster, more consistent, and more scalable SCN detection for integrated nematode management.

Determining optimum timing for planting biofumigant crops to manage root-knot nematodes in cucurbit systems

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Abstract Root-knot nematodes (RKN; *Meloidogyne* spp.) are a major threat to cucurbit production in Delaware, where sandy soils favor their proliferation. Biofumigation to manage RKN involves growing and incorporating glucosinolate (GSL)-producing crops like mustards to release isothiocyanates, which have nematicidal properties. Spring establishment of biofumigant crops can delay planting of cash crops such as watermelon beyond the optimal window. Consequently, growers have demonstrated interest in utilizing fall planting for RKN management instead. A field with history of RKN was planted with the biofumigant mustard *Brassica juncea* variety 'Caliente Rojo' for a two-year study, with field location varying by year. Year 1 compared fall 2023 and spring 2024 planting and incorporation to a fallow treatment, and year 2 compared fall 2024 and spring 2025 plantings to fallow with each treatment plot split to grow pumpkin and watermelon. To comprehensively evaluate differences among planting dates, several variables were assessed. Biofumigant biomass and soil GSL concentrations were measured at eight to ten timepoints after incorporation. Soil RKN populations were monitored before incorporation, at cash crop planting, and after harvest. Yield was recorded from each plot in 2025. Across both years, biofumigant plant counts were not significantly different between fall and spring. However, the fresh weight was significantly higher in fall in year 1, and significantly lower in fall in year 2. Interestingly, GSL concentration followed the opposite trend of fresh weight. In year 1, GSL concentration was higher in spring, averaging 25 nmol g⁻¹ compared to 19.2 nmol g⁻¹ in fall at 9 days post-incorporation (DPI). In year 2, GSL concentration was higher in fall, averaging 40.6 nmol g⁻¹ versus 24.4 nmol g⁻¹ in spring at 9 DPI. Overall, RKN pressure was low, and soil RKN counts did not differ significantly among fallow, spring, and fall biofumigant plantings for both years. In year 2, spring biofumigation did not differ from the fallow treatment in pumpkin count or yield, whereas fall biofumigation produced significantly higher pumpkin count (2,162,020 vs. 1,390,530 per hectare) and yield (4,371,160 vs. 2,745,949 kg per hectare) than the fallow treatment. No differences were observed in watermelon. Collectively, these results suggest that fall biofumigation better aligned with cash crop planting timing, and GSL concentration was negatively correlated with biomass. Fall planting showed potential to improve cucurbit crop performance, particularly for pumpkin, even under low RKN pressure.

Nontarget effects of endemic entomopathogenic nematodes on soil-surface arthropods and the soil nematode community

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Abstract Entomopathogenic nematodes (EPNs) are ideal biological control agents. EPNs have no effect on nontarget vertebrate organisms, have limited effects on foliar insects, and little interaction with fungi. Hawai'i effectively prohibits the importation of many commercial EPNs through quarantine regulations. However, possibly adventive or native populations of EPNs are found in Hawai'i but with limited distributions. These EPNs often have wide insect host ranges in laboratory, raising concerns about infections of nontarget and beneficial arthropods. EPNs' effects on soil-surface arthropod and soil nematode communities have been assumed to be minimal, despite the inundative releases of millions to billions of EPN/ha for insect pest control. Utilizing a sweetpotato cropping system targeting management of the sweetpotato weevil *Cylas formicarius*, EPNs from Hawai'i were evaluated for nontarget effects on soil-surface arthropods and soil nematodes. In one experiment, monthly applications of *O. tipulae* Oa-12 (0.5 billion IJ/ha) were compared to carbaryl applications and an untreated control. In another experiment, *Steinernema feltiae* MG-14 and *Heterorhabditis indica* OM-160 were applied separately (2 billion IJ/ha) as a single application and compared to an untreated control. In a third experiment, *S. feltiae* MG-14 (0.5 billion IJ/ha) was applied monthly and compared to an untreated control. Soil surface arthropod populations were monitored via pitfall traps weekly. The nematode soil community was monitored through preplant and harvest samples. No differences were found in soil-surface Dermaptera, Hymenoptera, Orthoptera, Arachnida, nor Isopoda populations with *O. tipulae* compared to the untreated plots. The number of sweetpotato weevil recovered from plots treated with *O. tipulae* was lower than in the untreated plots, but other coleoptera populations were unaffected. Carbaryl decreased the numbers of Coleoptera and Isopoda but had no effect on the other arthropod orders. Soil nematode trophic groups were not affected by *O. tipulae* or carbaryl compared to the untreated plots. With single applications of *S. feltiae* or *H. indica*, no differences were observed in the soil-surface arthropod populations of Dermaptera, Hymenoptera, Orthoptera, Arachnida, or Isopoda populations compared to the untreated plots. The number of Diptera recovered was higher in plots treated with either *S. feltiae* or *H. indica* compared to the untreated control. The nematode soil community at sweetpotato harvest was not different under the single application of *S. feltiae* or *H. indica* as compared to the untreated control. The monthly applications of *S. feltiae* also had no effect on soil-surface arthropod populations or on the nematode soil community. The inundative release of endemic *O. tipulae*, *S. feltiae*, and *H. indica* had no effect on nontarget soil-surface arthropods nor on the nematode soil community associated with sweetpotato. Why the EPNs do not have effects on soil-surface arthropods or the nematode community becomes a question worth answering.

Effects of nitrogen and biomix amendments on nematode communities in potato production soils of western guatemalan highlands

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Abstract The global significance of soil health degradation and the role of beneficial nematodes (BNs) in influencing soil health are well established. However, the precise impact of soil health degradation and the role of BNs on agricultural production of emerging economies remains largely unknown. This collaborative study among Michigan State University, University of Hawaii, and University of San Carlos was conducted in small-holder potato grower fields located in the Huehuetenango and Xela regions of the Western Guatemalan Highlands over two potato growing seasons. The Huehuetenango fields lay over Mollisol at 3,200 m to 3,353 m and the Xela fields over Andisol at around 2,896 m altitude. The experiment, repeated in each region, tested the effects of amending soils either with or without biomix and 0, 318, or 454 kg composted animal manure at eight locations. The Mollisols received composted horse manure while the Andisols received composted chicken manure. The biomix (BioCopia®) consisted of Guatemalan isolates of *Purpureum* and *Bacillus* applied at 1.8 kg/m² to suppress harmful nematodes. Soil samples were collected at planting, midseason and at harvest. Nematodes were extracted from 100 cm³ of soil subsample, fixed in double TAF solution at USAC, and enumerated at MSU. Predator and omnivore nematodes tended to increase with time in Andisols more than in Mollisols. As described by the Soil Food Web model, the combined effect of the amendments resulted in resource-limited conditions in both soil groups, suggesting the need for biological activities to release nutrients. The Soil Food Web structure was stable over time in both soil groups. The data points in Andisols were similar between years while data associated with the Mollisols were not similar between years. Farm size in the Andisols was larger than farm size associated with the Mollisols. The higher altitude of the Mollisols resulted in a shorter growing season compared to the Andisols. A PCA demonstrated that the two soil groups behaved differently and may have different biological properties. The amendments and by applied were unable to modify Soil Food Web biological properties in the two growing seasons to move the soils out of the resource-limited conditions.

Impacts of root-knot nematodes (*Meloidogyne chitwoodi* and *M. hapla*) on potato yield and quality

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Abstract The root-knot nematodes *Meloidogyne chitwoodi* and *M. hapla* are economically significant pests of potato in the Pacific Northwest. These nematodes are well adapted to the region's cool climate and threaten both yield and tuber quality. There is zero tolerance for nematode-infected tubers resulting in substantial losses for growers. The objective of this research was to quantify the effects of varying initial population densities of *M. chitwoodi* and *M. hapla* on potato yield and quality. A three-year field study was conducted at the Oregon State University's Hermiston Agricultural Research and Extension Center. A randomized complete block design evaluated *M. chitwoodi* at 0.01, 0.1, 1, 5, 10, 25, 50, and 200 eggs per 250 cc soil and *M. hapla* at 50 and 200 eggs per 250 cc soil on three cultivars: 'Ranger Russet', 'Clearwater Russet', and 'Russet Burbank'. Total yield and tuber number varied due to the interaction of initial nematode density and cultivar. High initial *M. chitwoodi* densities reduced yield in 'Clearwater Russet', whereas 'Ranger Russet' produced a greater proportion of large tubers under high nematode pressure. In contrast, *M. hapla* initial density did not significantly affect yield or tuber number; observed differences were attributable to cultivar. For both species, the primary effect was reduced tuber quality. Severe galling from *M. chitwoodi* occurred at initial densities as low as 5 eggs/250 cc soil, with tuber symptoms observed at 1 egg/250 cc soil. Although *M. hapla* infection did not produce visible galling on the tubers, infection indices per tuber confirmed nematode presence. These findings demonstrate that severe tuber quality losses can occur at very low initial population densities, and that cultivar responses to *M. chitwoodi* vary under nematode pressure. This work provides critical information for nematode risk assessment, cultivar selection, and targeted management to sustain quality potato production in nematode-affected regions.

Morphological and molecular characterization of *Dolichodorus heterocephalus* Cobb, 1914 from silver springs, Florida and other locations

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Abstract The awl nematode *Dolichodorus heterocephalus* was first described by Cobb in 1914 based on a female collected from Silver Springs, Florida, and a male from Douglas Lake, Michigan. Subsequently, Golden *et al.* in 1989 designated as lectotypes a female and a male collected by Cobb from Silver Springs, Florida, providing limited morphometric data while incorporating more detailed morphological observations from additional populations in Florida and Massachusetts. This work highlighted substantial intraspecific variability and noted discrepancies in body size between the female lectotype and other Florida populations. In the present study, a topotype sample from Silver Springs, Florida, was collected in 2025 and examined using an integrative approach combining morphology and molecular data including analyses of sequences from the 18S rRNA, D2–D3 expansion segments of 28S rRNA and partial *COI* genes. This effort aimed to establish robust reference standards for species identification, addressing the growing need for accurate diagnostics for the implementation of effective phytosanitary measures and nematode management practices. In addition, one population from north Florida was analyzed morphologically and molecularly and several other populations from Florida were sampled and analyzed only molecularly. Microphotographs of selected morphological features were taken. Morphological comparisons revealed that some adult females from the topotype sample exhibited a body length of more than three mm like the lectotype and greater than that reported for other populations. Both topotype female and male stylets were longer than those reported for the lectotypes, falling within the range observed in other Florida populations. Despite these differences, combined morphological and molecular analyses did not support significant differentiation among populations. Some populations from south Florida showed more morphological differences from the topotype specimens than those from central Florida. These differences were reflected by notable intraspecific variability in *COI* gene sequences. Overall, our findings confirm morphological and genetic variability within *D. heterocephalus* while supporting its status as a single species and provide updated reference data to improve diagnostic accuracy.

A tripartite symbiosis among the entomopathogenic nematode *Steinernema monticolum* KHA701, *Xenorhabdus hominickii*, and *Serratia marcescens*

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Abstract Entomopathogenic nematode (EPN) have evolved an unique lifecycle in which they harbor entomopathogenic bacteria and exploit these symbionts to kill insect hosts and efficiently acquire nutrients. Species of *Steinernema* are known to maintain highly specialized associations with *Xenorhabdus* bacteria, characterized by the presence of a dedicated organ “receptacle” for bacterial storage and transmission. *Xenorhabdus* bacteria also produce antibacterial metabolites that suppress competing microbes, enabling the nematode-bacterium partnership to monopolize host resources. *Steinernema monticolum* KHA701, isolated from forest soil in Ena City, Gifu Prefecture, Japan using the *Galleria* bait trap, harbors its specific symbiont *Xenorhabdus hominickii* but also *Serratia marcescens* and other pathogenic bacteria. These additional bacteria synergistically enhance the entomopathogenicity of the EPN (Sugiyama & Hasegawa, Sci. Rep. 15, 22550, 2026). However, the ecological and mechanistic basis of the interaction among *S. monticolum* KHA701, *X. hominickii*, and *S. marcescens* remains poorly understood. To elucidate the tripartite relationship, we generated GFP-tagged strains of each bacterium using a *Tn7* transposon system and confirmed that the labeled strains retained wild-type levels of insecticidal activity. When introduced into infective juveniles (IJs) of *S. monticolum* KHA701, *X. hominickii* localized specifically within the receptacle. In contrast, *S. marcescens* was unable to colonize IJs on its own, but when co-introduced with *X. hominickii*, it was observed in the intestine, on the cuticle surface, and inside the molted cuticles, although there was some individual variation. Co-culturing assays in LB liquid medium revealed that *S. marcescens* proliferates without inhibition from *X. hominickii*, while the growth of *X. hominickii* is suppressed in the presence of *S. marcescens*. Although *S. monticolum* KHA701 can be maintained in monoxenic culture on *X. hominickii*, it cannot grow on *S. marcescens* and rapidly dies. Moreover, the addition of *S. marcescens* after establishment of the nematode-*X. hominickii* symbiosis enhance nematode virulence, indicating that maintenance of the primary symbiosis is essential for this EPN strain. These findings suggest that *S. marcescens* may coexist with *X. hominickii* and its host nematode, *S. monticolum* KHA701, through resistance to *X. hominickii*-derived antibacterial compound and by maintaining an appropriate density balance. We propose that this tripartite association enable *S. monticolum* KHA701 to exploit insect hosts more efficiently, representing a flexible and ecologically advantageous symbiotic strategy.

Impact of field pennycress on the life cycle and reproduction of soybean cyst nematode (*Heterodera glycines*)

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Abstract Sustainable incorporation of pennycress, *Thlaspi arvense*, into Midwest cropping systems as a winter annual cover crop requires careful evaluation of its potential effects on existing crops and pests. Based on greenhouse studies, pennycress is moderately susceptible to soybean cyst nematode, *Heterodera glycines*, a major yield-limiting pest of soybean in the United States that is estimated to cause approximately \$1.5 billion in yield losses annually. We examined the potential for pennycress to support *H. glycines* development in the field, laboratory, and in growth chamber studies mimicking field conditions. In hatching experiments, pennycress root exudates did not stimulate *H. glycines* egg hatch relative to soybean root exudates at both 15°C and 25°C. In our growth chamber trial, we inoculated pennycress at 25°C followed by a steady reduction in temperature to 4°C and a subsequent increase of temperature back to 25°C. Our data suggests that following infection, *H. glycines* development pauses at reduced temperatures and then resumes once temperatures increase, highlighting the potential for development of *H. glycines* even under reduced soil temperatures. In field experiments, while pennycress did not statistically increase *H. glycines* egg density, we did observe new *H. glycines* females and eggs from pennycress field plots. These results suggest that while pennycress can be incorporated into soybean-corn production systems with minimal risk of exacerbating *H. glycines* populations, there is potential for some *H. glycines* reproduction. Together our results support the use of pennycress as a sustainable winter annual cover crop for Midwest production systems, although its use should still be accompanied by appropriate *H. glycines* management practices.

The spatial distribution and population densities of root-knot (*Meloidogyne* spp.) nematode species in north carolina vegetable production

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Abstract Root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the most economically damaging plant pathogens to North Carolina (NC) vegetable production. These obligate biotrophs feed inside the root, causing visible symptoms (galls and root swelling) that reduces yield. The heterogeneous spatial distribution of these pathogens often makes detection difficult. Information about the predominant RKN species affecting NC vegetable production is out of date, which creates difficulty in creating general management recommendations. The goal of this study is to survey various solanaceous and cucurbit crops across NC to identify prominent and emerging species, like the emerging RKN, *M. enterolobii*. Soil samples were collected from 1 acre divided field sections from May to August 2025 on solanaceous and cucurbit crops. Approximately 0.5 kilogram of soil was collected per subsample and soil cores were collected 15 to 20 centimeters deep, within the planting bed. Whitehead tray extractions were performed to extract nematodes from 100 cc of soil. Morphological analysis under the microscope identified nematodes to the genus level. Samples that were found positive for RKN were individually plucked for further species identification. DNA from the nematodes were extracted and assayed with RKN universal primers to ensure sample integrity. Real Time qPCR with species-specific primers was used to screen samples for three major species (*M. enterolobii*, *M. hapla*, and *M. arenaria*) and PCR aided the identification of *M. javanica*. Samples that were not positive for these species were assessed via Sanger sequencing targeting the mitochondrial DNA for species identification. Throughout the peak growing season, 115 samples from 14 solanaceous and cucurbit vegetables were collected across 14 counties. RKN was identified in 39.1% of the samples collected and present in 11 counties. Across all samples, population densities ranged from 17 to 15,100 RKN per 100 cc soil. Among solanaceous crops, 44.4% of samples were found to have RKN, while among cucurbit crops, 29.0% of samples were found to have RKN. *Meloidogyne enterolobii* was present in mixed populations and found in 2 samples from Lenoir County, which has previously been reported positive for this species. *Meloidogyne enterolobii* was not detected in any new counties in NC during this survey. Other root-knot nematode species including *M. hapla*, *M. javanica*, *M. arenaria*, and *M. incognita* were found in higher prevalence during this survey. The results from this survey show that RKN continues to be a predominant pathogen in NC vegetables and no evidence of *M. enterolobii* was found in western counties of NC. This study gives a first, updated insight into which RKN species are present and predominant in NC vegetable production, which helps increase the implementation of management strategies that are effective.

Synergistic effects of root-knot nematode and take-all root rot on hybrid bermudagrass decline

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Abstract High densities of plant-parasitic nematodes, particularly root-knot nematodes (*Meloidogyne* spp.), are often detected in turfgrass exhibiting symptoms of take-all root rot (TARR), a crown and root disease caused by a complex of ectotrophic root-infecting fungi. Although these two important soilborne pathogens may produce similar above- and belowground symptoms, their ecological interaction remains poorly understood. This study investigated the relationship between root-knot nematode and *Gaeumannomyces graminis* (GG) infection in hybrid bermudagrass and evaluated their combined effects on turfgrass performance. Hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* (Burt-Davy) cv. 'Miniverde'] pots were maintained under greenhouse conditions and assigned to one of four treatments: non-inoculated control (C), nematode only (N), fungus only (F), or combined nematode and fungal inoculation (NF). Treatments N and NF each received approximately 600 second-stage juveniles (J2) of *Meloidogyne graminis*. Ten weeks later, treatments F and NF were inoculated with two 5-mm potato dextrose agar plugs infested with GG. Visual turf quality and percent green cover (PGC; i.e., digital image analysis) were assessed weekly over the course of the experiment. At study termination, basal necrosis and final nematode populations were quantified. Area under quality progress curve (AUQPC) was calculated for each treatment after nematode inoculation. Lower AUQPC and PGC was associated with hybrid bermudagrass inoculated with *M. graminis* and GG than the non-inoculated control and either of the single-pathogen inoculated treatments. However, temporal changes in turf quality and PGC varied between experimental runs. In the first run, the combined inoculation treatment caused a twofold faster decline in PGC from the non-inoculated control compared to single-pathogen treatments and resulted in ~15–20% lower turf quality values by the end of the study. In the second run, turf quality and PGC of the combined inoculation treatment trended lower than the single-pathogen treatments for most of the study, yet all remained significantly lower than the non-inoculated control. Collectively, these findings indicate that simultaneous pressure from multiple pathogens can accelerate turfgrass decline and intensify reductions in overall turf quality and vigor compared with infection by a single pathogen alone.

Differential susceptibility of hop cultivars to hop cyst nematode, *Heterodera humuli*

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Abstract The hop is an important crop of the United States of America, with 41,654 acres harvested and production value of approximately \$447 million in 2025. Among plant parasitic nematodes (PPNs), hop cyst nematode (HCN) *Heterodera humuli* has appeared one of the most prevalent nematodes with infestation reported about 65% hopyards in the Pacific Northwest and 40–50% in the Midwest. HCN significantly affects plant growth resulting in reduction in dry hops per bine (38%), root length (68%), bine height (40%), and as well as inducing nutrients deficiencies. Host plant-resistance is one of most used strategies for managing PPNs. To date, susceptibility of different cultivars of hops to HCN is still largely unknown. To address this issue, we evaluated ten most grown commercial cultivars of hops in Michigan (Cascade, Centennial, Saaz, Chinook, Tettnanger, Comet, Crystal, Magnum, Cashmere, and Nugget) for their response to HCN in a greenhouse trials. Hops were grown in 1.5-gallon pots filled with 3:1 ratio of field infested soil with HCN and sterilize soil. Experiment was arranged in completely randomized block design with six different replicates for each cultivar and experiment repeated with a second time. Fertilizer and water were applied on regular basis throughout the study period. Post twelve weeks experiment setup, soil samples were collected to quantify HCN population density, additionally plant height (inch) and biomass (g) were counted. HCN population density varied among cultivars with maximum HCN population density was recovered from Centennial. Significantly lower populations were recorded from Cashmere ($p < 0.05$) while Nugget ($p = 0.05$) and Magnum ($p = 0.08$) were showed marginal differences compared with Centennial. All other remaining cultivars did not differ significantly from Centennial. Plant height differed significantly ($p < 0.05$) among different cultivars with highest plant height was recorded in Comet, followed by Cashmere, and Cascade while lowest in Crystal, Tettnanger, and Centennial. Similarly, plant biomass differed significantly ($p < 0.05$) among cultivars with highest biomass recorded from Magnum followed by Cascade while Tettnanger produced least. Based on HCN population density and plant growth parameters, Centennial, Tettnanger, Crystal, and Chinook were classified as more susceptible while Cashmere and Magnum showed comparatively lower susceptibility, while remaining cultivars showed intermediate responses. This study represents the first systemic evaluation of hop cultivars responses to HCN. Future research validation under the micro-plot trial is needed to confirm these findings and support cultivar selection for HCN management in hops.

Influence of irrigation and mulching on soil nematode communities in apple orchards

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Abstract Plant-parasitic nematodes are important soilborne pests in apple (*Malus domestica* Borkh.) production systems, yet the influence of orchard floor management practices such as irrigation and mulching on nematode population dynamics remains poorly understood. This study evaluated the effects of soil moisture management and mulching on plant-parasitic and beneficial nematodes in apple orchards. A field experiment was conducted during the 2025 growing season at the West Central Horticultural Research Station in Michigan using a randomized complete block design with four treatments: (1) non-irrigated mulch, (2) irrigated mulch, (3) soil moisture maintained at approximately 50% of field capacity, and (4) non-irrigated control without mulch. Each treatment was replicated nine times. Two apple cultivars, 'Honeycrisp' and 'Gala', were included in the study. Soil samples were collected on May 6 and September 6, 2025, to assess nematode population dynamics. Nematodes were extracted from 100 cc soil samples using a modified centrifugal flotation method and identified into plant-parasitic groups, primarily root-lesion nematodes (*Pratylenchus* spp.) and American dagger nematodes (*Xiphinema americanum*), as well as beneficial groups including bacterivorous and fungivorous nematodes. Population changes were quantified using reproduction factors ($RF = Pf/Pi$). Treatment effects on plant-parasitic nematodes varied by cultivar and nematode group. In 'Honeycrisp', RF values of root-lesion nematodes were significantly lower in the irrigated mulch and 50% soil moisture treatments compared to the non-irrigated mulch treatment, while the non-irrigated control showed intermediate values. For American dagger nematodes, the non-irrigated mulch and 50% soil moisture treatments resulted in significantly lower RF values compared to the non-irrigated control, with irrigated mulch showing intermediate responses. In contrast, no significant treatment effects were detected in 'Gala' for either nematode group, although irrigated mulch consistently resulted in numerically lower RF values for root-lesion nematodes. Beneficial nematode populations showed less pronounced responses to treatments. However, the irrigated mulch treatment consistently resulted in the highest numerical RF values for bacterivorous and fungivorous nematodes in both cultivars, suggesting that improved soil moisture and organic inputs may enhance soil biological activity. Overall, treatment effects were more evident in 'Honeycrisp' than in 'Gala', indicating cultivar-specific responses to soil moisture and mulching practices. These findings suggest that irrigation and mulching can influence nematode population dynamics in apple orchards, with potential to suppress plant-parasitic nematodes while maintaining or enhancing beneficial nematode communities. This approach may provide a practical and sustainable strategy for managing nematodes in perennial fruit production systems. Further multi-year and multi-location studies are needed to validate these findings and support the development of integrated orchard floor management recommendations.

Effectiveness of cover crops for reducing populations of hop cyst nematode, *Heterodera humuli*.

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Abstract The United States is the world largest producer of hops with Pacific Northwest (PNW) (Washington, Oregon, and Idaho) - representing primary hop producing region. Michigan ranks fourth nationally and is the only state outside PNW that contributes significant number of acres under hop cultivation. Hop cyst nematode, *Heterodera humuli*, is one of the most economically important nematode pests of hop and is established in approximately 65% and 40–50% hopyards of PNW and Michigan, respectively. Numbers of studies have documented substantial yield loss and growth suppression of hops associated with HCN infestation resulting in reductions in dry hops per bine up to 38%, lowers root length by 68%, decreases bine height about 40%, and nutrients deficiencies as well compared with non-infested hops. Currently, growers have limited management options for HCN other than relying on preventative measures such as use of clean machinery and disease-free planting materials. The use of cover crops for sustainable management of plant parasitic nematodes is gaining increasing attention among growers in United States because of multiple agronomic and soil health benefits. To date, efficacy of cover crops to reduce population of HCN has been underexplored. In the present study, eight different cover crops or cultivars including marigold (cv. Nema-Gone), white mustard (cv. Master), oat (cv. Go-T), daikon radish (cv. Eco-Till), and oilseed radish (cv. Defender, Control, Concorde, and Carwoodi) were assessed for their potential in reducing the populations of HCN in greenhouse conditions. Fallow (non-planted soil) and hops (cv. Cascade) were included as controls. Bioassay arena consisted of 1.5-gallon pot filled with 3:1 (v/v) ratio of field infested HCN soil and sterilized sandy soil. Experiment arranged in completely randomized design with five replicates for each cover crop or control group and experiment repeated for second time. Four months later, the soil samples collected to quantify the HCN population density. HCN population differed significantly ($p < 0.05$) among different cover crops along with control groups (fallow and hops). The lowest HCN population density recovered from marigold that was significantly different from oat, daikon radish, oilseed radish (cv. Defender and Control), and hop. Compared with fallow control, numerically lowered HCN population density observed in marigold, white mustard, and oilseed radish (cv. Carwoodi), while increased in population observed in oilseed radish (cv. Defender and Control), daikon radish and oat. Current findings demonstrated that certain cover crops marigold, white mustard, and oilseed radish (cv. Carwoodi) have potential to reduce the population of HCN, whereas increase in population was observed from oat and daikon radish. These findings underscore importance of careful selection of cover crops for sustainable management of HCN in hop production system. Future micro-plot trials are required to confirm the current findings before making any recommendations to hop growers.

Response of hemp (*Cannabis sativa*) cultivars to root-knot and sting nematodes

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Abstract Industrial hemp (*Cannabis sativa*) with <0.3% delta-9-tetrahydrocannabinol (THC) is now an agricultural commodity in the US. This versatile crop is grown for fiber, seed and oil as well as medicinal cannabinoids such as cannabidiol (CBD), cannabigerol (CBG) and other essential oils. In Florida, recent studies have shown that hemp can be associated with diverse plant parasitic nematodes, including root-knot nematode (*Meloidogyne* spp.), sting nematode (*Belonolaimus longicaudatus*), reniform nematode (*Rotylenchulus reniformis*), among others, which could cause potential damage to hemp growth and yield. Especially, root-knot and sting nematodes are of concern, as they are very prevalent in Florida's sandy soils, and responsible for the bulk of nematode crop damage in Florida. Previous research in Florida has shown that all tested hemp cultivars were excellent host to different root-knot nematode species, but studies in Tennessee have shown that the CBD cultivar Wife was a poor host to *M. incognita*. For sting nematode, no published data exists, but preliminary data from Florida indicated poor reproduction of sting nematode on hemp. A series of greenhouse experiments was therefore conducted to evaluate the response of different hemp cultivars to root-knot and sting nematodes. The root-knot nematode experiments include two CBD hemp cultivars (Wife and Sunset) which were screened against three root-knot nematode species (*M. javanica*, *M. incognita* and *M. enterolobii*). The sting nematode experiments included CBD cultivars (Sunset, Cherry Blossom, Early Bird and Maverick), and fiber/oil cultivars (Eletta Campana, Puma-3, X-59 Hemp Nut, SS Beta, Bialobrzieskie, Bama, Tygra and Yuma). Data collection on nematode reproduction and plant growth is ongoing, and results will be presented at the conference. Our findings will provide valuable information to hemp growers in Florida and abroad to select the best cultivars to minimize nematode damage, and to hemp breeders to select new lines for nematode resistance.

Integrated nematode management to control the reniform nematode (*Rotylenchulus reniformis*) in upland cotton

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Abstract The reniform nematode (*Rotylenchulus reniformis*) is a major pest of upland cotton (*Gossypium hirsutum*) in the U.S. Cotton Belt, causing significant yield and lint quality losses. In Louisiana, yield losses due to reniform nematode were approximately 2.25% in 2025, costing producers approximately \$2.2 million dollars. Management has primarily relied on crop rotation and nematicides, but recent commercialization of reniform nematode-resistant cotton cultivars offers new opportunities to combine host resistance with chemical control in infested fields. This study aimed to evaluate the effectiveness of combining resistant cotton varieties with various nematicide chemistries (fluopyram, aldicarb, and abamectin) for suppression of reniform nematode populations and enhancement of cotton seed yield in infested Louisiana fields. Field trials were conducted in 2024 and 2025 at two locations with reniform nematode infestations. Results showed that the resistant variety PHY 411 W3FE supported nematode soil population densities that were 58–61% lower than those of the susceptible variety PHY 340 W3FE, regardless of nematicide treatment. Integrated treatments combining the resistant variety with nematicides yielded up to 28% higher cotton seed production than the untreated susceptible control under high nematode pressure, suggesting potential yield-protection advantages. However, these benefits were not consistent across all fields tested. Ongoing field experiments aim to verify treatment effects and explore interactions among host resistance, nematicides, and the future integration of crop rotation.

ATR-FTIR spectroscopy for biochemical analysis and identification of entomopathogenic nematodes

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Abstract Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) provides information on the molecular composition and structure of samples. The use of ATR-FTIR was evaluated for biochemical analysis and taxonomic differentiation of entomopathogenic nematodes (EPNs). Spectra were obtained from a small sample (pellet) of a nematode population recovered from commercial EPN packages, which was placed directly on the ATR plate. Differences in signal intensity at multiple peaks associated with biomolecules critical to the survival of EPN (trehalose, glycogen, and triglyceride) were measured and visualized using Non-Metric Multidimensional Scaling (nMDS) and Principal Component Analysis (PCA). Statistically significant differences in peak signal intensity were observed between EPN species for each biochemical parameter, providing a basis for assessing the likelihood of success in the field conditions. The present study also evaluated FTIR analysis of EPN for taxonomic differentiation. Results demonstrate that FTIR can be used to identify and differentiate *Steinernema* and *Heterorhabditis* genera/species, offering a potentially faster, less expensive alternative to molecular identification techniques. Ultimately, this study demonstrates the efficacy of ATR-FTIR as a reliable method for assessing the biochemical suitability of EPN products for field applications and differentiating between EPNs.

The European reference laboratory for plant parasitic nematodes: A beacon for diagnostics of regulated nematodes

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^{1,2}EURL for Plant Parasitic Nematodes

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Abstract The EU Reference Laboratory (EURL) for Plant Parasitic Nematodes serves as a central hub for diagnostic excellence of nematodes that are regulated in the European Union (EU). It is one of five EURLs in Plant Health established to ensure that all 27 EU Member States use reliable, harmonized, and high-quality diagnostic methods for detecting and identifying quarantine pests and pathogens. The main tasks of the EURLs are to strengthen diagnostic capacity and consistency across the EU. This includes the development and/or validation of diagnostic methods, organization of trainings on an as-needed basis, and providing reference materials. The Member States became increasingly involved over the years as their national reference laboratories (NRLs) are required to participate in proficiency tests - for which EURLs provide a follow-up in case of non-conforming results - and to attend annual workshops organized by each EURL. Exchange of knowledge and information, between Member States and with the EURLs, is achieved via these workshops, but also via e-mail contacts and informative EURL websites. The EURL for Plant Parasitic Nematodes, is a collaboration between ANSES (France) and ILVO (Belgium). Our EURL produced detailed diagnostic protocols for several high-priority nematode pests: *Meloidogyne chitwoodi*, *M. fallax*, *M. graminicola*, *Bursaphelenchus xylophilus*, *Globodera rostochiensis*, *G. pallida*, accessible for everybody via the EURL website. A working group has been initiated in 2026 to come up with morphological and molecular diagnostic protocols for the seven EU-regulated *Xiphinema* species. All recommended protocols must be validated and, when not available, new molecular assays are developed and evaluated through interlaboratory test performance studies to ensure reproducibility and robustness. Through these protocols, the EURL also supports laboratories in achieving and maintaining ISO/IEC 17025 accreditation for official diagnostic methods, required by EU legislation. Another main activity of the EURL is the establishment of a reference collection of live nematodes, preserved specimens, photographs and DNA, that can be shared with NRLs. This is crucial for their method development, validations, and set-up of own reference collections. The EURL also offers diagnostic support during pest outbreaks and confirmatory analyses. Scientific and technical assistance is given to the European Commission, and there is collaboration with organizations, e.g. EPPO and EFSA, within the scope of the EURL mission. The efforts of all five EURLs collectively strengthen plant health systems and biosecurity, not only across the European Union, but worldwide as countries are interconnected more than ever before.

Insights into a new landscape of effector genes within the anguinidae

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Abstract Nematodes belonging to the family Anguinidae are responsible for crop and forest damage across North America, posing a substantial threat to agricultural productivity and ecosystem stability. Their parasitic relationship with plants is driven by intricate biological processes, including the induction of cell hyperplasia and hypertrophy within host tissues. Despite their importance, the molecular mechanisms underlying the formation of these specialized feeding structures, and the effector proteins responsible, remain poorly understood. To address this gap, we conducted comparative analyses of genomic and transcriptomic datasets across multiple species. This approach revealed a previously uncharacterized repertoire of effector gene families within the Anguinidae, and more broadly across the phylum Nematoda. Of the 45 newly validated effectors, 40 were classified as pioneer genes, lacking prior functional annotation yet exhibiting strong expression during plant infection. Further functional characterization of one such pioneer effector demonstrated its localization to the nucleus and nucleolus, indicating a potential role in host nuclear processes. Transcriptomic profiling of leaves expressing this effector revealed widespread upregulation of genes associated with regulation of DNA-templated transcription, associated with ectopic cell division, a defining feature of Anguinidae-induced feeding sites. The discovery and characterization of these novel effectors provide valuable insight into the molecular basis of nematode parasitism and feeding site establishment. These findings lay the groundwork for developing innovative strategies to mitigate the impact of Anguinidae on agriculture and forestry scenarios.

Identification of turfgrass associated nematodes using deep learning algorithms

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Abstract Plant-parasitic nematodes are important threats to turfgrass. Left unmanaged, they can reduce the quality and playability of golf courses and athletic fields. Effective nematode management relies upon accurate identification of nematode genera extracted from soil samples. Recent advancements in deep learning models offer promising solutions for the future of nematode identification. In this study, we evaluated the performance of EfficientNet V2-S, MobileNetV3-L, ResNet101, and Swin Transformer V2-B convolutional neural network model architectures in the classification of seven nematode taxa associated with turfgrass. Balanced classification accuracy on the test set was highest for EfficientNet V2-S and Swin Transformer V2-B at 94.63% and 94.34%, respectively. MobileNetV3-L and ResNet101 had lower balanced accuracies of 90.83% and 86.33%, respectively. Testing the models on an additional user-end platform derived dataset showed that EfficientNet V2-S achieved the best performance, with 82.47% balanced accuracy and was subsequently integrated into a demonstration web application to showcase its feasibility for diagnostic workflows. The findings of this study demonstrate the potential application of deep learning tools for accurate nematode identification to aid in diagnostics.

From data to decisions: Integrating artificial intelligence diagnostic nematology into extension

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Abstract Extension and diagnostic nematology are under increasing pressure to deliver rapid, consistent, and scalable recommendations in the face of a limited pool of specialized expertise. While advances in imaging, molecular tools, and data collection have increased the quantity of available information, the ability to process, interpret, and communicate those data remains a critical bottleneck. Artificial intelligence (AI) may reshape how diagnostic workflows are organized and delivered. This presentation examines how AI can be embedded across the diagnostic pipeline, from sample intake and image-based screening to standardized report generation and grower-facing recommendations. AI-assisted systems can streamline preliminary identification, harmonize diagnostic language across laboratories, and synthesize historical and regional datasets into actionable outputs. These capabilities enable a shift from highly individualized, expert-dependent diagnoses toward reproducible, scalable, and data-informed decision support systems. Importantly, AI does not replace nematological expertise; rather, it augments it by increasing throughput, reducing variability, and enabling broader access to high-quality diagnostics. AI has potential to support screening of complex samples, generate consistent risk categorizations, and produce clear, stakeholder-oriented summaries. Key challenges include validation, transparency, and maintaining biological accuracy will be addressed within the context of real-world implementation. Positioned within the broader transformation outlined by the symposium, this talk demonstrates how AI moves from concept to practice serving as the operational bridge between emerging technologies and the day-to-day realities of nematode diagnostics, Extension delivery, and agricultural decision-making.

Beech leaf detection using computer vision tools

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Abstract Beech leaf disease (BLD) is an emerging threat to forests across the eastern United States and Canada. Current surveillance relies on visual identification of characteristic leaf banding, a method that can miss early infections. To address this limitation, we developed deep learning neural network models capable of distinguishing between BLD symptomatic leaves and asymptomatic leaves. A primary dataset of symptomatic and asymptomatic leaves collected in Maryland was used for model development and an independent set of images collected in North Carolina, Ohio, and New England provided real-world validation. In the primary dataset model testing, EfficientNetV2-Small was the most accurate model (100%), followed by ResNet50 (99.32%), MobileNetV3-Large (97.95%), and InceptionV3 (94.88%). Testing on the independent dataset also identified EfficientNetV2-Small as the most accurate model (96.55%). Grad-CAM visualizations confirmed that EfficientNetV2-Small focused on banded regions of BLD leaves that are a main characteristic of the disease. These findings demonstrate the potential of deep learning and computer vision approaches to support more efficient monitoring of BLD in forested regions.

Strengthening diagnostics for regulated nematodes: PPCDL's role in method development and federal confirmation

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Abstract Protecting U.S. agriculture from invasive and high-consequence plant-parasitic nematodes requires coordinated survey efforts, reliable diagnostic tools, and timely regulatory actions. USDA APHIS Plant Protection and Quarantine (PPQ) provides national leadership in survey coordination, regulatory oversight, and emergency response. Within PPQ's Science and Technology core functional area, the Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL) serves as the laboratory responsible for confirmatory biochemical and molecular diagnostics for federally regulated plant pathogens, including nematodes of concern. Although initial detections originate from programs such as the Cooperative Agricultural Pest Survey (CAPS), port-of-entry inspections, and state regulatory laboratories, PPCDL strengthens the diagnostic foundation that supports these activities. With more than 120 nematode taxa listed in the U.S. Regulated Plant Pest Table, the laboratory develops, evaluates, and refines molecular methods for accurate identification of regulated nematodes, including conventional PCR, real-time PCR, Loop-mediated isothermal amplification (LAMP), Recombinase Polymerase Amplification (RPA), next-generation sequencing, and whole-genome sequencing. Method development emphasizes sensitivity, specificity, reproducibility and fitness for use appropriate for regulatory decision-making. Validated diagnostics are shared with partner laboratories through standardized documentation, collaborative reviews, and targeted laboratory training. PPCDL further promotes national diagnostic competency by conducting proficiency-testing, maintaining quality-controlled reference controls, and providing technical guidance to state, federal, and academic laboratories participating in PPQ-supported detection programs. When a sample is suspected to contain a regulated or potentially actionable nematode, diagnosticians work with State Plant Health Directors (SPHDs) and State Plant Regulatory Officials (SPROs) to initiate the APHIS confirmation process. Required steps include completion of PPQ Form 391, tracking through the Agriculture Risk Management (ARM) system, and adherence to APHIS-approved packaging, biosafety, and chain-of-custody procedures. PPCDL performs confirmatory diagnostics using validated assays and quality-managed procedures, producing results that support regulatory decisions such as quarantine actions, trace investigations, and pest status determinations. Through integrated method development, diagnostic training, maintaining proficiency, and authoritative federal confirmation, PPCDL provides a critical link between field detections and regulatory actions. This work strengthens national capacity to detect and manage regulated nematodes, safeguarding agriculture and natural resources while facilitating safe trade.

Velvet bean as a nematode-suppressive rotational cover crop for sweetpotato production across five farms in Hawaii

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Abstract Field trials with farmers were conducted to evaluate the benefits of velvet bean as a rotational cover crop for sweetpotato production in Hawai'i. Velvet bean (*Mucuna pruriens*) is a common tropical cover crop valued for its high biomass, nitrogen fixation (4.7% tissue nitrogen), weed suppression, drought tolerance, and nematode suppressive properties. We conducted on-farm trials at five sweetpotato farms across O'ahu, Hawai'i, and Kaua'i islands to evaluate a bristle-free, bushing-type velvet bean (VB) against buckwheat (BW, *Fagopyrum esculentum*) as a rotational cover crop for (1) suppression of reniform nematode (*Rotylenchulus reniformis*), (2) soil health enhancement, and (3) sweetpotato yield improvement. Farms spanned a gradient of organic management history: long-term organic with high inputs, organic with low inputs, organic following a fallow period, newly initiated organic, and a non-organic sweetpotato-pasture rotation (which did not complete the trial). Three replicated plots per treatment were evaluated at cover crop planting, sweetpotato planting, and sweetpotato harvest at each site. All parameters were calculated into response ratio ($rr = \bar{x}_{(VB)} / \bar{x}_{(BW)}$) for each farm site. Across the three farms with established organic practices, reniform nematode populations were reduced in VB plots at sweetpotato harvest relative to BW with rr of 0.88 ($p = 0.60$), 0.39 ($p \leq 0.001$), and 0.45 ($p = 0.25$). In the newly initiated organic farm, VB had higher reniform nematode populations at sweetpotato harvest compared to BW ($rr = 1.69$, $p = 0.02$); however, at this farm the VB plants were under a row cover while the BW plants were not, which may confound the results. At cover crop termination, the long-term organic farm showed VB-driven increases in bacterivorous ($rr = 2.94$, $p \leq 0.01$) and fungivorous ($rr = 3.25$, $p \leq 0.01$) nematodes relative to buckwheat, indicating stimulation of the soil decomposer community by VB, though these differences were not sustained through sweetpotato harvest. Marketable yield was approximately 57% higher under VB at the low-input and previously fallowed organic farms ($rr = 1.58$ and 1.57 ; $p = 0.047$ and $p \leq 0.01$, respectively) but was not improved ($p > 0.05$) at the long-term high-input farm ($rr = 0.93$) or the newly initiated farm ($rr = 0.86$). Soil nitrogen, aggregate stability, and microbial respiration showed no consistent directional responses to cover crop treatment across farms. These on-farm trials show that VB's effect on plant-parasitic nematodes and sweetpotato yield is variable and possibly related to degree of organic management. Lack of reniform nematode suppression at the newly established organic farm may reflect difference in the soil biology at that site, pointing to an underlying biological mechanism of how VB suppress plant-parasitic nematodes that required further investigation. These on-farm trials demonstrate that the agronomic performance of VB varies by farm history and management context, and careful decision making is warranted before VB should be recommended.

Limitations and potentials of anaerobic soil disinfestation as a practical method in perennial cropping systems

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Abstract Almond, grape, pistachio, and walnut are grown on over 1.3 million ha of productive land in California. These crops are vulnerable to infections caused by plant-parasitic nematodes and other soil-borne pathogens. The development of resistant and tolerant rootstocks to specific nematode and pathogen species has provided some relief, but developing resistance to multiple soil-borne pathogens remains challenging. Soil fumigation with methyl bromide until its ban in 2005, and afterward with 1,3-dichloropropene, often combined with chloropicrin, has reduced the impact of soil-borne plant diseases. Increasing restrictions on soil fumigation require alternative preplant soil treatments. In anaerobic soil disinfestation (ASD), easily decomposable organic matter (often rice bran) is spread on the soil surface, incorporated, and the soil is kept near moisture saturation with drip irrigation under a totally impermeable film (TIF) for about one month. In several field experiments involving *Pratylenchus vulnus*, *Meloidogyne incognita*, and various perennial crops, optimal rates of rice bran per ha and watering requirements were determined. Typically, the most consistent nematode suppression was achieved with 20.2 metric tons of rice bran per ha. Similarly, efficacy was most consistent when initial soil saturation with 150 L m² was followed by 25 L m² every other day for the incubation period. In these studies, nematode suppression was measured when ASD treatments were initiated in July, August, September and October. The efficacy was different according to the source of inoculum of *M. incognita*: ASD effectively reduced root gallings on post treatment planted susceptible Prunus rootstock when inoculum source was infested soil or infected Prunus roots but was less effective when infected grape roots were the source. The use of nematode-resistant cover crop, e.g., Sudangrass ‘Piper’, biomass as a substrate for ASD was tested in walnut plots. After using traditional incorporation with a plastic tarp cover, walnut tree growth and early yield were comparable to plots pretreated with fumigation. In almond, an alternative method was tested using a moldboard plow to bury the substrate at plow depth and to utilize the moisture-saturated soil layer above the substrate to exclude atmospheric oxygen. Almond tree growth in these treatments was comparable to that in fumigation comparatives. In summary, substrate amount, watering amounts, population densities left behind from previous crops, and expenses due to the use of plastic were identified as limitations of ASD, and new, more cost-effective protocols were developed and have potential to enhance the utility of this biorational method.

Deciphering biological suppression of Reniform nematode (*Rotylenchulus reniformis*) in sweetpotato production following velvet bean cover crop

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Abstract Velvet bean (*Mucuna pruriens*) is widely cited as a nematode-suppressive cover crop, yet the biological basis of suppression has not been fully explored. Two studies were conducted to evaluate effects of velvet bean on the soil microbiome and suppression of reniform nematode (*Rotylenchulus reniformis*). The first study assessed soil health factors and nematode community dynamics in a cover crop-sweetpotato rotation, contrasting velvet bean with sunn hemp, sorghum, and bare ground in a previously fallow field. Multivariate analyses of nematode communities, soil physical indicators, and phospholipid fatty acid (PLFA) profiles showed that soil biological structure was strongly season-dependent, with cover-crop effects emerging primarily in the first season. Velvet bean suppressed reniform population growth during the first cover-crop cycle ($P \leq 0.05$; response ratio, rr, relative to bare ground = 0.33), and the suppression coincided with elevated bacterivorous (rr = 3.4) and fungivorous (rr = 2.8) nematodes during the sweetpotato season and elevated total fungal (rr = 8.11) and saprophytic fungal (rr = 5.6) PLFA biomass at sweetpotato planting ($P \leq 0.05$), indicating a transient shift toward a more fungal-dominated soil food web. During the second season, reniform populations converged across treatments to a suppressed level, and soil biological indicators were driven largely by seasonal environmental variation rather than cover-crop treatment. These results indicated that velvet bean can enhance fungal-associated soil processes and suppress reniform nematodes under certain field conditions. To further examine whether velvet-bean-associated suppression was linked to shifts in the soil microbiome, a second experiment was conducted at a nearby location to evaluate microbial community structure and nematode suppressiveness during two rotations of cover crop and sweetpotato. Velvet bean, cowpea (a non-suppressive legume), and bare ground were compared in a randomized complete block design with four replicates, and rhizosphere microbiomes, nematode communities, and nematode suppressiveness were assessed at cover-crop planting and termination, mid sweetpotato season, and harvest. During the cover-crop phase of the first season, reniform nematode populations increased in cowpea plots relative to bare ground ($P \leq 0.05$, rr = 4.1) but remained low in velvet bean plots (rr = 0.8). During the first sweetpotato season, treatment effects were not statistically significant, but cowpea plots consistently supported higher reniform populations than bare ground (midpoint rr = 1.51; harvest rr = 1.48), while velvet bean plots remained lower (mid-season rr = 0.74; harvest rr = 0.86). Nematode community analyses showed elevated bacterivore populations in both cowpea (rr = 4.2) and velvet bean (rr = 4.6) after cover cropping. A greenhouse bioassay testing reproduction of reniform nematode on sweetpotato grown in field soil showed all field soil suppressed nematodes relative to sterilized soil, with no difference in suppression driven by cover crop treatment. The second season is underway, and forthcoming microbial barcoding analysis will examine whether suppression correlates with microbiome shifts.

Root lesion nematode effectors target distinct host cellular compartments to facilitate host parasitism

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Abstract Root lesion nematodes (*Pratylenchus* spp.) are globally destructive migratory root endoparasites with limited sustainable management options to mitigate their negative impacts on high-value staple crops. Despite their economic importance, the effector biology of root lesion nematodes (RLNs) remains poorly characterized and thought to lack complexity relative to sedentary plant-parasitic nematodes. Through transcriptomic profiling and functional analyses, we uncover the largest RLN effector repertoire reported to date, comprised of over 50 gene “families”, including a considerable number of pioneer genes for *Pratylenchus fallax* and *P. penetrans*. Transient expression of effector-fluorescent protein fusions in *Nicotiana benthamiana* reveals that RLN effectors target distinct host cell compartments, including the cytoplasm, endoplasmic reticulum, nucleus, nucleolus, vesicle-like structures, and organelle-like compartments, indicating diverse subcellular sites of function. As a result of effector functional characterization, we identified *Effector2901* as a critical gene required for parasitism. Gene expression profiling indicates that *Effector2901* is expressed during the early stages of root infection, while *in planta* subcellular localization assays demonstrated nuclear and nucleolar localization. Stable *Effector2901* overexpression lines of *Solanum tuberosum* resulted in host transcriptional regulation, impacting molecular pathways related to hormone signaling, ribosome biogenesis, growth and development, and defense-associated pathways. These overexpression lines also supported significantly higher densities of nematodes, indicating increased host susceptibility. Conversely, RNAi-mediated silencing of *Effector2901* markedly reduced nematode infection success, confirming its essential role in virulence. These results provide novel and more intricate insights into the molecular components that drive RLN parasitism, highlighting key effector targets that could be exploited in RNAi-based management strategies across different RLN species.

Meloidogyne konaensis (= *M. paranaensis* jr. syn) an international problem: Its current status, virulence profile and ongoing efforts for control

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Abstract *Meloidogyne konaensis* is a devastating international pest of coffee. A recent study determined that *M. paranaensis* is a junior synonym of *M. konaensis*. *Meloidogyne konaensis* was previously thought to be restricted to the island of Hawaii. However, with the synonymization with *M. paranaensis*, *M. konaensis* has a distribution throughout Central and South America, Hawaii, and possible incursions in the United States. In addition, emerging evidence suggests natural hosts of *M. konaensis* may include ornamental and weedy plants such as *Brugmansia* sp., *Caladium* sp., *Amaranthus* sp. and numerous other species. Further, *M. konaensis* has a unique ability to change esterase phenotype (F1-S1 or S2) from a single egg mass population (F1). The change was often associated with a reduced virulence on coffee and increased virulence on tomato, and eggplant. However, a wild type alternative esterase phenotype (F1-S1) from Guatemala was more aggressive on coffee and the overall virulence mechanisms of the species remain unknown. To improve nematode control, several approaches are being approached simultaneously. A long-established practice is to graft the desirable *Coffea arabica* cultivar Guatemala typica onto resistant or tolerant rootstock such as *C. canephora* or *C. liberica*. More recently, screenings of Ethiopian *C. arabica* varieties have shown resistance to *M. konaensis*. To reduce the cost of resource intensive and time-consuming whole plant evaluations, a current initiative is to search the transcriptome for upregulated and down regulated genes to identify gene variants that may suggest nematode resistance in coffee. Control of *M. konaensis* may be accelerated with work previously conducted with *M. paranaensis* (junior synonym).

Nitrogen-fixing rhizobia influence their host plant's response to the parasitic root-knot nematode *Meloidogyne hapla*

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Abstract Nearly all plants rely on microbial symbionts that provision key nutrients. For example, nearly all plants in the bean family (family Fabaceae) recruit nitrogen-fixing bacteria (rhizobia), from which they receive bioavailable nitrogen in exchange for carbon. In a series of experiments, we found that nitrogen-fixing rhizobia influence the plant *Medicago truncatula*'s response to the root-knot nematode *Meloidogyne hapla*. We performed three complementary experiments: First, we performed a dual-RNAseq experiment to measure how nematodes influence gene expression in the root nodules of plants inoculated with different rhizobia. We identified 475 and 1,465 differentially expressed genes in nodules occupied by the two different strains, respectively, indicating that root-knot nematodes modify gene expression in this symbiotic organ. Furthermore, we found that for 85% of differentially expressed genes, their response to nematode infection depended on the rhizobia strain that occupied the nodule. Second, we performed a quantitative genetic experiment to estimate the contribution of the rhizobia genome to their host's parasite resistance, tolerance, and virulence. We found that nematode resistance and nematode virulence differed among plants inoculated with different rhizobia strains, indicating that rhizobia influence the outcome and cost of infection by this parasite. Finally, we tested whether nematode infection impacts resource exchange between the host plant and its rhizobia. We compared the nitrogen-for-carbon exchange rate between nematode-infected and uninfected plants. We found that the impact of parasite infection on the N:C exchange rate strongly depended on the plant genetic background. In one plant accession, nematode infection resulted in a more plant-beneficial exchange rate (higher N:C), while in another, nematode infection resulted in a more rhizobia-beneficial exchange rate (lower N:C). Our work suggests that microbial symbionts are major mediators of their host plant's response to parasitic nematode infection.

Impact of pesticide exposure on the nematode community in turf soil

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Abstract Turfgrass covers approximately 2% of U.S. land and plays an essential role in urban and suburban habitats. Like other cultivated plants, turfgrass is often subject to management efforts alleviating abiotic and biotic stresses, including pest and disease infestations. The management of turfgrass health has primarily relied on chemical control, especially in high-input turf such as golf courses that have high expectations for turf quality and low tolerance to damage. Such practices may interrupt the balance of microbial communities, including nematodes, in the soil environment. The objective of this study was to evaluate the impact of pesticide exposures on the nematode community in turf soil with different pesticide application regimes. Soil samples were taken from greens, fairways, roughs, and unmanaged weedy areas, representing high, moderate, low, and no pesticide exposure, respectively, at two golf courses. Using the baiting method with *Galleria mellonella* larvae, greens had the lowest number of dead insects infected with entomopathogenic nematodes (EPNs), which were mostly identified as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* stains molecularly. In addition, nematodes were extracted from soil samples using the centrifugal sugar floatation method and identified morphologically. Different from the trends of EPNs, the number of bacterivores was the highest (averaging 752–1008/100 cm³ soil) in greens, significantly higher than roughs (280–450) and weedy areas (208–342), indicating the presence of other bacteria-feeding nematodes in addition to EPNs, likely associated with rich organic matters and thatch. Types of plant-parasitic nematodes (PPNs) varied with locations; however, spiral (35.9–41.6%) and ring nematodes (43.9–60.0%) were the predominant types at both courses. In general, most types of PPNs did not differ with sampling sites, except that at one course, weedy areas had lower total PPNs, especially spiral nematodes, than other sites. These findings confirm the negative impact of pesticide applications on nematode communities, especially EPNs and possibly PPNs, and support the use of more eco-friendly tactics for sustainable turfgrass health management.

Potential of rotational cover crops for managing *Heterodera schachtii*

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Abstract Sugar beet cyst nematode (SBCN), *Heterodera schachtii*, is a major constraint to sugar beet production in the United States, and the use of cover crops represents a promising strategy for sustainable nematode management. This study evaluated the host status and management potential of twelve cover crop species and cultivars under greenhouse and microplot conditions. The cover crops included oilseed radishes ‘Concord’, ‘Control’, ‘Defender’, ‘Nitro’, ‘Image’, ‘Select’, ‘Respect’, daikon radishes ‘Eco-Till’, ‘Enricher’, white mustard ‘Master’, black oat ‘Pratex’, Wheeler rye, susceptible sugar beet, and a fallow control. Greenhouse experiments were conducted using a randomized complete block design with five replications. Each pot was inoculated with approximately 2,000 SBCN eggs, and cyst, egg, and juvenile populations were assessed two months after inoculation. In the greenhouse, oilseed radish cultivars ‘Concord’ and ‘Control’, as well as daikon radish ‘Eco-Till’, consistently exhibited non-host status, with no cyst or egg production observed. In contrast, white mustard ‘Master’ supported higher cyst and egg production, indicating susceptibility. Although statistical differences among treatments were not significant, clear biological trends were observed among cultivars. Microplot trials were conducted over two growing seasons to evaluate the effects of these cover crops under semi-field conditions. Treatments included all cover crops listed above and a fallow control, arranged in a randomized complete block design. In 2023, daikon radish ‘Eco-Till’ was the only treatment that reduced egg and J2 numbers compared to the fallow control, with a 3.7% decrease. In 2024, numerical reductions in SBCN populations were observed for several treatments. Daikon radish ‘Eco-Till’ reduced egg and juvenile densities by 65.8%, and white mustard ‘Master’ reduced them by 67.0% compared to the fallow control. However, due to variability among replicates, treatment effects were not statistically significant. Overall, host status varied substantially among cover crop species and cultivars, even within closely related groups. Oilseed radish cultivars ‘Concord’ and ‘Control’, along with daikon radish ‘Eco-Till’, demonstrated the greatest potential for inclusion in SBCN management programs due to their non-host or suppressive characteristics. These findings highlight the importance of cultivar-specific selection when integrating cover crops into crop rotation systems for nematode management. Further field-scale evaluations are needed to validate these results and to develop region-specific recommendations for growers seeking sustainable SBCN management strategies.

Effects of fluopyram and organic amendments on nematodes and tree performance in cherry orchards

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Abstract Plant-parasitic nematodes, particularly *Pratylenchus penetrans*, are persistent constraints to cherry (*Prunus* spp.) production in the Great Lakes region, and effective post-plant management options remain limited. This study evaluated the effects of fluopyram (Velum[®] Prime), organic amendments, and their combinations on nematode communities, tree growth, and yield in established cherry orchards. Field trials were conducted at two Michigan research stations using a randomized complete block design with six treatments: (1) Velum[®] Prime soil drench, (2) Dairy Doo[®] compost + straw mulch, (3) Layer Ash Blend[®] compost + straw mulch, (4) Dairy Doo[®] compost + straw mulch + Velum[®] Prime, (5) Layer Ash Blend[®] compost + straw mulch + Velum[®] Prime, and (6) an untreated control. Each treatment was replicated four times. Nematode populations, including plant-parasitic species (*P. penetrans*, *Mesocriciconema* spp., and *Xiphinema americanum*) and non-parasitic groups (bacterivores and fungivores), were assessed using reproduction factors (RF), and trunk diameter growth and fruit yield were measured. In the first year, compost-based treatments and fluopyram reduced RF of *P. penetrans*, although differences were not always statistically significant, whereas significant reductions were observed for *Mesocriciconema* spp. No significant treatment effects were detected for *X. americanum*, likely due to high spatial variability. Fluopyram alone significantly reduced bacterivorous and fungivorous nematodes initially, while compost-based treatments maintained or enhanced these groups, indicating improved soil biological activity. In the second year, treatment effects on nematode populations were generally less pronounced, suggesting stabilization of the soil system over time. Tree growth responses varied by site and year. At the Northwest site, Velum[®] Prime and its combinations with Layer Ash Blend[®] compost resulted in greater trunk growth in the first year, while compost-only treatments, particularly Layer Ash Blend[®] + straw mulch, showed stronger effects in the second year. At the West Central site, cultivar-specific responses were observed, with ‘Emperor Francis’ showing significant treatment effects and ‘Ulster’ showing no significant differences. Yield responses were limited in the first year but became more evident in the second year. At the Northwest site, the Layer Ash Blend[®] compost + straw mulch + Velum[®] Prime treatment produced the highest yield, significantly exceeding the untreated control. At the West Central site, yields were low due to the young age of the orchard, but Layer Ash Blend[®] + straw mulch significantly improved yield compared to the control. Overall, these findings demonstrate that integrating fluopyram with organic amendments can provide effective post-plant nematode suppression while supporting soil biological function and improving orchard performance. This integrated approach offers a promising and sustainable strategy for managing nematodes in established cherry production systems.

QTL mapping and kasp marker development for resistance to *Pratylenchus neglectus* in triticale

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Abstract The root-lesion nematode, *Pratylenchus neglectus*, is a significant constraint to wheat production worldwide; however, effective host resistance in wheat remains limited. Triticale, a wheat-rye amphiploid, offers a promising but underexplored source of resistance due to its rye-derived defense traits. The objective of this study was to identify quantitative trait loci (QTL) associated with resistance to *P. neglectus* in triticale and to develop molecular markers for efficient introgression of resistance into wheat via marker-assisted selection. A mapping population of 137 triticale recombinant inbred lines (RILs), derived from a cross between the susceptible cultivar Siskiyou and the resistant cultivar Villax St. Jose, was evaluated for resistance to *P. neglectus*. Genotyping-by-sequencing generated 1,054 high-quality single-nucleotide polymorphism (SNP) markers, which, together with seven simple sequence repeat (SSR) markers, were used to construct a genetic map comprising 21 linkage groups covering the triticale genome. QTL analysis consistently identified a resistance locus on the rye-derived chromosome 5R, explaining approximately 20% of the phenotypic variation across experiments. A Kompetitive allele-specific PCR (KASP) marker (*fcp1070*) was developed from the SNP strongly associated with the QTL, enabling rapid and high-throughput selection of the resistance allele. This marker reduces reliance on labor-intensive phenotyping and facilitates efficient screening for *P. neglectus* resistance. To our knowledge, this is the first report of a mapped major QTL for root-lesion nematode resistance in triticale. These findings provide a valuable genetic resource and a practical molecular tool to facilitate introgression of the 5R resistance allele into wheat via marker-assisted selection combined with chromosome engineering, to broaden the genetic basis for nematode resistance in cereal crops.

Identifying protein interactions involved in root-knot nematode feeding tube assembly

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Abstract Root-knot nematodes (RKN, *Meloidogyne* spp.) represent the most economically damaging group of plant-parasitic nematodes, parasitizing most crop plants and cause billions of dollars of yield losses annually. To effectively parasitize host plants, RKNs use a mouth stylet to secrete effectors into root vasculature cells to induce elaborate feeding sites called “giant-cells” from which adult females obtain nutrients to develop and produce hundreds of eggs. To facilitate efficient nutrient uptake, adult female stylet secretions produce a unique crystalline tube-like structure within the giant-cell cytoplasm called a “feeding tube” (FT), which functions as an extension of the nematode’s stylet. Upon formation, FTs are immediately enveloped by host endomembrane system, which is hypothesized to facilitate the formation of the FT and increase nutrient uptake. Recent work in our lab has identified a family of *M. incognita* dorsal gland-produced stylet-secreted effectors, including Minc03784, that is localized to FTs, representing the first identified component of RKN FTs. We hypothesize that Minc03784 interacts with itself or family members, other effectors localized to the FTs, and/or host endomembrane proteins to assemble the FT. To identify interactions of FT proteins with host proteins, we are coupling FT-enriched proteomics data analyses with co-immunoprecipitation studies. Gene ontology enrichment analysis of the FT-enriched host proteome indicated several high-confidence proteins localized to the ER/Golgi. For co-immunoprecipitation studies, *Minc03784* without its signal peptide (Δ SP) has been cloned into an overexpression vector and is being used to generate composite plants. In preliminary composite plant experiments, no significant difference in root growth was observed in roots expressing *Minc03784* ^{Δ SP} compared to the empty vector controls. These plants are being tested to determine how Minc03784 may affect RKN infectivity and will be used for co-immunoprecipitation of interacting proteins using an anti-Minc03784 antibody. A better understanding of FT protein interactions will provide insight into the assembly and function of these structures.

Nematode advisory service in North Carolina department of agriculture & consumer services

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Abstract The Agronomic Division of the North Carolina Department of Agriculture & Consumer Services has a publicly-operated nematode assay lab providing nematode diagnostic, advisory, and management support to protect the state's agricultural productivity from plant-parasitic nematodes. The lab started from 1974 and has 12 full-time employees analyzing approximately 45,000 samples annually. From March 2, 2026, a new fee (\$10 per sample for routine sample, \$40 for molecular diagnosis) has been implemented. This service plays a critical role in identifying nematode species affecting a wide range of crops, including sweet potato, tobacco, soybean, cotton, turfgrass, vegetables, fruit trees and horticultural plants. Through systematic soil sampling, laboratory analysis, and accurate species identification, the program delivers timely and science-based recommendations tailored to growers' specific field conditions. By integrating research-based thresholds and region-specific data, the Nematode Advisory Service helps farmers implement effective management strategies such as crop rotation, resistant varieties, nematicide applications, and cultural practices. The service also contributes to ongoing surveillance and data collection, enabling better understanding of nematode distribution and population dynamics across North Carolina. In addition to direct grower support, the program collaborates with extension agents, researchers, and industry stakeholders to promote education and outreach on nematode management. Ultimately, the Nematode Advisory Service enhances sustainable agricultural practices, reduces crop losses, and supports the economic viability of North Carolina's farming systems.

Emerging threat of virulent root-knot nematodes to high tunnel tomato production

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Abstract Root-knot nematodes (RKN; *Meloidogyne* spp.) are among the most damaging soilborne pests of vegetable production, particularly in high tunnel systems where elevated soil temperatures and moisture favor their overwintering and rapid population buildup. While growing RKN-resistant tomato cultivars or grafting onto RKN-resistant rootstocks is effective in managing RKN, current options rely almost exclusively on a single resistance gene, *Mi-1*. We identified three *Mi-1*-virulent *Meloidogyne incognita* populations from galled resistant tomato rootstocks in Indiana high tunnels between 2021 and 2024. These isolates caused severe galling and reproduced on *Mi-1*-resistant tomato cultivars, but they failed to reproduce on both “susceptible” pepper cultivars lacking the *N* resistance gene and resistant pepper cultivars carrying the *N* gene. Root penetration assay showed that second-stage juveniles (J2) penetrated “susceptible” and resistant pepper roots at similar rates, indicating that resistance in peppers acts post-penetration. Microscopic observation showed hypersensitive response-like tissue damage in pepper root-tips inoculated with the *Mi-1*-virulent isolate, with nematode development arrested at the early parasitic J2 stage. This reciprocal virulence pattern suggests a fitness cost associated with overcoming *Mi-1* resistance in tomato. In parallel, we evaluated 13 commercial tomato rootstocks in greenhouse trials inoculated with both virulent and avirulent *M. incognita* populations. All rootstocks effectively suppressed the avirulent *M. incognita* population. However, none provided complete control over the virulent populations. We then grafted tomato ‘BHN589’ onto each of the 13 rootstocks and evaluated their performance in a commercial high tunnel where virulent *M. incognita* was identified. In the virulent *M. incognita* infested high tunnel, severe galling occurred on all plants, with no significant differences among rootstocks. Overall, our findings highlight the vulnerability of relying solely on grafting for RKN management in high tunnel tomato production. Integrated strategies, including rotation with pepper crops, even susceptible cultivars, may provide an effective and economical approach to suppress virulent *M. incognita* populations and prolong the durability of *Mi-1*-mediated resistance.