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**Project Title:** Variability, Adaptation, and Management of Nematodes Impacting Crop Production and Trade

**State:** Alabama Kathy Lawrence

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**W4186 Meeting Participants:**

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**Objective 1:** Characterize genetic and biological variation in nematodes relevant to crop production and trade.

In California, *Mi-1* is the only commercially available root-knot nematode resistance gene in tomato. Mi-*1* confers resistance to three commonly occurring, damaging species of RKNs belonging to the MIG group (*M. arenaria*, *M. incognita*,and *M. javanica*) in tomato. In addition to nematodes, Mi-1 also confers resistance to some isolates of potato aphid (*Macrosiphum euphorbiae*) and whitefly (*Bemisia tabaci*). The increased reliance on Mi-1 due to the restricted use of nematicides has led to the emergence of resistance-breaking nematode isolates in tomato fields, posing a serious threat to tomato production. The objective of this work was to elucidate the genetic mechanisms and identifying genetic factors that allow nematodes to break Mi-1-mediated resistance and to investigate the potential fitness cost associated with breaking this resistance. We used two closely related strains of *M. javanica*, VW4 and VW5, which differ in their ability to parasitize tomato carrying Mi-1. VW4 cannot reproduce on tomato plants harboring Mi-1. By contrast, VW5 can reproduce on tomato carrying the Mi-1 gene. To assess whether the ability of VW5 to reproduce on Mi-containing tomato was associated with a loss of fitness, we inoculated tomato that does not carry Mi-1 with VW4 or VW5 and counted the number of eggs at 35 dai. We observed a significantly reduced egg number on plants inoculated with VW5 compared to VW4 indicating that fitness was reduced on tomato for the resistance breaking strain. To see if this loss of fitness extended to additional hosts, we compared egg production of VW4 and VW5 on cucumber [*Cucumis sativus*] and rice [*Oryza sativa*]). For both of these hosts, the fitness loss was greater than on tomato and very few eggs were produced. The quality of current published reference genome for *M. javanica* is not sufficient to allow resolution of the homeologous genomes. To address this limitation, we sequenced and assembled a high-quality reference genome for VW4 using a combination of HiFi, Hi-C, Iso-seq, and NanoPore sequencing. The scaffold numbers of our reference genome are close to those of the *M. javanica* chromosome indicating that several of the scaffolds are likely to span entire chromosomes. In addition, we have also sequenced the genome of VW5 using PacBio’s HiFi technology. Future work will focus on using a long-read aligner to map VW5 reads to the genome of VW4 and identify regions that differ between them.

States in the Northwestern region of the USA (WA, OR, ID) produce more than 50% of the potatoes in the country. *Meloidogyne chitwoodi* (also known as the Columbia root-knot nematode) is a major problem for potato producers in this region because the nematode infects tubers and causes blemishes. There is little tolerance for *M. chitwoodi* damage in the potato industry. To easily identify *M. chitwoodi* from field soils, the Gleason lab (WSU) developed a LAMP assay. The LAMP assay is a quick DNA-based detection method for potato-infecting root-knot nematodes (Zhang and Gleason 2019). The LAMP assay reliably detects two root-knot nematodes: *M. chitwoodi* and its close relative *M. fallax*. Because *M. fallax* is not found in the USA, a positive result in the LAMP assay would indicate the presence of *M. chitwoodi*. Nevertheless, a report of *M. fallax* in California turfgrass has raised the alarm about the potential introduction of this virulent, quarantine nematode into potato growing areas of the US (Nischwitz et al., 2013). Another related root-knot nematode called *Meloidogyne minor* was found in a Washington golf course (Nischwitz et al., 2013). *Meloidogyne minor* can also infect potatoes, although it has not been detected yet in US potato fields. In order to develop a single tube nematode assay that can distinguish *M. chitwoodi, M. minor,* and *M. fallax*, the Gleason lab has developed a molecular beacon assay. This PCR assay utilized the small genetic variation of the heat shock protein 90 (*HSP90*) gene to generate species specific probes. The molecular beacon assay can determine the species of a single nematode. This is the first single tube assay that can distinguish these three potato-infecting nematodes, two of which are of regulatory importance.

Within the species of *M. chitwoodi,* there are three major strains in the Pacific Northwest, and they are called Race 1, Race 2, and Roza. The different strains of *M. chitwoodi* are an on-going issue because all can infect potatoes. However, there are important differences between them. For example, Race 1 and Roza cannot reproduce on alfalfa, but can reproduce on carrot. Meanwhile, Race 2 can reproduce on alfalfa but not carrot. In addition, Roza is able to overcome the nematode resistance found in the potato breeding line PA99N82–4, but Race 1 and Race 2 cannot. Using the genetic variability between races, we developed PCR markers that can differentiate these three races. Using this PCR-based assay, the Gleason lab found that Race 1 and Roza were the predominant strains in Eastern Washington. This information has implications for future resistance breeding efforts.

In Idaho, they have recently initiated a project to determine the genetic diversity found in *Globodera* spp. so that we can target appropriate sources of resistance to encompass the genetic diversity found in *Globodera* spp. for development of resistant potatoes suitable for US growers and potato industry. The pathotype of 10 populations from Peru is being characterized through the use of a set of potato differential lines containing different resistance genes. One experiment has been terminated and is not being repeated. In addition, samples of Bolivian *Globodera* spp. are being genetically characterized. We also have set up one experiment in Bolivia to phenotype for resistance to 3 populations of *Globodera* (1 *G. rostochiensis*, and 2 *G. pallida*).

In Michigan they are working to understanding interactions of the northern root-knot (NRKN) and other plant-parasitic nematodes’ (PPN) parasitic variability (PV) in the same environment as beneficial (bacterivore, fungivore, predator and omnivore) nematodes in diverse cropping systems with varying degrees of soil health degradations is difficult. In this regard, the community analysis-based soil food web (SFW), fertilizer use efficiency (FUE), and integrated productivity efficiency (IPE) models are important decision-making tools for translating basic and complex science into practical application. The SFW model describes soil conditions of a given environment or in response to agricultural practice treatments by measuring changes in trophic and colonizer-persister (cp) groups (functional guilds) relative to food and reproduction against resistance to disturbance. With the SFW model, we established that NRKN was distributed in mineral and muck soils with disturbed and/or degraded soil health conditions and isolated 9 populations for PV test (Lartey et al., 2021). The degraded soils were characterized with low N. Knowing that there is link between NRKN presence and soil health conditions provides a foundation for formulating potential management strategies.

At University of Nebraska we have been developing metabarcoding methods and testing them against single nematode, Sanger sequencing methods for community diagnostics. This comparison has included cultivates, restored, and unplowed prairie land. For aquatic nematodes we have compared nematode communities from western Nebraska lakes with pH values ranging from 7.4 to 10.5. With regards to nematodes as environmental indicators, we are working on a set of soil samples that were affected by a major contamination event associated with an ethanol production facility that specialized in extracting ethanol from unsold, coated seeds in North America.

In New Mexico, the turf industry is an important partner in monitoring the movement of plant parasitic nematodes. Historically, root-knot nematodes in turf have only been isolated from locations in southern New Mexico. Species determination is currently underway for a root-knot nematode isolated from a commercial bentgrass turf sample taken from northern New Mexico. Of interest is if this is a new species to New Mexico or perhaps the beginnings of a northern expansion of our southern root-knot nematode as climate patterns shift. We continue to stay vigilant about *Meloidogyne enterolobii,* which has not yet been detected in New Mexico.

**Objective 2:**  Determine nematode adaptation processes to hosts, agro-ecosystems and environments.

In Michigan, they tested 2 populations (8 and 13) from degraded and Population 2 from disturbed mineral soil and 3 populations each from disturbed (4, 6 and 10) and degraded (5, 14 and 16) muck soil, and revealed highest (Population13), medium (Population 8) and lowest (Populations 2, 4, 5, 6, 10, 14 and 15) PV (Lartey et al., 2022). In addition to expanding our understanding of why NRKN PV is higher in mineral than in muck soils, these results show that a) there is specificity within a category of soil health degradation and b) provide a foundation for formulating hypotheses that test deeper levels of interactions between nematode PV and the biophysicochemical environment.

**Objective 3**: Develop and assess nematode management strategies in agricultural production systems.

In Alabama,we determine the yield potential of the new *M. incognita* resistance variety PHY 360 W3FE and the *R. reniformis* resistant variety PHY 332 W3FE in nematode infested fields and if there was an additional benefit of adding nematicides to the resistant varieties. In 2020 and 2021, eight field trials were established in nematode infested fields and arranged as a RCBD with five replications. A Vydate® C-LV and Fluazaindolizine mixture was applied at planting as an in-furrow spray across two resistant cultivars, PHY 360 W3FE and PHY 332 W3FE, and a susceptible cultivar, PHY 340 W3FE to further reduce nematode population levels. Field trials indicated that both *M. incognita* and *R. reniformis* eggs per gram of root were significantly (P > 0.05) lower on the resistant cotton cultivars, PHY 360 W3FE and PHY 332 W3FE, at 45 days after planting compared to the control PHY 340 W3FE without nematicides. *M. incognita* population levels were 84% lower on PHY 360 W3FE compared to PHY 340 W3FE and *R. reniformis* populations were 78% lower on the PHY 332 W3FE variety compared to PHY 340 W3FE. Nematode eggs per gram of root were further reduced after addition of Vydate® C-LV and Fluazaindolizine to both susceptible and resistant varieties. In the *M. incognita* tests, PHY 360 W3FE with TRiOT M + Vydate® C-LV + Fluazaindolizine at the high nematicide rate supported the greatest lint yield (1571 kg/ha), which was increased by 419 kg/ha over the lowest yielding treatment, PHY 340 W3FE + TRiOTM (1152 kg/ha). The addition of the nematicides improved yield by 34 and 15 kg/ha for PHY 340 W3FE and PHY 360 W3FE, respectively. In the *R. reniformis* tests, PHY 332 W3FE with TRiOTM + Vydate® C-LV + Fluazaindolizine at the medium nematicide rate, supported the greatest yields (2137 kg/ha) which was increased by 1288 kg/ha over the lowest yielding treatment, PHY 340 W3FE. The addition of the nematicides improved yield by 572 and 293 kg/ha for PHY 340 W3FE and PHY 332 W3FE, respectively. Overall, the use of the resistant varieties significantly increase yield while limiting nematode population density; the addition of the nematicides also further enhanced yields of the PHY 360 W3FE and PHY 332 W3FE, nematode resistant varieties.

Further studies in Alabama integrated additional fertilizer and nematicide combinations to establish economical nematode management strategies while promoting cotton yield and profit. Field trials were run to evaluate fertilizer and nematicide combinations applied at the pinhead square (PHS) and first bloom (FB) plant growth stages to reduce nematode population density and promote plant growth and yield. Cost efficiency was evaluated based on profit from lint yields and chemical input costs. Data combined from 2019 and 2020 suggested a nematicide seed treatment (ST) ST + (NH4)2SO4 + Vydate® C-LV + Max-In® Sulfur was the most effective in increasing seed cotton yields in the *R. reniformis* microplot trials. In *R. reniformis* field trials, a nematicide ST + (NH4)2SO4 + Vydate® C-LV at PHS supported the largest lint yield and profit per hectare at $1176. In *M. incognita* field trials, a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB supported the largest lint yields and profit per hectare at $784. These results suggest that combinations utilizing fertilizers and nematicides in addition to current fertility management show potential to promote yield and profit in *R. reniformis* and *M. incognita* infested cotton fields.

In Hawaii, management of plant-parasitic nematodes in pineapple typically involves fallow followed by fumigation with 1,3-dichloropropene (1,3-D) with unintended effects on soil health. Allyl isothiocyanate and crustacean meal to stimulate soil biological activity. A trial was established in a commercial field in collaboration with Dole Fruit Hawaii. CrabLife Flake (25% chitin) was applied at 0 or 785 kg/ha and incorporated. 1,3-D (300 l/ha) and allyl isothiocyanate (290 l/ha) were applied in the bed. An unfumigated, no CrabLife Flake area served as an untreated control. Soil samples were collected at pineapple planting, 3 months, and 6 months after planting. The ratio of fungi to bacteria in the at-planting samples was greatest in the untreated control and the fumigated plots receiving the CrabLife Flake. Plant-parasitic nematode populations were near 0/250 cm3 soil at planting and 3 months after planting. At 6 months after planting, the population of reniform nematodes remained low in the 1,3-D treated plots (133/250 cm3 soil) and increased in the untreated and allyl isothiocyanate treated plots (650 and 733/250 cm3 soil, respectively). Fungal feeding nematodes predominated over other free-living nematodes. Plant growth was not different among the treatments. The chitin amendment had positive effects on the fungi in the soil. Allyl isothiocyanate was effective in reducing plant-parasitic nematode populations for the first 3 months and may offer an alternative to improve soil health in pineapple production.

Hawaii also works with EPN’s on sweet potato weevil, *Cylas formicarius,* which severely damages sweet potato by burrowing through the tuber and depositing fecal pellets resulting in an unpalatable tuber to humans and livestock. Sweet potato yield losses can be up to 100%. Entomopathogenic nematodes (EPN) have potential use as an organic alternative to synthetic pesticides for the control of sweet potato weevil. Two local EPN isolates (*Steinernema feltiae* MG-14and *Oscheius sp.* Oa-12) were evaluated for efficacy against *C. formicarius*. Five larvae were placed in a petri dish lined with Whatman filter paper #1 (inoculation courts) and inoculated with either 100 Infective Juveniles (IJ) of either *S. feltiae* MG114 or *Oschieus* Oa-12 per larvae and exposed for 72 hours. Water served as a negative control. Infection courts were checked every 24 hours for larval mortality and dead larvae were transferred to White traps. EPN emergence was evaluated 21 days later. The experiment was replicated 6 times and repeated once. Larvae exposed to *S. feltiae* MG-14 had a mortality of 93% after 72 hours. Larvae exposed to *Oscheius* OA-12 had an average mortality of 43%. The negative control had a mortality of 10%. Emergence of IJ was observed from most larval cadavers from both treatments. *Steinernema feltiae* MG-14 inoculation resulted in greater mortality on the weevil larvae than *Oscheius* OA-12. The lack of emergence observed in some cadavers from inoculated plates may be due to natural death as reflected by the 10% mortality seen in the controls. Future research should examine the effectiveness the strains in field trials.

Currently, in Idaho, resistance to *Globodera pallida* in commercially relevant potato varieties for US growers does not exist. Through collaborations with breeders and plant geneticists, research has focused on breeding resistance into russet type potatoes for the US industry. Clones with partial resistance have been developed, and future efforts are focused on incorporating pyramiding different sources of resistance to achieve higher levels of resistance to *G. pallida*. We continue to screen the clones that are being developed to determine their resistance level to *G. pallida*. In addition to development of resistance through traditional breeding efforts, we have initiated a project to understand plant defenses of the trap crop, *Solanum sisymbriifolium*. We have found that exposure to different plant root exudates changes the behavior of *G. pallida*. Exposure to *S. sisymbriifolium* root exudates decreases mobility and infection rates of *G. pallida*. A transcriptome analysis revealed differences in expression of virulence and detoxification genes. Field trials have been established to develop a 3-year rotation plan for use in *G. pallida* infested fields. The rotation compares use of resistance or a trap crop in rotation with a susceptible potato variety and evaluates *G. pallida* population decline for each of the rotation plans. Our second-year rotation indicates that low levels of *G. pallida* reproduction occurred when a resistant potato was followed by a second year of a resistant potato, but no reproduction was observed when a resistant potato was planted in rotation with the trap crop S. sisymbriifolium. The third-year rotation will be planted to the susceptible potato Russet Burbank. We continue to evaluate potential nematicidal compounds isolated from the trap crop S. sisymbriifolium. High levels of solamargine have been extracted from roots of this trap crop. Fractions extracted from this plant have been found to be highly toxic to *G. pallida*, and reduce hatch, infection and reproduction of the nematode. Further fractionation and purification of this butanol and hexane fractions are being conducted and evaluated to determine their impact on *G. pallida*.

Michigan has developed an IPE model that a) expands the weighted abundance of functional guilds (WAFG) of the SFW, and integrates b) a soil health indicator (SHI) and c) the concepts of the FUE model to identify sustainability of soil health outcomes as: i) sustainable if SHI and WAFG increase (*best case*), ii) unsustainable if SHI and WAFG decrease (*worst case*) and iii) need additional measures to increase either SHI or WAFG to get to a sustainable outcome (Habteweld et al., 2022). *This is the first model to quantitatively link SHI and WAFG to a specific soil health value and the only one of its kind*. *In addition, the IPE model provides a platform for integrating a broad range of SHIs in ways that will lead to identifying soil health from a single core of soil*. The concepts of these models are being applied on on-farm studies (Kakaire et. al., 2022) accompanied by socio-cultural analysis of stakeholders (Widanagea et al., 2022) and actively promoted at national and international professional conferences (Melakeberhan and Habteweld, 2022a and b).

Maintaining the only nematode diagnostic lab for the state of New Mexico is a beneficial tool in staying informed about new introductions of plant parasitic nematodes relevant to commercial agriculture in our state. Potato tuber samples received from the Navajo Nation were recently confirmed to be infested with *Meloidogyne chitwoodi*. This nematode causes tuber defects that can significantly diminish the value of the crop and impinge on exportable destinations. Managing it is necessary. Research reported from Washington State University found that different isolates of *M. chitwoodi* differ in virulence and host range, which affects nematode management strategies. Efforts will continue to learn more about this New Mexico isolate and how/if it may vary from other isolates in the region. Root-knot nematodes are of economic significance in New Mexico agriculture, predominately we are concerned with *Meloidogyne incogntia* in the south and east where the bulk of our state agriculture is located. We do however have *Meloidogyne hapla* and *M. chitwoodi* in the northern agricultural fields of the state and *M. partityla* has been recovered from our pecan orchards.

Because access to irrigation water and arable land can be limiting factors in New Mexico, our annual row-crop fields tend are smaller on average. To be profitable on such limited acreage, farmers grow specialty crops that can withstand our climate, such as chile peppers and onion. With limited success in breeding *M. incognita* resistance into chile peppers, chile growers maintain a reliance on chemical control options to manage their persistent root-knot nematode populations. Several new chemistries have been developed in recent years that necessitate assessment in our region. A field study was conducted this year on a cayenne chile pepper crop grown in a sandy loam soil under buried drip irrigation with a history of severe root-knot nematode problems (*Meloidogyne incognita*). The objective was to assess yield and *M. incognita* population responses to different rates and timings of fluazaindolizine (Salibro® ), fluopyram (Velum® Prime) and fluensulfone (Nimitz®). Data are still being analyzed, but average *M. incognita* J2 populations at the end of the season were 28% and 13% lower in plots that received single doses of 61.4 and 30.7 fl. oz. /acre Salibro® at planting respectively, compared to untreated control plots. Comparative root gall rating results at harvest did not correlate with the soil *M. incognita* J2 data at harvest. While average gall rating scores in the 61.4 fl. oz. /acre Salibro® treated plots were the lowest among treatments assessed (46% reduction compared to the untreated control); there were several other treatments that had much lower gall ratings than the single 30.7 fl. oz. /acre Salibro® treatment that produced the successful soil J2 reduction. A double dose treatment of 30.7 fl. oz. /acre of Salibro® (at planting and again 14 days later) and the Nimitz® treated plots resulted in a 39% and 35% reduction in root galling compared to the untreated control. Despite suppressing *M. incognita* J2 populations and reducing host plant root galling, Salibro® treatments when applied singly, regardless of the rate, did not effectively produce yields differing from the untreated control. Average fresh red chile yields were greatest in the 30.7 fl. oz. /acre of Salibro® + Vydate® treated plots with a 28% increase in yield compared to the untreated control followed by Nimitz®, which resulted in a 22% increase in yield on average.

After ten years of chemical trials to control *Meloidogyne incognita* in vineyards across southern New Mexico with little success, an effort is underway to look closely into the soil population characteristics of *M. incognita* under typical vineyard management throughout the year. Temporal studies of *Meloidogyne incognita* on *Vitis vinifera* initiated in 2021 continued this year with the objective to determine if there is a seasonality or temperature correlation to *M. incognita* J2 populations in the soil that might help determine a most effective timing for chemical nematicide applications in vineyards. Soil and roots from multiple sites in a vineyard are being sampled biweekly. Soil temperatures are continuously recorded and will be used to determine the probability of a degree day reference for nematicide applications in vineyards. This study is unfunded and is being conducted on a commercial vineyard in collaboration with the grower. Data are communicated to growers at university sponsored field days and will ultimately be published.

**Impact Statements:**

Investigations into the seasonality of *Meloidogyne incognita* populations in drip-irrigated, wine-grape vineyards in southern New Mexico will aide in determining the most effective timing for nematicide applications.

Working directly with local commercial producers to evaluate new nematicides in locally relevant cropping systems aides my constituency in making informed management decisions.

Continued work in detecting and diagnosing the presence of new plant parasitic nematodes provides valuable management insights to the New Mexico and regional agricultural industry

A high-quality reference genome for *M. javanica* has been sequenced and assembled.

The genome of resistance-breaking nematode strain has been sequenced.

Several PSY-like peptides (MigPSYs) were identified and functionally characterized in root-knot nematodes.

Increased understanding of genetic diversity of *Globodera* spp will lead to development of broad spectrum resistance to this economically devastating pest of potato.

Develop commercially relevant potato varieties with resistance to G. pallida for use by growers of infested fields.

Develop novel nematicidal compounds for use in fields infested with G. pallida in support of eradication efforts.

Establishes the first link among soil health, *M. hapla* distribution and parasitic variability.

Provides basis for exploring links between soil biophysicochemical conditions and *M. hapla* parasitic variability.

New cotton cultivars with root knot and reniform resistance are doubling cotton yields in nematode infested fields.

Allyl isothiocyanate holds promise as a preplant treatment for management of reniform nematode.

The entomopathogenic nematode *Stienernema feltiae* Mg-14 kills sweet potato weevil adults and larvae quite effectively under laboratory conditions.

**Publications:**

Book chapters:

Lawrence, Kathy S. 2021. Reniform nematode (*Rotylenchulus reniformis*) and its interactions with cotton (*Gossypium hirsutum*) Chapter 14: pages 94-100 *in* Integrated nematode management: state of the art and visions for the future. eds Richard Sikora, Johan Desaeger and Leendert Molendijk for CABI. DOI: 10.1079/9781789247541.0014

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