

# W\_TEMP\_5186: Variability, Adaptation and Management of Nematodes Impacting Crop Production and Trade

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Admin [lsgouhi Kaloshian]  
Advisors:

NIFA Reps:

## Statement of Issues and Justification

### The Need as Indicated by Stakeholders:

Estimates over the last 35 years indicate that plant parasitic nematodes cause 10-14% average annual yield loss among the world's major crops [1], and within the United States' major crops, there are losses ranging from minimal in some localities to as high as 50% in other areas [2, 3]. More recent surveys of nematode-associated crop losses indicate that these estimates remain at similar levels. In economic terms, these estimated annual crop losses translate to at least \$8 billion in the United States and \$80 - \$157 billion worldwide [4-6]. These numbers are likely to be under-estimates since plant parasitic nematodes are belowground and invisible to the naked eye, and because the disease symptoms are often non-specific, growers may be unaware of the presence of these pests [4]. Therefore, in many production systems incipient losses are probably missed. In addition, there are significant economic losses associated with the costs of nematicides. A recent cost estimate from a representative commercial potato farm in central Michigan, USA, indicated that nematicides can cost \$1,000 or more an acre [7, 8]. The costs associated with nematode management include the price of the nematocidal products, the labor costs for application, and their efficacy [8]. In addition to the production costs incurred by nematode management, the presence of plant parasitic nematodes may be associated with trade embargos due to actual or suspected quarantine nematode infestations.

Increasingly, scientific evidence and public awareness have heightened concerns about environment quality, food quality, and human health and safety relative to pest management in agricultural production. The need for alternative, integrated nematode management has been propelled by the actions triggered by the Montreal Protocol and the Food Quality Protection Act (FQPA) of the 1990's. The phase-out of methyl bromide and the loss of several highly effective organo-phosphate and carbamate nematicides has occurred over the last decade [9, 10]. In addition to methyl bromide, the use of fenamiphos and aldicarb have become severely restricted [11]. Moreover, the soil fumigant nematicide 1,3-dichloropropene (Telone II) is a B2 carcinogen, reviewed under FQPA and must be used under more rigorous restrictions. Together with another fumigant, metam-sodium, the fumigant nematicides have been identified by California EPA as the largest agricultural source of VOC (volatile organic compound) that contribute to air pollution by ground level ozone formation. US EPA Phase 2 labeling for all soil fumigants, implemented in 2012, establishes mandatory buffer zones surrounding treated fields that further restricts their use. More recently, safer nematicide chemistries have become available and can be tested as components of integrated nematode management programs [9]. Both locally and nationally, the agricultural production community (our stakeholders) have a critical need for viable, sustainable alternatives to traditional chemical-based nematode control.

World travel and commerce have accelerated the dissemination of pest species, including plant parasitic nematodes. Climate change is contributing to enhanced disease severity in crops, and it is also leading to the northward migration of plant parasitic nematodes into new regions and agricultural systems [12, 13]. Development and application of new diagnostic protocols for accurate identification of nematode species are imperative for national and international regulatory and quarantine agencies relative to free trade and economics [14]. For example, the presence of quarantine-status of cyst or root-knot nematodes in US potato production areas has led to trade restrictions on international trade and movement of potatoes within the country [15, 16]. It is imperative that quarantined nematodes are not spread or that new plant parasitic nematodes are not introduced to the USA. The nematology community has repeatedly advocated the need for funding support focused on the basic and applied research required to advance agro-ecologically sustainable alternative management approaches and accurate nematode detection and diagnostic tools. Building on advances made in the W-4186 project over the last 5 years, this proposed project renewal, called W-5186, addresses these needs directly for important groups of plant parasitic nematodes.

### Importance of, and Consequences Without, the Work:

Cyst, root-knot, root-lesion and other nematode species included in this project are the most important groups of plant parasitic nematodes in the United States and globally [4]. Management of these nematodes in US agriculture has been largely via the application of broadly efficacious nematicides [17]. Nematicidal activity, especially of soil fumigants, is generally non-discriminating between nematode species and genera. Historically, growers would “*spray and pray*” that the chemicals they used would solve the problems with nematodes and other soil borne pathogens, while also sterilizing the soil of beneficial organisms [18]. A more targeted approach towards nematode control would be beneficial to soil health, and such an approach is now possible in the “omics” age. An understanding of the genetic variability and adaptation potential among nematodes will be important for effective nematode control, such as the development of target-based agrichemicals [9]. Additionally, desirable alternative nematode management approaches involve combinations of crop rotation, host plant resistance, cultural manipulations, and biological control. All of these tactics may have specific genotype-level interactions with nematodes and are influenced by production practices and environmental conditions. Preliminary evidence indicates that the promising new nematicide products show differential efficacy among main nematode groups (e.g., root-knot versus cyst nematodes). Hence, variability and adaptation in nematode populations must be considered to successfully develop and deploy new management strategies.

This multistate project was initiated because the membership recognized the increasing importance of characterizing the genetic variation in nematode populations and its influence on the success of alternative management strategies. For example, many years of research went into the development of cyst-nematode resistant soybeans and root-knot nematode resistant tomatoes, cotton, and potatoes but in some of these cases rapid selection of resistance-breaking nematode isolates has eroded their management efficacy. For instance, in the case of potatoes, the root-knot nematode resistance that was bred into potato was lost due to the rise of a virulent population within a few years [19]. The biological processes in nematodes are complex and ultimately will influence the development of effective management strategies. The multiple generations and interactions between nematode, host plant and environment can finally be addressed using a combination of applied and basic research. A renewal of the project as requested herein is critical for continuing the research and application required to meet the overall project goals outlined below.

An important difference from traditional broad-spectrum nematicides is that the alternatives are influenced directly by genetic variability existing in field populations of the target nematodes. This project will advance the assessment and characterization of variability and adaptation extant in nematode populations which in turn will assist in the successful development and application of alternative management approaches. For example, knowing the frequency of virulence genes in a nematode population will allow breeders to screen for broad-spectrum resistance and deployment of corresponding resistance genes, and promote the durability of genetic nematode resistance.

Research conducted under the current W-4186 multistate project has provided considerable evidence that genetic variability in nematode populations is important in nematode management systems. Results from current work indicate that genetic variability and adaptation potential in nematode populations are responsible for the aberrant and inconsistent results of many experiments assessing resistance, crop rotations, host ranges, cover/trap cropping, biological control, and new nematicides. The plasticity of nematode responses to environmental factors such as the microbiome, soil structure, temperature/moisture, host nutrient status, and other biotic/abiotic factors stems from genetic variability. Nematode variability and population genetics require additional characterization. Greater understanding of nematode genetic response and adaptation to biotic/abiotic factors will be important in optimizing the design of cultural management tactics such as manipulations of planting and harvest times, wet or dry fallow, and soil solarization. The potential for invasive nematode pests to establish in our agricultural production systems also can be better determined from studies on genetic responses and adaptation to local environments.

Without the proposed work continuing in a coordinated manner, the participants believe that effective nematode management alternatives will be developed more slowly, with success coming more on an *ad hoc* basis and with economic inefficiencies and a high likelihood of short-term failure of new products or management approaches. Knowledge gained from our focus on cyst, root-knot and other significant nematodes will be applied and tested between nematode groups within the project matrix (see Table 1 in Attachments). This will strengthen the overall scientific scope of the research activities and will broaden the impact of the findings to benefit agriculture in multiple states.

### **Strength of the Group in Technical Feasibility of the Research:**

Recent advances in molecular and genetic methodologies and knowledge will facilitate the study of nematode genetic variability and adaptation and promote diagnostic protocols with much greater resolution than has been possible. Some of these protocols have been developed and tested under the current project. For example, shared root-knot and cyst populations led to characterization of a mitochondrial cytochrome oxidase I (COI) gene that promises to alleviate current ambiguities in molecular species identification within these difficult-to-identify genera [20, 21]. In addition, development of several diagnostic tools for *Meloidogyne chitwoodi* were developed under the current project, including markers for a virulent, resistance-breaking pathotype [22]. Rapid advances in DNA-based diagnostics and genetic analyses generated by the current and other projects and their coupling to on-line databases and knowledge-based systems will assist in information transfer to user groups in the relevant agricultural communities.

The root-knot and cyst nematodes are distributed throughout the United States and are damaging root pests, parasitizing a wide range of important crops. Three groups of nematodes are the primary focus for this project: Group I - The warm-temperature root-knot species (*Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii*); Group II - The temperate root-knot species (*M. chitwoodi* and *M. hapla*); Group III - The cyst species (*Globodera*, *Heterodera*). These nematodes are the subject of research efforts in the designated participating states. Current research is addressing many areas of management for these three groups, including: development and deployment of nematode-resistant plants; rotation to reduce population densities of these pathogens; cover crops, trap crops and soil amendments including green manures to reduce population densities; characterization of resistance genes and host resistance responses; nematode comparative genomics for differential host and pathogenicity ranges, the development of molecular diagnostic protocols for nematode identification and the reference databases necessary for their implementation. Thus, the project participants share strong common interests that will provide the central focus for both project members and other collaborators. In addition, parallel studies will be made by some participants on other endoparasitic nematodes, such as reniform nematode (*Rotylenchulus reniformis*), sting nematode (*Belonolaimus longicaudatus*), and root-lesion nematodes (*Pratylenchus* spp.). This will maximize both the scientific scope of the project and its multi-state impact in agriculture and natural ecosystems.

### **Characterizing genetic variability – requisite for novel management strategies:**

The unifying theme of this project is that genetic variability is a critical biological feature that complicates management and enhances the pest status of nematode species and populations. W-4186 participants and others have been documenting the extent of genetic variability within populations, and the agro-environmental factors that influence it. Rapid developments in genomic techniques and their application through this project will continue to increase our understanding of the genetic processes involved. Failure of current nematode management practices, such as breakdown of resistance, can be resolved through greater understanding of the underlying genetic and biological processes in parasitic nematode populations *vis a vis* management. For example, the importance of mutation compared to maintained variability in field populations is unclear, and this is among the research areas that will be pursued.

Genetic variability can impact both the effectiveness and longevity of alternative nematode-management strategies based on host plant resistance, crop rotation, cultural manipulations, and biological control. Therefore, continuing the knowledge development in these systems should provide rational guidance for the design and development of nematode management strategies. The project focus is on understanding nematode variability and adaptation, such that it can be identified, characterized, and managed or manipulated to benefit agricultural production systems. This requires research on the phenotypic and genotypic characterization of variability and gene frequencies, including aspects of stability and adaptability, of host range, response to resistance, response to environmental conditions, biological processes (e.g. fecundity) and morphology. This approach is being complemented and aided by the development of markers to identify variability by molecular, histochemical, and morphological polymorphisms. The development of molecular techniques with greater efficiency, predictability and ease of use will expedite nematode genetic analyses and design of management strategies.

Current and previous work under W-4186 has allowed participants to make advances on these research goals. However, this work cannot be considered “complete,” and pressure for alternatives to existing nematicides has increased. Regarding our objectives, it is exciting that the arsenal of established and new tools used to address our applied research questions is increasing rapidly.

Four key considerations based on nematode genetic variability are central to development and deployment of alternative management strategies as proposed under this multistate project:

- **Host plant resistance** – The genetic composition of nematode populations is changed by the selection pressure imposed by growing resistant cultivars. The changes include shifts in species composition and shifts in presence and frequency of nematode virulence alleles matching specific resistance genes in crop cultivars [23-26]. Similar potential shifts may occur in response to nematode-resistant trap crops [27]. Little is known of the existing frequency of virulence alleles, the frequency with which new alleles are generated, or the underlying mechanisms that regulate changes in genetic variability in root-knot, cyst and other nematode populations. New genomic resources have provided information about nematode populations, the genes underlying nematode parasitism, and the molecular pathways that link the nematode and host plant [28, 29]. In addition, as more sources of resistance are bred into cultivars, knowledge of gene frequency and stability effects assumes greater importance in determining the direction and requirements of breeding for nematode resistance, and the effective long-term deployment of available resistant cultivars [27, 30, 31].
- **Host range for rotations and cover-cropping** - The host ranges of important nematode species have been defined within general limits, but the extent of variability in host range among populations within species is not well-characterized. Thus, although most cyst nematodes have narrow host ranges and are amenable to control by non-host rotation programs, less is known about the extent of reported hosts outside the typical host taxa, the likelihood of shifts in host range, or the host ranges of new species. For example, although sugar beet cyst nematode hosts are found almost entirely within the Brassicaceae and Chenopodiaceae, tomato (Solanaceae) has been reported to host this nematode in California and Utah, with potentially serious consequences for rotation planning in western sugar beet production areas.

In contrast to cyst nematodes, root-knot nematode species have host ranges of more than 2000 plant species from diverse plant families [32]. Much of this host range information has been compiled from numerous tests and observations based on non-standardized host testing procedures, and in most cases with only one or a few isolates per species. Standardized conditions are needed to determine whether differences are due to variability in nematode populations or to differences in susceptibility in the plant lines used. For example, there is evidence that local weed populations influence the behavior of root-knot nematode populations on subsequent crops [33]. In addition, recent work stemming from this project has shown that a virulent root-knot nematode population exhibited a shift in its host range; the virulent population can no longer readily infect cucumber. Resolving the true levels and stability of host range relationships will be critical to development and implementation of non-host crops in rotation and cover-cropping programs, and for determining the role that host weed species play in maintaining nematode population levels.

- **Cultural and Biological controls** - Manipulating abiotic effects on nematode populations to suppress nematode activity or infection are key strategies. Examples include wet or dry fallowing, so nematodes starve while active (wet fallow) or die from extreme moisture stress (dry fallow). Soil solarization involves natural heating of soil under plastic cover to attain the thermal death point of nematodes. Avoidance may include changes in planting and harvest dates, such as delaying planting in the fall to avoid infection activity, and early planting or late harvest of crops to avoid additional nematode generations. Genetic variability in nematodes for response to temperature and moisture has been demonstrated, but little is known about underlying mechanisms or stability. Soil amendments (compost, green manures, various bio-products) show promise for nematode suppression and improving soil health in some systems and require further study [34-37].

Potential biological control agents of cyst and root-knot nematodes are known to have specific host ranges among target nematode species. For instance, the bacterium *Pasteuria penetrans* differs in its effectiveness in binding to the nematode cuticle among root-knot nematode species and populations [38]. Such specificity may be controlled through surface protein binding and recognition between bacterium and nematode, suggesting variability in *Meloidogyne* may influence the potential of this and similar organisms as useful biological control agents.

- **Nematicide controls** – Several new nematicides have been shown by our current group and others to have high nematicidal efficacy and are in final testing phases to support new use registrations. Active ingredients include fluopyram, fluensulfone, fluazaindolizine, spirotetramat, and is cyclobutrifluram, among others. These materials offer much lower mammalian and avian toxicities than the current suite of fumigant, organo-phosphate and carbamate nematicides, thus being safer to apply, less damaging to the environment, and with less re-entry and residue issues. They represent important tools for integration into nematode management programs, including use as seed treatments and in combinations with bio-products and biocontrol organisms.

#### **Advantages of a Multistate Effort:**

Under the current project, research to apply emerging methodologies to obtain knowledge of the variability and adaptation potential in nematode populations is in various stages of advancement. The W-4186 membership proposes to continue and extend these efforts to identify and characterize the variability in important nematode pests. The participants share research interests on primary nematode pests and bring complementary expertise and resources to the project. The group is a cross section of nematologists that span both basic and applied research on nematodes of major agronomic importance. Our research expertise will help to determine gene frequencies, genetic stability, and adaptation and fitness, such that genetic variability can be managed and manipulated in agricultural production systems by appropriate alternative management strategies. The cyst and root-knot species are of primary importance as major nematode pests and as actual or potential invasive nematodes in most agricultural production areas and cropping systems, as reflected in the proposed contributions from participating states across the country. The diversity in cropping systems and rank of importance of nematode groups among participating states clearly provides opportunities for conducting meaningful collaborative research on major nematode pathogens. The participants utilize the opportunity to collaborate in ways that enhance the benefits accrued from the research, as opposed to pursuing individual projects within limited geographic boundaries. For example, the warm climate root-knot species will be studied by participants from a majority of the participating institutions (see Attachment Table 1) – a group effort that will pay large dividends in understanding nematode variability relative to management.

Despite retirements of key members in the W-4186 working group, this multistate project has attracted several new members. These members include nematologists asking very basic research questions about variability in nematode biology, effectors, and host resistance. In addition, several new members are extension specialists or have extension appointments. Their goal in the multistate effort will not only be to contribute to the excellent scientific research, but to also enhance the group's outreach to growers and stakeholders, which is a focus of this project.

This team approach enables a pooling of scientific expertise and resources to maximize the amount and quality of the information that can be generated. The resources available to researchers working within the Agricultural Experiment Stations have been declining in recent years, especially for nematology programs. Conversely, the demands and expectations for new, environmentally friendly management tactics have never been greater. This multistate project is unique in that it provides a necessary forum for rapid scientific advancement in aspects of both basic and applied research directed toward nematode diagnostics and development of management strategies. For example, some participants have programs devoted to molecular research on nematodes, which can be applied across all states for diagnosis and to assess nematode genetic variability. This project has ongoing molecular-based programs in some states (e.g. California, Hawaii, Mississippi, Nebraska, Florida, Washington) that can facilitate research by other participant states. In turn, those states focusing on phenotypic differences in nematode populations can provide nematode populations and isolates for molecular analysis. This coordinated approach minimizes unnecessary duplication of research programs, and provides fertile opportunities for a seamless, interactive approach to advancing nematode management. The multistate effort has been key to establishing collaborations and sharing data, particularly during the lively annual meetings. Research strategies, partnerships, and grant writing opportunities have arisen from this multistate effort. The end result is that the project has enhanced the quality and applicability of the research findings across geographic locations and agricultural production systems.

#### **Likely Impacts of Work:**

The stakeholders (production agriculture) will be positively impacted by application of the project findings in expediting the development of new, environmentally benign management strategies to minimize economic losses from nematodes. This in turn should help boost the international competitiveness of our agricultural production systems. The project will also benefit the diagnosis and response process required when invasive nematode pests are suspected or found in production fields or in traded agricultural products.

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## Related, Current and Previous Work

#### **Related, Current and Previous Work: Accomplishments (2018-2023):**

A full listing of the 224 research publications of the W-4186 project from 2018-2022 and Annual Progress Reports for W-4186 are available through the NIMSS site for more in-depth descriptions of related work progress. The following is a summary of examples that highlight areas of significant impact in addressing project goals, with comments concerning the need for additional research.

Overall, the project scientists have been very productive in efforts to develop improved techniques for nematode diagnostics, understanding of nematode diversity and genetic variability, processes of nematode fitness and adaptation, and the incorporation of this knowledge into the design and analysis of improved integrated nematode and soil health management strategies. These findings directly benefit all participating states and more broadly other states whose crop production systems are compromised by cyst, root-knot and other nematode infestations and invasions. Moreover, many of the accomplishments have resulted from our close collaboration within W-4186, through which shared knowledge, techniques and materials provide important synergies.

Under **Objective 1** (characterize genetic and biological variation in nematodes relevant to crop production and trade), nematode (a)virulence and plant resistance gene interactions were elucidated in several important nematode crop combinations. These included root-knot nematode interactions with resistance gene(s) and nonhost determinants in coffee, cotton, cowpea, common and Lima beans, potato, tomato, wheat, and chile pepper. For example, resistance markers were developed against *M. hapla* in carrot cultivar "Homs." Although the *M. hapla* avirulence gene(s) in this interaction need to be identified, the new knowledge on natural host resistance traits can be adopted by plant breeding programs and the seed industry to benefit growers by producing nematode resistant crop varieties.

In Idaho, a project to investigate the genetic diversity found in *Globodera* spp. was initiated to 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. Variation in virulence and pathogenicity of *Globodera pallida* is known to occur, and different populations are characterized into pathotypes based on their ability to reproduce on a set of potato genotypes with known resistance genes. The pathotypes of 10 populations from Peru were characterized using a set of potato differential lines containing different resistance genes. According to the pathotype scheme, the Idaho *G. pallida* population has been identified as a pathotype 2/3 (Pa2/3) [39]. Pathotypes with different virulence is a constraint towards development of fully resistant potato varieties. Continuous use of resistance has selected for populations with increased virulence. Resistance derived from *Solanum tuberosum* spp. *andigena* is more readily overcome than resistance from *S. vernei* [40, 41] and may select for more virulent populations [42]. Moreover, recent evidence suggests individuals within a cyst may have different virulence characteristics and these individuals may contribute to erosion of resistance. Understanding genetic factors that contribute to virulence would help to maintain durability of resistance genes.

Studies on root-knot nematode and host genes have led to insights on the genetic factors controlling nematode parasitism. Transcriptome analyses of *M. chitwoodi* was performed to identify nematode parasitism genes (i.e. effector genes) that facilitate infections of potato [43]. Other accomplishments include a high-quality reference genome for *M. javanica* as well as a genome of a resistance-breaking *M. javanica* strain. The genetic variation between these *M. javanica* strains will help elucidate how the nematode has evolved to avoid immune detection in resistant plants. Altogether, the information will be key in understanding variations in plant nematode interactions, how nematodes manipulate plant responses, and changes in nematode populations that contribute to their virulence on previously resistant plants.

Molecular approaches to nematode identification within and between species have also been advanced under this objective. Molecular techniques have been applied to develop identifying targets for a nematode species, races and populations [14, 44, 45]. Some of the major accomplishments include simple molecular assays for the identification of *M. chitwoodi*, including specific races/pathotypes of the nematode [46]. This information will help growers make management decisions in potato production. Other major accomplishments include advances in nematode identification by molecular 'barcoding' approaches (e.g., [47, 48]). Continued efforts are being made to refine and define the conditions and limitations of DNA barcoding using the COI mitochondrial gene. Barcoding surveys on a large geographic scale will help establish species boundaries of plant parasitic nematodes. A DNA barcoding called NemaTaxa was developed as a reference data base of major plant parasitic nematodes that impact US agriculture [47]. Work from Nebraska has supported a field device for rapid identification of cyst nematode juveniles, which can accelerate the time of species identification and reduce expenses. Continued work in detecting and diagnosing the presence of new plant parasitic nematodes provides valuable management insights to regional agricultural industry.

Under **Objective 2** (determine nematode adaptation processes to hosts, agro-ecosystems and environments), several systems have been analyzed, revealing high levels of nematode adaptation to parasitic ability on resistant host plants, alternative hosts (weeds), seasonal climatic differences, co-infection of plants by fungal pathogens, and soil conditions. Variation in parasitic ability among root-knot nematodes was described for species parasitizing grain legumes such as cowpea and Lima bean [49], common beans, cotton, tomato, and potato cultivars and wild relatives. For example, in California, *Mi-1* is the only commercially available root-knot nematode resistance gene in tomato. Work within this project found that the host-range of the *Mi*-resistance breaking *M. javanica* strain seems to be different from its progenitor strain. Resistance-breaking nematodes are becoming increasingly problematic to growers. These studies demonstrate the need for broad-based forms of resistance for use in crop cultivars and cover and trap crops. Members of the project also continue to screen the potato clones that are being developed to determine their resistance level to *G. pallida* [50, 51]. In addition to development of resistance through traditional breeding efforts, a project was initiated to understand plant defenses of the trap crop, *Solanum sisymbriifolium* [52]. This will lead to new knowledge on natural host resistance traits to manage nematodes in field and vegetable crops.

Understanding nematode parasitic variability (PV) in diverse cropping systems and developing integrated soil health and nematode management strategies therein has been limited by lack of decision-making tools and models that can translate basic and complex science into practical applications. Therefore, one accomplishment of the W-4186 project focused on modeling the PV of soybean cyst nematodes and Northern root-knot nematodes in various soil conditions [53]. Field studies in Michigan showed that soil texture influences *H. glycines* population density [54] and *M. hapla* reproductive potential, with adaptation to mineral or muck soils [53-55]. It was shown that there are clusters and specificity of PV in *M. hapla* populations isolated from mineral and muck soils with disturbed and degraded soil health conditions. Populations from degraded mineral soils had significantly higher PV than those from disturbed mineral and disturbed and degraded muck soils, and PV within the populations from the degraded mineral soils varied [56]. The information derived from this research has been utilized to develop models of nematode populations in various soil types, and in particular, it will help scientists understand how nematodes adapt to changes in cultural practices and in environmental factors.

Nematodes can also adapt to new environments and a major issue is that plant parasitic nematodes can be introduced into new areas where they adapt and infect important crop plants. Identifying such infestations is critical; potato cyst nematodes *G. rostochiensis* and *G. pallida* are significant economic threats to potato production, and a comprehensive detection survey documented absence of *G. rostochiensis* but confirmed the presence of *G. pallida* in Idaho. Project members also determined that a new ring nematode species, *Mesocriconema nebraskense*, endemic to the Central Plains has adapted to cause serious damage to bent grass on numerous golf courses in New Mexico. Lastly, within this objective, new nematode records have been reported. There was a first report of *Ditylenchus dipsaci* from alfalfa in New Mexico [57]. At a value of \$125 million, New Mexico alfalfa hay production is ranked as the fourth largest cash commodity in the state. It is a critical agricultural commodity that supports the two top agricultural industries, dairy and cattle production, in New Mexico. In addition, the alfalfa cyst nematode *Heterodera medicaginis* was found in samples from Kansas, Montana, and Utah. This is the first time that the alfalfa cyst nematode has been found in North America [58]. The cactus cyst nematode, *Cactodera cacti*, was found in Idaho and Colorado, a first for these two states [59]. There was a first report of *Cactodera milleri* from Quinoa fields in Colorado [60].

Another aspect of objective 2 has been to look at distribution of nematodes in terms of adaptation to potential new hosts. For example, the University of Hawaii has surveyed native plants in areas around University of Hawaii Campus for susceptibility to root-knot and reniform nematodes. For reniform nematodes, the native plant *Ipomoea* appeared to be a good host, but most native plants seemed to be poor hosts to these nematodes. The observed variation of resistance in seedlings may reflect the variation in the seed germplasm.

Under **Objective 3** (develop and assess nematode management strategies in agricultural production systems), root-knot, cyst, and other nematode management systems have been studied, developed, or improved for multiple cropping systems and production areas. Several examples highlight nematode-cropping systems that have been and will continue to be a project focus, targeting the most damaging nematode problems.

In Idaho, cropping systems using a combination of resistant varieties and trap crops are being developed for control of *G. pallida*. Toxic metabolites from the trap crop *S. sisymbriifolium* are being purified and assessed for development of a 'smart' nematicide against potato cyst nematodes [61]. There is a continued need for new safe, effective, and inexpensive nematode control options in potato and beyond.

Work on root-knot nematode management has included host plant resistance studies. In carrot, high resistance was found in carrot lines from Brasilia, South Africa and India to *Meloidogyne incognita* and *M. javanica*. Resistance to *M. hapla* in a carrot entry from Syria was found effective against nine out of ten different *M. hapla* isolates collected from different cropping systems and agro-ecologies. In cowpea, analysis was continued to study the genome-level organization of root-knot nematode resistance traits, and the study identified resistance loci on four of the 11 cowpea chromosomes. Work in Southern US has shown the new cotton cultivars with root-knot and reniform resistance are doubling cotton yields in fields infested with these nematodes.

Nematicides offer an effective means of nematode management, but as companies develop new nematicides, it is important to investigate the effectiveness of their formulations or their application methods. Working directly with local commercial producers to evaluate new nematicides in locally relevant cropping systems aids growers in making informed management decisions. For example, investigations into the seasonality of *M. incognita* populations in drip-irrigated, wine-grape vineyards in southern New Mexico has aided in determining the most effective timing for nematicide applications. For management of reniform nematode, allyl isothiocyanate holds promise as a preplant treatment. However, the reniform nematode reduced susceptible cotton yield potential in half even with nematicide applications. Numerous studies were also performed on *M. chitwoodi*, allowing for specific recommendations be made to potato growers such as using Velum Prime with in-furrow application rather oxamyl [62].

Developing predictive tools to aid in nematode management has also formed part of the project effort, including improving a fertilizer-use efficiency model by incorporation of concurrent changes in nematode community population density, plant growth and soil environment [63], and use of spatial information regarding nutsedge and *M. incognita* populations as predictors in modeling the nematode/nutsedge pest complex [64].

These are selected examples of many advances in nematode management being made under W-4186 participation in which the collective knowledge gained in one system is being applied to other systems. These nematode management systems are at various stages of development and require additional study and modification under a project renewal.

### **Areas Needing Further Investigation:**

Many of the cropping systems we are studying involve perennial crops or multi-year rotations requiring several years of experimentation to test the various permutations of cropping sequences and tillage systems, durability of resistance, and nematode adaptation to environment and to other control measures. Efforts under W-4186 have laid the foundations for integrating new approaches to nematode management that consider the genetic variability in nematode species. Additional studies and modifications of these systems for managing nematodes are necessary in order to design and optimize new integrated nematode management strategies. Coupled with these efforts, DNA sequence-based technologies have developed rapidly in the last several years and their versatility and knowledge value has increased tremendously, while their costs of application to diagnostics and research into nematode variation has dropped considerably. These advances in molecular protocols are also applicable for selection of nematode resistance traits in crop plants for plant breeding. We must take advantage of these new resources and apply them to the nematode-crop systems and invasive nematode threats that the project members are familiar with and for which representative samples are available. A new generation of novel, highly efficacious and less toxic nematicides has become available for testing, and these need to be researched and adapted into our focus nematode management systems. Farmers' limited knowledge of the cause-and-effect relationships between agricultural practices, soil health, and nematodes adversely affect crop yield. Therefore, this multistate project has expanded to increase the number of members with extension programs. This will help promote our research efforts and contribute to higher rates of adoption of 'best' practices by livelihoods.

**Other Regional Projects:** There are three multistate projects that are regional in scope and with minimum overlap with the current multistate proposed project. NC-1997: Practical Management of Nematodes on Corn, Soybeans and Other Crops of Regional Importance. This project is mostly focused on the US Midwest. S1092: Biology, ecology, and management of emerging nematode threats in the Southern United States. This multistate project focuses primarily on nematode threats to agriculture in the Southern region. It is much more limited in regional scope. It emphasizes invasive nematodes and infestations of endemic nematodes on new crops. Thus, while portions of its goals are similar to the current proposal, it is limited to a defined geographical region and focuses on approaches to mitigate emerging nematode problems. The other multistate project with potential overlap is NE-2140: Sustainable Management of Nematodes in Plant and Soil Health Systems. However, this project is focused on developing ecologically based nematode control practices rather than understanding the genetic variability of the host and nematode parasites that our multistate project does.

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## Objectives

1. Characterize genetic and biological variation in nematodes relevant to crop production and trade.
  2. Determine nematode adaptation processes to hosts, agro-ecosystems and environments.
  3. Develop and assess nematode management strategies in agricultural production systems.
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## Methods

The research focus of each participating state is given as a matrix in Table 1. The nematode group and main crop areas are indicated, together with the procedural research emphasis, as covered under the three objectives. Research coordination will ensure that standardized procedures are generally followed, and research findings can be compared within and across nematode, plant and subject area categories. Plant germplasm (accessions, breeding lines, cultivars), nematode isolates of representative species and populations with GPS location data, and DNA primer and other sequence information for markers will be available among the participants.

Experimental protocols and procedures are structured according to the main target nematode groups under the three objectives. All objectives have a common focus of addressing critical aspects of nematode variability, adaptation, and management, with the application and extension of the findings tailored to meet individual state and sub-regional needs, in addition to those at the project-wide level. While findings under all objectives are considered important to each state, duplication will be avoided by partitioning individual state research activities. This structure will also ensure that all phases of the objectives are being met, while addressing local state needs, a structure that has worked well in the W-4186 project and its previous versions. Following are examples: tropical root-knot and reniform nematodes will be considered in Alabama, Arkansas, California, Louisiana, Florida, Maryland (USDA-ARS), and Mississippi. Cool-climate root-knot species (*Meloidogyne chitwoodi* and *M. hapla*) will be studied in detail in California, Michigan, Nebraska, New Mexico, and Washington. *M. hapla* will also be studied in Florida, where it is commonly found in strawberry fields during winter. Cyst nematodes will be studied in Indiana, Florida, Hawaii, Michigan, Arkansas, and Nebraska. Idaho will consider impacts specifically of *G. pallida* on potato production systems.

### **Objective 1:**

Phenotypic assessments will be made on isolates of nematode populations that are collected from field sites. The assessments will include nematode host range and virulence/avirulence on known resistant crops. This information will provide important background considerations for the level and nature of any phenotypic differences detected in comparative experiments. In many programs, investigators either have a partial or nearly complete collection of live cultures for genetic comparisons. For example, for Group I warm-climate root-knot populations, 40 isolates have been assembled at California-Riverside and a collection of group II cool-climate root-knot nematodes *M. chitwoodi* and *M. hapla* isolates are maintained and expanded in Washington. For cyst nematodes, numerous populations of *H. glycines* are being cultured in Michigan and Nebraska, and a collection of *H. schachtii* and *H. cruciferae* geographic and host-selected isolates is under culture in Hawaii. The Idaho population of *G. pallida* is being cultured and maintained in Idaho, and additional populations from South America and Europe are being assembled. Work in Michigan applying various models that identify the soil environment in which the nematodes exist and how, will help W-5186 members relate soil health to nematode populations across the study areas. Based on the outcomes, Michigan will establish nematode cultures for testing parasitic variability for *M. hapla* and a database accessible to all collaborators.



Some of the questions to be addressed are how nematode populations respond to changes in the hosts. Assays of (a)virulence responses to resistant lines and cultivars and of host range will be made under greenhouse and controlled environment conditions using well-established experimental procedures. The host range and virulence testing for the root-knot species (Groups I and II) will include standard sets of host differential plants and also differential cultivars and crop plants applicable to their local cropping systems, e.g., on potatoes in the Pacific Northwest. A specific example of the future research under this project is based on a recent survey which detected resistance-breaking isolates of group I nematodes (*M. incognita* and *M. javanica*) in 27 out of 30 tomato fields exhibiting symptoms typical of nematode infection throughout California [65]. Comparative genomics will be used to identify genetic factors that contribute to resistance outbreaks in tomato fields. Host range assays will be made under greenhouse and controlled environmental conditions. The genetic factors that contribute to a potential fitness cost of virulence will also be investigated. In addition to root-knot nematodes, *Heterodera*, *Globodera* and other nematode populations will be typed phenotypically for (a)virulence with respect to resistance genes from different crop plant species and close wild relatives, for host range, and for biological traits including responses to abiotic soil conditions. In all cases, the genomic data will be used to develop rapid reliable methods to identify resistance-breaking nematode isolates from fields. The availability of diagnostic tools for species and pathotypes within a species will facilitate the development of alternative management strategies including the identification of appropriate rotation crops.

Members of W-5186 are in a uniquely advantageous position to develop rapid and robust molecular diagnostic tools for simultaneous analysis of multiple nematode species. Molecular analyses of these populations may lead to stable marker systems for nematode (a)virulence phenotypes, host range determinants (including changes in phenotypic response associated with prior parasitism of weeds), and geographical variants for diagnostic purposes, using mtDNA, ribosomal DNA, and nuclear DNA sequence and markers [66, 67]. They also will provide stronger and more accurate diagnostic protocols. This will allow us to monitor changes in gene frequency in fields, as we subject field populations to different cropping sequences. From a perspective of diagnostics, high resolution DNA markers will facilitate standardization of identifications and aid in analysis of hypothesized pathways for dispersal that may explain current species or haplotype distributions.

W-5186 will play a central role in the development of new markers and identification assays based on genetic variations within and between species. It necessitates a team approach due to the need to identify multiple species/or haplotypes from multiple geographic regions, coupled with the testing of multiple genetic markers. Different states will focus on particular species in a collaborative framework: e.g., *M. incognita* populations from states, including Alabama, Arkansas, California, Indiana, and New Mexico; *M. chitwoodi* and *M. hapla* in the potato-small-grain-alfalfa systems of Washington and Idaho; *H. schachtii*, *H. glycines*, and *H. cruciferae* from California, Hawaii, Michigan and Nebraska. Excitingly, development of diagnostic systems that feature the simultaneous analysis of multiple species based on DNA extracted from soil provides new opportunities. To be effective, the DNA reference database of plant parasitic nematodes must be enhanced and validated as a goal of the project. In the future, this level of analysis will no doubt be required for phytosanitary certification. As costs of regulatory surveys and identifications are increasing with the demands for greater specificity, high-throughput approaches to nematode diagnostics that can identify multiple species could eventually counteract those cost increases.

One of the exciting aspects of looking at genetic variation in plant parasitic nematodes is its potential usefulness in developing novel biotechnology for crop protection [68, 69]. For example, members will study nematode parasitism gene diversity for the identification of nematode genes that could be specifically targeted for silencing by a process called RNA interference (RNAi) [70]. Parasitism genes (namely effectors encoding nematode secreted proteins) are usually genes whose inactivation/targeting impair the success of parasitism. Several members of W-5186 (Washington, California, Indiana, Florida, Maryland [USDA], and Arkansas) are in the unique position in having the knowledge and expertise in using sequencing and molecular approaches that leverage genetic diversity between parasitic and non-parasitic nematode in order to develop targeted nematode controls using biotechnology, including RNAi.

One important way to control *Meloidogyne* and other plant parasitic nematodes is the deployment of nematicidal compounds that are either natural or synthetic in origin. The use of these compounds may enhance the sustainability of planting nematode-resistant crop varieties by interfering with the ability of nematode populations to evolve a resistance-breaking phenotype. However, to know whether this will indeed be the case, we need a better understanding of the genetic and phenotypic variation in their populations with regards to their responses to natural and synthetic nematicidal compounds and how populations may respond evolutionarily to the deployment of such compounds. Our goal is to understand patterns of phenotypic and genome-wide genetic variation in nematode responses to natural and synthetic nematicidal compounds such as glucosinolates and their isothiocyanate derivatives, glycoalkaloids, and other compounds through considering natural variation in these traits across populations. The diversity of root-knot and cyst nematode populations will be useful. The cyst nematodes are excellent "model" nematode systems for genetic studies because they reproduce sexually. Conversely, many root-knot species are parthenogenetic (asexual). Their genetics will be studied using isofemale or single descent lineages to track inheritance, variability and adaptation, and via Mendelian approaches using species that can reproduce sexually, such as *M. hapla* and *M. chitwoodi*. The knowledge of costly trade-offs, for example by diminishing the success of toxin-insensitive nematodes in infecting a variety of potential host plants, will be key for developing new management approaches and minimizing loss of crop yield.

## **Objective 2:**

Phenotypic characterization and genetic markers developed under Objective 1 will provide the basis for selecting candidate populations that show adaptation to increased virulence and parasitism, and for variants associated with hosts, geographic (climatic) and soil (abiotic) adaptations. The fitness of isolates virulent for specific resistance genes will be assessed by controlled culturing on susceptible plants for multiple generations. Effects of non-agronomic hosts on fitness will also be examined, such as studies on the effects of weed hosts on root-knot nematodes in cropping systems, which will be expanded to include weed populations from other geographic areas to better understand the implications of this adaptation. Participants also will include comparisons with invasive root-knot nematode species, such as *M. enterolobii* (Group I), which have heightened aggressiveness and broad host range. Adaptation and fitness also will be studied by imposing continuous selection for virulence in wild-type populations. Changes in reproductive capacity will be assessed to measure virulence frequencies and genetic stability of virulence. Tests of selected isolates will be made on resistant or nonhost plants other than those used to impose the original selection pressure. For example, studies of resistance breaking populations of *M. javanica* [71] and *M. chitwoodi* [72] will be conducted to investigate how a genetic adaptation to plant resistance may influence the nematode's host range and determine whether there is stability of virulence with and without host selection pressure.

A combination of controlled, large field scale studies will be conducted to understand nematode adaptation for nematodes such as *M. incognita*, *M. javanica*, *M. hapla*, *H. glycines* and *R. reniformis*, in cropping systems. We will evaluate survival and reproduction of *M. javanica*, *H. glycines* and *R. reniformis*, and potential to cause damage to their hosts under different temperature, moisture and other factors associated with climate change.

Quantifying nematode adaptability to changes in soil-nutrient environments will be extended as a major emphasis of work in Michigan, enhancing understanding of possible factors contributing to nematode adaptations. For example, in order to inform potential mechanisms of parasitic variability in *M. hapla* and basis for testing other nematodes with known parasitic variability, we will explore links or variations in biophysicochemical conditions across the clusters of parasitic variability [73] and data to be generated under Objective 1. Associations and links to parasitic variability will be identified using proteomic and genetic approaches for *M. incognita*, *M. javanica*, *M. hapla*, *H. glycines*, and *R. reniformis* [74]. Second tier follow up experiments will determine the implications of what this variation does and could play in nematode adaptation to changes in cultural practices and in environmental factors. In addition, we will continue with our long-term study on *H. glycines* establishment subjected to typical Midwest APs [75] and effects of agricultural practices on nematodes soil health and nutrient cycling. We will apply a combination of the concepts of the "soil food web", "Fertilizer Use Efficiency," and "Integrated Production Efficiency" models as diagnostic and decision-making tools to describe the experimental soil conditions and/or sustainability of the outcomes [56, 73, 76, 77].

### **Objective 3:**

A diversity of features and methods will be considered in the design of nematode management strategies encompassed in Objective 3. This objective will address integrated nematode management including host plant resistance, tillage and reduced to no tillage practices, new pre- and post-plant nematicides, and impact of biological antagonists in crop rotation sequences, cover, trap, and green manure crops will be considered in practical combinations and multiple locations. These field-based studies will employ standard experimental designs (randomized complete block, split-block, split-plot) with positive and negative control treatments and 4 to 6-fold replications under current production practices. Parameters measured in these experiments will be expanded to include soil properties, nematode community structure and function, sampling strategies tied with opportunities afforded by precision agriculture technologies for site-specific management. The development of biotyping schemes for cyst and root-knot nematode populations for reaction to host and nonhost crops and to resistant cultivars and accompanying practical marker systems under Objective 1 will provide important information for resistance and rotation implementation in annual crops. For example, soybean cyst nematode, reniform nematode and root-knot nematode rotation experiments will be conducted with contemporary highly resistant, resistant and susceptible soybean, cotton, and vegetable varieties, because new varieties are coming to market every year and we must continually evaluate new varieties in production systems. Crop rotation utilizing only nonhosts in production or cover crops that are grown in rotation with hosts will be evaluated for success in nematode management and profitability. Knowledge on nematode fitness and adaptability developed under Objective 2 will aid in the optimization of durable nematode management strategies. Climatic and soil conditions, different nematode species, and variability in host ranges among different populations will require unique rotations for different growing regions.

Several cropping systems being evaluated currently under W-4186 and described earlier have multi-year horizons and crop sequence combinations that require additional analysis. Rotation schemes will be assessed in microplots or infested field sites within the relevant localities. Techniques will be coordinated among participants to facilitate direct comparisons of results. The rapidly changing demands on crop production present new challenges to the use of crop rotation for nematode management. For example, demand for ethanol has resulted in the shift from wheat to corn in potato rotations. While wheat is a host for *M. chitwoodi* and *M. incognita*, it is harvested early in the summer and supports limited reproduction in northern areas but can increase populations in the southern regions. In some areas it can also be followed by green manure cover crops in the fall that significantly suppress nematode populations. In contrast, when corn is grown, it will support several generations of *M. chitwoodi* or *M. incognita* and eliminate the opportunity for suppression of population increases using cover crops. However, corn can vary greatly in its host status to *M. chitwoodi* and different cultivars need to be screened for resistance to inform growers about which varieties would limit population increases. Grower interest in green manure crops and cover crops for nematode suppression is increasing but little is known about the host status of many of these plants. This needs to be determined so that effective recommendations can be made. In the case of sugar beet cyst nematode, *H. schachtii*, and cereal cyst nematode *H. avenae*, and the soybean cyst nematode, fallow or planting with a green manure crop incorporated after eight weeks will be tested in rotations with non-host crops such as corn, bean, onion or potato between main crops. In Idaho, the trap crop *S. sisymbriifolium* has been found to greatly reduce populations of *G. pallida* [61]. A comprehensive management plan for deployment is being developed.

The long-term economic productivity of U.S. agriculture depends upon the ability to respond in a timely, economical, and environmentally sound way to invasive agricultural pests such as the recent introductions and discovery of the regulated cyst nematodes in the genus *Globodera* and *Meloidogyne*. Risks and damage from introductions of these types of invasive pests can be minimized when appropriate tools are available for their control. For invasive and potentially spreading pests, critical first steps toward deployment of effective control strategies, which includes rapid detection methods. There is also a need for a clear understanding of environmental pressures and parameters which contribute to genetic and biological variation so that effective control strategies can be implemented.

As stated earlier, there are several new pre- and post-plant nematicides with reduced environmental toxicity available to growers across production systems. The W-5186 research group is uniquely situated to evaluate these nematicides across a range of environments, crops, and nematode targets. For example, *Meloidogyne incognita* and *M. enterolobii* are the species of nematode field trials of Alabama, Arkansas, Florida, Louisiana and New Mexico, most often in cotton, corn, soybean, peppers, vegetables, and fruit crops. *Rotylenchulus reniformis* has also been spreading across the cotton belt but is also a production problem in Hawaii. Many new nematicide products that are characteristically lower in mammalian toxicity are becoming available. The active ingredients including cyclobutrifluram, fluopyram, fluensulfone, fluazaindolizine, spirotetramat, and others that are yet to be announced. They will be evaluated as tools for use in nematode management programs, including use as seed treatments and in combinations with bio-products and biocontrol organisms. New biological products are also increasingly available and often found in combinations with chemicals and varieties. The diverse crops and nematode pests investigated by project participants present a major opportunity for incorporation of these new tools into nematode management systems that have been a central focus of this project.

To enhance understanding of spatio-temporal relationships among nematode populations, soil conditions, biological antagonists, and production practices, a combination of the soil food web (SFW,[78]) and fertilizer use efficiency (FUE, [63]) will be utilized to assess agro-biological and ecological suitability of cultural practice-based nematode management strategies. The SFW and FUE models are nematode community analysis based and use quadrant systems to describe best-to-worst case scenarios for outcomes of soil biophysicochemical changes and potential sustainability of the agronomic practices, respectively. Both models will be utilized to assess how any specificity and variable outcomes observed under Objective 2 relate to agricultural and ecological efficiencies of cultural practice-based nematode management strategies. In doing so, we will be developing bridges suitable for multi-disciplinary approaches beyond nematology and critical to solving agricultural problems [79].

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# Measurement of Progress and Results

## Outputs

- Basic knowledge on biological, ecological and genetic processes underlying the success of cyst, root-knot, and other nematodes as parasites of crop plants.
- New or improved, and safer tactics for the management of nematodes that affect crop production and trade in US agriculture.
- Holistic approaches for integrating different nematode management strategies into IPM systems. A diagnostic DNA barcoding reference data base of major plant parasitic nematodes that impact US agriculture
- Economic impact and ecological assessment of management approaches for control of plant parasitic nematodes of major agricultural significance. Comments: Concerning the milestones indicated above, the research will provide information necessary to implement nematode management alternatives. Research in each nematode - cropping system combination is at various stages of maturity along a sequence of progressive steps based on the order of the objectives. These include developing new basic knowledge, from which a control strategy or diagnostic protocol is developed, followed by testing and implementation by field experimentation and demonstration, and transfer to stakeholders. For example, identification of resistance in a crop, advancement of the trait into commercial varieties and testing its effectiveness in a cropping system rotation will be made. Each nematode-crop plant combination and cropping system will have its own timeline for completion, but typically will require completion of the objectives in the order presented; thus, Objective 3 will require completion of Objectives 1 and 2 for a given nematode-crop system.

## Outcomes or Projected Impacts

- Increased knowledge base in plant-nematode biology, ecology and genetics for use in identifying novel targets for nematode control.
- Implementation of nematode management tactics that complement reduced or softer pesticide usage, benefitting human health and the environment.
- Promotion of sustainable farm management practices through new nematode management tactics.
- More efficient and effective response and mitigation capabilities for invasive and trade product contamination issues.
- Economic benefits to producers and consumers through reduction in nematode management costs and food production.

## Milestones

**(2025):**Development of new research-based knowledge on nematode variability and adaptation relative to abiotic and biotic parameters in target cropping systems.

**(2023):**Development and validation of new nematode diagnostic and detection protocols.

**(2026):**Evaluation of new nematode management tactics under appropriate experimental conditions.

**(2027):**Transfer of knowledge to stakeholders on new nematode management tactics and new diagnostic and detection protocols.

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## Outreach Plan

Throughout these efforts, researchers will interface with growers and other agricultural professionals through outreach efforts to partner in managing nematode diseases. The project has a successful track record of disseminating the new knowledge and information created by participants and co-operating colleagues. It is planned that the traditional outlets for transferring project results will continue to be utilized, including peer-reviewed journals, annual progress reports, scientific meeting presentations, and websites. Basic aspects of this research (i.e., resistance/parasitism gene discovery and characterization) will be published in refereed journals such as the *Journal of Nematology*, *Nematology*, *Phytopathology*, *Molecular Plant-Microbe Interactions*, *Plant Molecular Biology*, and *PLoS ONE*. Applied research will be published in refereed journals such as *Plant Disease Management Reports* and *Plant Health Progress*. Social media, blogs, and trade journal articles will be used to disseminate educational material and recent findings to a mass audience in the national and international community.

Outreach and extension goals also include translating research findings into recommendations for agricultural professionals and engaging in dialogue with agricultural professionals to improve and direct research efforts. In order to increase the dissemination of the project accomplishments, we have invited several new researchers with extension appointments to join as W-5186 members. This includes new members from Florida, Louisiana, Indiana, and Arkansas. Extension presentations and publications also will be made, both by participants with extension appointments, and by the AES and ARS participants, most of whom have a strong applied research component to their programs and who routinely participate in extension-based activities (meetings, presentations, publications) with our agricultural stakeholders. The project members will use web-based delivery vehicles to reach the broad stakeholder base that will benefit from objective, science-based knowledge and information about important plant parasitic nematode biology, diagnosis, management and regulation. Examples include GenBank for depositing most DNA sequences, the Barcode of Life Database (BOLD) for DNA sequences of COI barcodes together with associated metadata, NemaTaxa (new ITS nematode barcoding database), and the international database of quarantine organisms (QBOL). Among our stakeholders we have identified growers and food processors, national and international trade partners, USDA-APHIS and state regulatory agencies, national and state legislators, fellow scientists, and K-16 teachers and students.

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## Organization/Governance

The organization and governance of this Multistate Research Project will conform to the guidelines presented in the United States Department of Agriculture's publication "Manual for Cooperative Regional Research". Committee officers include a Chair, a Vice-Chair, and a Secretary. A new secretary will be elected at each annual meeting of the Technical Committee with the current secretary assuming the position of Vice-Chair. The Vice-Chair assumes the position of Chair at the end of the annual meeting. In the event that an Executive Committee is needed, the officers are authorized to serve in that role. For organization of each annual meeting, one member of the Technical Committee will be selected to coordinate and oversee local arrangements for the meeting. The meeting coordinator will be chosen based on the consensus selection of the meeting site, and typically will be the member from the state or institution hosting the meeting. The Technical Committee will rotate the annual meetings between representative Experiment Stations who will be participating in the project. Members will benefit from site visits of research and extension facilities at the host Experiment Station institution. Administrative guidance will be provided by an assigned Administrative Advisor.

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## Land Grant Participating States/Institutions

NM, ID, WA, CA, OR, AL, MI, NE, HI, FL, AR, LA

## Non Land Grant Participating States/Institutions

### Participation

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Beacham, Jacqueline	Yes	New Mexico - New Mexico State University	3	212	3130	1120	0.10	0.00	0.00	0	0
Dandurand, Louise-Marie		Idaho - University of Idaho	3	212	1310	1120	0.20	0.00	0.00	0	0



Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Desaeger, Johan		Florida - University of Florida	1,2,3	212	1122	1120	0.10	0.00	0.00	0.1	212
DiGennaro, Peter		Florida - University of Florida	1,2,3	203 201 206 201 212	2499 0 0 0 0	1160 1120 0 0 0	0.20	0.00	0.00	0	201 206 203
Faske, Travis R.		Arkansas Cooperative Extension	3	212 212 212 0	1710 1820 2410 0	1120 1120 1120 0	0.10	0.00	0.00	0.1	212
Gleason, Cynthia	Yes	Washington - Washington State University	1	212	1310	1120	0.10	0.00	0.00	0	0
Groen, Simon		California -Riverside : University of California, Riverside	1,2	212	2499	1120	0.25	0.15	0.10	0	0
Kaloshian, Isgouhi	Yes	California -Riverside : University of California, Riverside	2	212 212	1460 2499	1040 1040	0.10	0.10	0.00	0	0
Kud, Joanna	Yes	Arkansas - University of Arkansas	1,2,3	212	2499	1120	0.10	0.00	0.00	0	0
Lawrence, Kathy S	Yes	Alabama - Auburn University	2	205 215 216	1499 1710 2499	1120 1160 0	0.10	0.10	0.00	0.05	205
Melakeberhan, Haddish	Yes	Michigan - Michigan State University	1,2,3	212 212 212 215 215 215	1418 1499 1820 1418 1499 1820	1010 1120 1120 1010 1120 1120	0.20	0.00	0.00	0	212
Powers, Thomas O.	Yes	Nebraska - University of Nebraska	1,3	212	3130	1120	0.20	0.50	0.50	0	0
Roberts, Philip		California -Riverside : University of California, Riverside	1,2,3	212 212 212	1410 1499 3130	1120 1120 1080	0.20	0.50	0.50	0	0
Siddique, Shahid		California -Davis : University of California, Davis	1,3	212 201 216 216	1212 1410 1460 3130	1040 1120 1030 1030	0.10	0.00	0.00	0	0
Sipes, Brent	Yes	Hawaii - University of Hawaii	1,2,3	212 212 216	1020 1099 2299	1120 1120 1120	0.10	0.00	0.00	0.07	212

Participant	Is Head	Station	Objective	Research						Extension	
				KA	SOI	FOS	SY	PY	TY	FTE	KA
Watson, Tristan		Louisiana - Louisiana State University	3	212	3130	1120	0.10	0.00	0.00	0	0

## Combined Participation

Combination of KA, SOI and FOS	Total SY	Total PY	Total TY
212-3130-1120	0.1	0	0
212-1310-1120	0.2	0	0
212-1310-1120	0.1	0	0
212-2499-1120	0.25	0.15	0.1
212-2410-1120	0.1	0.1	0.1
212-1310-1120	0.1	0	0
212-1460-1040	0.05	0.1	0
212-2499-1040	0.05	0.1	0
205-1499-1120	0.03	0.1	0
215-1710-1160	0.03	0.1	0
216-2499-0	0.03	0.1	0
212-1418-1010	0.03	0	0
212-1499-1120	0.03	0	0
212-1820-1120	0.03	0	0
215-1418-1010	0.03	0	0
215-1499-1120	0.03	0	0
215-1820-1120	0.03	0	0
212-3130-1120	0.2	0.5	0.5
212-1410-1120	0.07	0.5	0.5
212-1499-1120	0.07	0.5	0.5
212-3130-1080	0.07	0.5	0.5
201-1410-1120	0.03	0	0
212-1212-1040	0.03	0	0
216-1460-1030	0.03	0	0
<b>Grand Total:</b>	<b>2.25</b>	<b>1.35</b>	<b>1.10</b>

<b>Combination of KA, SOI and FOS</b>	<b>Total SY</b>	<b>Total PY</b>	<b>Total TY</b>
216-3130-1030	0.03	0	0
212-1020-1120	0.03	0	0
212-1099-1120	0.03	0	0
216-2299-1120	0.03	0	0
212-1131-1320	0.01	0	0
212-1211-1120	0.01	0	0
212-1410-1120	0.01	0	0
212-1450-1120	0.01	0	0
212-1461-1120	0.01	0	0
212-2130-1120	0.01	0	0
212-2310-1120	0.01	0	0
212-1122-1120	0.1	0	0
201-0-0	0.04	0	0
201-0-1120	0.04	0	0
203-2499-1160	0.04	0	0
206-0-0	0.04	0	0
212-0-0	0.04	0	0
212-2499-1120	0.1	0	0
212-3130-1120	0.1	0	0
0-0-0	0.03	0	0
212-1710-1120	0.03	0	0
212-1820-1120	0.03	0	0
212-2410-1120	0.03	0	0
<b>Grand Total:</b>	<b>2.25</b>	<b>1.35</b>	<b>1.10</b>

<b>Program/KA</b>	<b>Total FTE</b>
0	0
0	0
0	0
0	0
212	0.03
0	0
0	0
205	0.02
212	0
0	0
0	0
0	0
212	0.02
0	0
212	0.03
201	0
206	0
203	0
0	0
0	0
212	0.03
<b>Grand FTE</b>	<b>0.32</b>
<b>Total:</b>	

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