Testimony of Andrew B. Plant, Plant Pathologist, Maine Potato Board to the Committee on Agriculture, Conservation and Forestry
March 19, 2026

LD473 An Act to Support Agriculture, Conservation and Forestry in Maine

Senator Talbot Ross, Representative Pluecker and members of the Agriculture, Conservation and Forestry Committee, I am Andrew Plant, Plant Pathologist for the Maine Potato Board. I am here today to speak in favor of LD473.

Maine Chapter 30: Quarantine (Potato) rule which intends to regulate and prevent infested items (potatoes and related soil-bearing equipment) from entering Maine is antiquated and in need of updating.

Meloidogyne chitwoodi, commonly known as the Columbia Root Knot Nematode (CRKN) is a regulated, quarantine pest of potatoes for the state of Maine as well as for Maine's primary potato trade partner of Canada.

Historically, it appears as though Chapter 30 was written in response to CRKN, and the published findings of Nyczepir et. al. (1982). This report, which came out in July of 1982, was quickly responded to by Maine Department of Agriculture, Conservation, and Forestry (MDACF) with Chapter 30 being published months later in September of the same year. This initial survey in the Northwest US was conducted in portions of the states of Washington, Oregon, Idaho, California, and Nevada. All states reported finds of CRKN, and are assumed to be the originally listed states in the 1982 published Chapter 30 rule.

The following year, in 1983, surveys were conducted in adjacent states to these initial discoveries and positive finds were observed in Colorado (Pinkerton and McIntyre, 1987) and Utah (Griffin and Thomson, 1988). Finally, in 1985, it was discovered in Virginia (Eisenback et. al., 1986). However, it appears as though MDACF never amended Chapter 30 with these additional finds until May of 1991; nearly 10 years after Chapter 30's first publishing, and nearly eight years from these additional states making initial discovery of CRKN.

Since its last apparent amendment over 35 years ago, there have been three more states to report CRKN. Wyoming (Wy Pest Detection Program, 2006), Texas (Szalanski et al., 2007), and New Mexico (Thomas et. al., 2007). Additionally, since 1991 two more nematode species are regulated in the US and Canada, and a fungal disease caused by *Synchitrium endobioticum*, commonly known as Potato Wart, have been discovered and do not appear in this agency rule, yet should.

Neither these pests, being soilborne and seedborne, nor their geography of occurrence is static. Unfortunately, from a risk management standpoint, our regulatory barriers to their introduction into Maine, cannot be static either. It however appears that the MDACF has failed to keep this rule updated and is now a liable risk to Maine's $1.3 Billion potato industry.

Specifically for Columbia Root Knot Nematode, sole responsibility for its control and limitation of spread is by individual states. An introduction into Maine would single us out as the only seed producing state, and the only US Top-20 potato production state east of the Mississippi River with an infestation of this nematode.

To quote Handoo et. al. (2025) in their chapter on Root-knot nematodes in "Practical Plant Nematology, 2nd Edition": "The basic objective in any pest management is to increase both quantity and quality of crop yield, and increasing the yield quality is sometimes the most important objective for *Meloidogyne*. Targeting species as quarantine organisms reduces the risk of spread through international trade. *Meloidogyne chitwoodi* is a serious pest of economically important crops, such as potatoes and carrots, and is a prohibited pest in many countries."

They further elaborate on control and prevention, stating: "In view of the high multiplication rate of root-knot nematodes and difficulty in determining occurrence of low population densities, previously infected land should always be considered infested, even if the presence of *Meloidogyne* cannot be demonstrated by soil analysis."

This highlights an important aspect: how cultural methods of soil and equipment sanitation, hygiene, and disinfestation—or the lack thereof, or lack of oversight and documentation of such activities—contribute to the perceived risk of contamination via local dissemination field-to-field through seed, shared equipment and machinery, or shared storage areas. This necessitates strict quarantine rather than conditional regulation of potatoes and equipment entering our state from these infested areas.

Compliance with regulations to prevent introduction and spread of Columbia Root Knot Nematode is not easy. Although the transport of infected tubers through fresh or seed potato markets has been responsible for many infestations, asymptomatic tubers and low-density populations and consequent infections limit the efficacy of detection by random field and tuber sampling and examinations. Once in a field, eradication by any means short of soil sterilization and long fallow periods is near impossible and financially burdensome (USDA-ARS, 2013).

A hierarchy of pest management might include: exclusion, followed by eradication, and then management based upon measurable action thresholds. Prophylactic quarantines to prevent colonization and establishment of nematodes, plant pathogens, and insects, such as described above are foundational to the premise of Integrated Pest Management, which serves to reduce our pesticide usage while improving environment and economy. These practices serve even greater prominence for our organic farming sector as curative or maintenance measures such as synthetic fumigants, nematicides, insecticides, and fungicides are restricted (Briar et al., 2016).

Thank you for your time and consideration on this important matter. I hope that you will vote in favor of LD473, and I am happy to try and answer any questions you may have of me.

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Distribution of Regulated Potato Pests in the Continental United States

September 16, 2022

State	PCN [1]	CRKN [2]	PRN [3]
Arkansas (AR)			✓
California (CA)		✓	✓
Colorado (CO)		✓	
Idaho (ID)	✓	✓	✓
Indiana (IN)			✓
Nevada (NV)		✓	
New Mexico (NM)		✓	
New York (NY)	✓		
Oregon (OR)		✓	✓
South Carolina (SC)			✓
Texas (TX)		✓	
Utah (UT)		✓	
Washington (WA)		✓	✓
West Virginia (WV)			✓
Wisconsin (WI)			✓
Wyoming (WY)		✓	

1 Potato Cyst Nematodes (*Globodera pallida* and/or *Globodera rostochiensis*)

2 Columbia Root Knot Nematode (*Meloidogyne chitwoodi*)

3 Potato Rot Nematode (*Ditylenchus destructor*)

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Recovery Plan For

Root-Knot and Cyst Nematodes

Parasites of Agronomic and Horticultural Plants Throughout North America

March, 2013

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This recovery plan in one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks and capacity required to mitigate the impact of high consequences plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Root-knot and cyst nematodes are two large groups of sedentary endoparasitic nematodes. They are distributed globally, affect thousands of plant species, and include some of the most devastating plant pathogens on the planet. Arguably, *Meloidogyne incognita* is the single most important plant pathogen based on global yield loss and the resources spent in attempts to control it. *Globodera pallida* and *G. rostochiensis*, the potato cyst nematodes may be the most highly regulated of all plant pathogens. It is difficult to think of an agronomic plant species that is not infected by either a root-knot or cyst nematode. Many of the most damaging species already reside in the U.S. The soybean cyst nematode *Heterodera glycines*, which costs U.S. producers over $300 million annually occurs in virtually all regions in the states that grow soybeans. *Meloidogyne chitwoodi* infects potatoes throughout the Pacific Northwest, and vegetable production in the southeast routinely applies nematicides to control *Meloidogyne incognita*, *M. arenaria*, and *M. javanica*. Given the constant struggle to manage the widespread root-knot and cyst nematode infestations that currently exist in the U.S., it may seem presumptuous to propose a recovery project for species that have not yet become established in U.S. soil. Furthermore, the differences among nematode species and the crops they infect may be so profound that recommendations that attempt to encompass all species may be too general to be meaningful. However, it is possible to outline some important issues in nematode mitigation that apply to both cyst and root-not nematodes. I refer to these as Recovery Plan Realities.

1. Recent history clearly indicates that new species will be introduced into the U.S.
2. It is highly likely that new species or genotypes already exist in the U.S. presently undetected.
3. There will be a significant lag time between nematode introduction and detection.
4. Early detection of established infestations is critical for mitigation success.
5. Many states to not have personnel trained to act as nematode infestation "first responders."
6. Opportunities for training in nematology are decreasing.
7. USDA APHIS/PPQ has the incidence command structure to rapidly respond to new nematode detections. States do not.
8. Once a nematode species is widely established it is practically impossible to eradicate.
9. The management tools of 2013 are essentially the same as they were 50 years ago, minus the variety of chemical nematicides.
10. The current management tools have greater precision but require an increased understanding of the site-specific nematode problem.
11. Genetic resistance is available for some crops and some regions and effective against some nematode genotypes.
12. No one knows how climate change will affect future nematode management.

A set of recommendations has been proposed for dealing with these realities. The most important recommendations are:

1. Increase and improve nematode survey efforts.
2. Create databases of pest species and geographic locations.
3. Improve taxonomic resolution of pest nematode identifications.
4. Train first responders.

5. Support studies of nematode biology that improve risk analysis models.
6. Establish a nationwide program of field nematology internships.
7. Support broad-based integrative approaches for nematode management.

Rationale and justification for these recommendations are presented below.

I. Introduction to Root-Knot and Cyst Nematodes

Root-knot and cyst nematodes are two taxa of sedentary endoparasitic nematodes, each containing nearly 100 species. Within both groups there are species that cause severe agricultural losses in a wide range of crops, from temperate to tropical habitat types. Until 1949, all root-knot and cyst nematodes were classified in the genus *Heterodera.* The creation of the genus *Meloidogyne* apart from *Heterodera* and all other cyst-forming nematodes was the first step in the process of recognizing the fundamental genetic and physiological differences between the two groups. Today cyst and root-knot nematodes are typically classified in separate families reflecting the tens of millions of years since they shared a common ancestor. Root-knot species belong to the monophyletic genus *Meloidogyne* existing as the only member of the family Meloidogynidae. Cyst nematodes are divided among six genera in the family Heteroderidae, which also include genera that morphologically resemble the cyst-forming species, but lack the hardened, resistant cuticle in the adult female stage. It is this character that primarily defines the subfamily Heteroderinae, and it is within this subfamily that exists most of the major agricultural pest species.

II. Symptoms and Physiology

Collectively cyst and root-knot nematodes are responsible for a large proportion of the estimated 10 billion dollars lost annually in the United States to plant parasitic nematodes (Chitwood, 2003). Potato cyst nematode alone is thought to account for losses of more than 12% of the average world potato crop yield (Urwin et al. 2000). Accurate economic estimates are hard to develop due to the complex nature of nematode induced plant disease. The site of cyst and root-knot nematode infection is the roots. Root penetration and establishment of a feeding site may facilitate interactions with other bacterial and fungal plant pathogens. The above-ground symptoms are not exclusively diagnostic of nematode infection since plants exhibit general symptoms of wilt, nutrient deficiency, stunting, and uneven growth of plants within a field. In some cases, significant yield losses occur without conspicuous above ground symptoms. There is one feature that sets root-knot and cyst species apart from most other plant parasitic nematodes. The female nematodes are large. The adult female stage of development can be observed in the field using a hand-held magnifier. Developing cysts can be detected on roots and the galls induced by root-knot nematodes can be examined to reveal the swollen female stage or gelatinous egg masses on the root surface. In a sense this constitutes "real-time" morphological confirmation of nematode infestation. Standard soil sampling methods for soil-dwelling nematodes are generally sufficient for detection of the infective juvenile stages of both root-knot and cyst nematodes. These assays, however, require laboratory extractions and microscopic examination.

From a developmental perspective, the similarities between the two nematode groups include the requirement of freshly hatched second-stage juveniles to migrate through soil to locate a suitable plant host. The juveniles must penetrate the root cortex, migrate internally, and establish a feeding site that is characterized by large multinucleate metabolically active cells. Feeding site development and ingestion of cell contents involve nematode secretions transmitted through the hollow nematode stylet. Once established the juveniles undergo a series of molts resulting in a swollen sedentary adult female or a migratory vermiform adult male. Both root-knot and cyst nematodes have evolved a complex relationship with their plant hosts that dramatically alters normal plant host physiology while avoiding plant defenses.

III. Life Cycle Similarities and Differences

Physiological and ecological differences between the two nematode groups highlight features that have been targeted in management strategies. The eggs of root-knot nematodes are often deposited in the soil, initially surrounded by a protective gelatinous matrix that can exhibit antimicrobial properties. While a portion of the eggs of cyst nematodes are produced within a gelatinous matrix, by the end of a growing season the eggs of cyst nematodes are encased in the highly resistant and easily dispersed cyst. Root-knot eggs readily hatch in the soil environment in the presence of adequate moisture and temperature. Cyst eggs typically require the additional presence of hatch inducing chemicals. Once hatched, the infective juveniles of both groups have limited energetic resources for locating and infecting a suitable host. Neither cyst nor root-knot juveniles can migrate much more than 100 cm under their own power. Yet root-knot nematodes may increase their chances of encountering a susceptible host, simply due to the large number of potential host species. Some *Meloidogyne* species have demonstrated successful development on hundreds of plant species, including monocots and dicots. By comparison the limited host range of cyst nematodes lessens the likelihood of encountering weed hosts adequate for sustaining nematode development. For example, corn cyst nematode *Heterodera zeae*, and carrot cyst nematode, *H. carotae* only exist on cultivated and wild forms of their respective hosts.

The infective juveniles of both cyst and root-knot species must navigate soil pore space that is inhabited by a wide range of predatory organisms. These include mites, tardigrades, amoebae, infectious fungi, bacteria, and predaceous nematodes. The root surface may be colonized by bacterial species that deter or impair host recognition and root penetration. Considered together, all soil organisms antagonistic to plant parasitic nematodes compose what has been termed nematode "suppressive soil". Recent observations of unexplained nematode suppression in fields otherwise untreated have led to a resurgence of research focused on identifying and exploiting the agents of suppression.

When a nematode feeding site has been established within the root, and the nematodes have molted to adult stages, most cyst species require fertilization by the migratory males. These males will emerge from the root and seek females that are exposed at the root surface. In contrast, many root-knot species are parthenogenetic and males, which may be produced under some conditions, have no role or a limited role in reproduction. Obligatory mating in amphictic species has also been identified as a potential stage in the nematode's life cycle amenable for disruption.

Undoubtedly the most active field of investigation in the science of Nematology is determining the molecular and biochemical pathways involved in initiating and maintaining a nematode feeding site within the root (Atkinson et al., 2012). An obvious target for future nematode management is the engineering of site-specific termination of nematode feeding. While remarkable progress has been reported, the goal of incorporating these desirable traits into publically available cultivars has not yet been achieved.

IV. Historical Case Studies

There have been several high profile nematode infestations that have, or had the potential to seriously impact U.S. agriculture. These case studies are instructive for the evaluation of mitigation strategies and overall impact of nematode infestations. Each of these case studies highlights a major issue in regard to mitigation.

A. Golden Potato Cyst Nematode in New York

Long recognized as a major economic pest of potatoes in Europe, *Globodera rostochiensis* was suspected to have been introduced to Nassau County, Long Island, New York through soil adhering to military equipment following the First World War (Brodie and Mai, 1989). Poor potato growth was noted in the region as early as 1934 in a 16-ha potato field. The nematode species was positively identified in 1942 (Chitwood et al., 1942). By 1944, a strict state quarantine was established to confine the nematode to that area of eastern New York. The New York State quarantine was shortly followed by the federal Golden Nematode Act in 1948 which established policy for protection of the potato industry. A new discovery of an infested potato field in Delaware in 1968 hastened the establishment of the federal Golden Nematode quarantine. Failure of the earlier New York State quarantine was evident when infestations in western New York were observed in Steuben County 1967, and subsequently discovered in three additional counties in the 1970's and early 1980's. Brodie (1984) has noted that the several decade lag-time between hypothesized introduction and nematode discovery closely parallels the timing of the discovery of potato cyst nematode in Europe. If it is assumed that the importation of potato breeding stock from South America following the potato late blight was largely responsible for the introduction and establishment of potato cyst nematode in Europe, then it took nearly 50 years before the nematode was widely recognized as a major pest. It is generally acknowledged that shortening the interval between introduction and pest detection will significantly aid mitigation efforts. The significance of this epidemiological timing will be explored further in following sections.

Containment of the Golden Cyst Nematode in the state of New York through the federal quarantine could be considered one of the major success stories in the history of nematode regulatory policies. Today the experience gained through the Golden Cyst Nematode has been instrumental in establishing a monitoring and control program for the Pale Potato Cyst Nematode, *Globodera pallida* in Idaho (see below).

- Mitigation point #1. There generally exists a significant lag-time between nematode introduction and nematode detection.

B. Soybean Cyst in Eastern and Central US

The soybean cyst nematode (SCN), *Heterodera glycines*, is found in nearly every soybean growing state in the US. Estimated annual losses in the US averaged over 2009-2011 exceed 110 million bushels (Unitedsoybean.org). SCN was first recorded in the US in 1954 in Hannover County, North Carolina where imported flower bulbs from Japan were grown (Noel, 1992). Within the next four years it was discovered in Arkansas (1957), Kentucky (1957), Illinois (1957), Missouri (1956) Tennessee (1956), and Virginia (1958). The rapid expansion of SCN in the 1950s-1960s suggested to some investigators that SCN distribution in North America was not due to a single introduction and subsequent dispersal via agricultural practices and commerce, but resulted instead from events occurring 50 years earlier. Noel (1992) outlines plausible sources of SCN introduction through the turn-of-the century practice of importing soil from Asia to enhance the natural populations of nitrogen-fixing rhizobia. Unlike regulations to curb the movement of potato cyst nematode, the establishment of state and federal quarantines designed to contain the movement of soybean cyst nematode were ineffective. The relatively recent spread of SCN in Nebraska illustrates the speed of dispersal in spite of efforts to prevent cyst movement. Discovered in 1986 in a single county in the southeastern corner of Nebraska, yearly surveys have tracked its apparent westward movement across approximately 200 miles, and it is now recorded from 54 counties. Significantly management protocols for minimizing SCN spread, operating within the confines of the traditional corn-soybean rotation, were established from the beginning of nematode discovery. There protocols did not prevent spread of the nematode. Clearly at this advanced stage of establishment SCN mitigation must depend on methods other than regulations attempting to restrict movement.

- Mitigation point #2. Once established, it is extremely difficult to prevent further spread of plant-parasitic nematodes.

C. Columbia Root-Knot Nematode in the Pacific Northwest

The Columbia Root-knot nematode, *Meloidogyne chitwoodi*, was first recorded from the Columbia River Valley of Washington in 1981. Its dramatic symptoms on potato tubers create a virtually unmarketable potato for fresh market and one unsuitable for chip production. Its wide host range includes cereals commonly grown in rotation with potatoes. Damage to potatoes starts with infective juveniles invading developing tubers where they establish feeding sites just beneath the potato surface. Late season infection may result in asymptomatic tubers that later express the characteristic pimple-like swellings while in cold storage. *M. chitwoodi* is adapted to development in cool, temperate climates, although isolates in Colorado, Nevada, New Mexico, Utah and Texas illustrate that it can exist in semiarid desert habitats with hot summers. In many production systems phytosanitary certification indicating the absence of *M. chitwoodi* is required for international potato transport. USDA/APHIS regulations regarding the Columbia root-knot nematode affect nine states in the US. Compliance with regulations to prevent introduction and spread of *M. chitwoodi* is not easy. Although it is acknowledged that transport of infected tubers through fresh or seed-potatoes markets has been responsible for many infestations, asymptomatic tubers and low density infections limit the efficacy of detection by visual examinations. Once in a field, eradication by any means short of soil sterilization and long fallow periods is near impossible.

- Mitigation point #3. Endoparasitism and asymptomatic infections by root-knot nematodes emphasize the need for soil surveys to detect infective juvenile stages.

V. Newly Emergent Case Studies

Several high profile nematode species have emerged within the last decade, although the precise timing of their introduction is unknown. They provide an indication of our ability to address a potential nematode threat to US agricultural given current understanding of the disease process and recent advances in technology. The profiles below emphasize the distinction between cyst and root-knot disease management.

A. Cyst Nematodes on Potato in Idaho and Oregon

The Pale Cyst Nematode, *Globodera pallida*, was first recorded in the United States in 2006. Its initial discovery at a potato processing facility in eastern Idaho sparked a chain of regulatory decisions that rapidly closed markets to Idaho potatoes by Canada, Mexico, and Korea, and prevented all US potato exports from entering Japan. In 2007, USDA APHIS PPQ and the Idaho State Department of Agriculture put into place a potato cyst eradication plan that continues today. Initial delimitation of the infestation identified nine fields within a one mile radius in two counties near the city of Idaho Falls. Today 17 infested fields representing 2,015 acres have been identified, expanding the radius of infestation to five miles.

The fortuitous discovery of the 2006 *G. pallida* cysts was made by sampling tare soil at the processing facility, not through standard in-field soil sampling. The soil was collected as part of Idaho Department of Agriculture's participation in the federal Cooperative Agricultural Pest Survey Program (CAPS). Sampling tare soil, the soil that accompanies the tubers following their removal from the field, is an effective method to detect cysts, but will not serve as a detection method for nematode species that are not protected by the resistant cyst stage. *Meloidogyne* species, for example, would not survive in the desiccated soils that accumulate in the processing facility. Another drawback to detection at this stage in potato production as evidenced by the 2006 discovery was the relatively lengthy time spent tracing the cysts to their field of origin due to the heterogeneous mix of tuber shipments at the processing facility.

In the original nine infested fields, a combination of annual fumigation in the spring with methyl bromide fumigation and in the fall Telone II, together with planting non-host crops has reduced egg viability to less than 1% according to the five year review report. Fumigation, given the availability of these highly toxic general biocides, would be expected to be a standard response to any newly discovered soil inhabiting nematode of quarantine status. The soil sterilization process would be complicated if the detection was within an orchard or forest. In those cases, fumigation would most likely have to be accompanied by tree removal and deep soil fumigation to ensure the nematode did not persist within roots. In the case of PCN and potato production, other methods can supplement eradication efforts.

Additional general tools for eradication include fallowing fields, solarization, and biofumigation. A Solanaceous trap crop that induces eggs to hatch but does not support nematode replication has been added to the Idaho PCN eradication plan. Trap crops are a management practice dating back to the earliest days of plant-parasitic nematode control. Recent improvements of the approach use specifically bred cultivars and a detailed understanding of host-parasite dynamics for management efficacy. Evidence of mitigation success has led to the reopening of markets for Idaho potatoes in all countries other than Japan. Eradication and monitoring efforts continue. An estimated $7 million dollars has been spent annually on the potato cyst eradication program.

Accompanying the eradication attempt has been a nationwide survey of all seed potato production fields including a significant percentage of table stock potatoes. There are no reports of additional infestations of *G. pallida* outside of the two counties in Idaho identified in the initial discovery. However, a new *Globodera* species, named *G. ellingtonae*, has been identified and described from Oregon. This discovery occurred in a valley near Powell Butte, Oregon that was used in a potato breeding program active since the 1970s. Because the cyst nematode population levels were relatively low, this new species was thought to be a recent introduction. Molecular analyses indicate that *G. ellingtonae* is also present in Caribou and Teton Counties in Idaho, well outside the range of the current *G.pallida* infestations. Host range tests are ongoing. Early results have demonstrated reproduction on potato although the question of pathogenicity is unresolved. The possibility of the existence of native North America *Globodera* species associated with Solanaceous weeds has not been excluded as a potential source of cyst isolates with the ability of reproduction on cultivated potato. These *Globodera* discoveries emphasize the importance of systematic surveys, monitoring and the earliest possible mitigation efforts.

- Mitigation point #4. Eradication, if possible, will require an expensive, highly regulated, large-scale operation that will include multiyear applications of general biocides.

B. *Meloidogyne enterolobii* in Florida

Meloidogyne enterolobii (synonym *M. mayaguyensis*) has recently been recognized as a cryptic nematodes species widespread in southern Florida (Brito et al.,2004). It is representative of a category of emerging pest species that are initially recognized based on their ability to reproduce on a host or cultivar believed to be resistant to the species. *M. enterolobii*, a phenotypically variable species, was most likely misdiagnosed in Florida as *M. incognita* or *M. arenaria* due to strong morphological similarity to both species. There are no clear morphological features that allow this nematode to be discriminated from other common species of *Meloidogyne*. It is not known how long *M. enterolobii* has existed in Florida. It was not until the reduction in use of general biocides like methyl bromide and the subsequent employment of more narrowly effective resistance genes, that species such as *M. enterolobii* were noticed. In the case of *M. enterolobii* it was reproduction and galling on Mi1-resistant tomato that led to investigations that revealed it as a cryptic species. Once molecular methods were developed to identify the species, it was shown to have a worldwide distribution. In addition to Florida, it has been reported from south, west, and eastern countries in Africa, China and Vietnam, Central and South America, Europe, and recently Mexico. *M. enterolobii* is a particularly aggressive species that can also infect *M. incognita* resistant soybeans and sweet potatoes, and peppers containing the N-resistance gene.

This species is now recognized as a major pest of many plant species throughout tropical and subtropical regions of the world. More than 50 host species are known, but many more species are expected to be suitable hosts. The host range includes many of the vegetables grown in the U.S., as well as ornamentals commonly transported by the nursery industry. *M. enterolobii* is thought of as a tropical or subtropical nematode species and it has been frequently intercepted on plant species shipped from tropical countries. The lower bounds on temperature necessary to complete nematode development have not been determined. A recent report from North Carolina confirms that the species exists north of its verified distribution in Florida (Ye et al., 2013). As a species that reproduces by mitotic parthenogenesis, a single infective juvenile could initiate an entire population. Since susceptible soybean and cotton are widespread in the southeast and south central U.S., it is easy to imagine *M. enterolobii* rapidly spreading across these regions.

There is a high probability that many more *Meloidogyne* species in North American species exist as cryptic species complexes. For example, *M. floridensis* which was originally considered a variant of *M. incognita*, was first recognized because of its reproduction on nematode- resistant Nemaguard and Okinawa peach root-stocks (Handoo et al., 2004). Molecular diagnostic techniques readily differentiate these resistance-breaking *Meloidogyne* species, but geographically comprehensive surveys need to be conducted to determine their U.S. distribution.

- Mitigation point #5. The next major nematode pest may emerge from populations already resident in the U.S.

VI. A Poll of Nematologists

As a means to assess expert opinion about the future of nematode management and the national capacity to address current and future management needs, an email poll was send to 56 professionally active plant nematologists in the US. Forty-two nematologists replied. The answers are summarized below.

1. **Do you think it is likely that within 5-10 years, novel species or races of nematodes will be encountered in the U.S. that are capable of causing economic damage to our agricultural or horticultural crops?**

Respondents were unanimous on this point. They all felt that it was likely, highly likely, or inevitable that US agriculture will be confronted with new economically damaging nematodes. The most frequently cited reasons include the global scope of trade, the volume of agricultural commodities coming from Mexico, and the inability of border and port inspectors to examine a significant portion of shipments. One respondent mentioned the statistical impossibility of conducting successful detections given the quantity of commodities and the endoparasitic life stages of many nematodes. Many nematologists cited the invasions of the last 10 years as evidence supporting the probability of future invasions. Over 50% of the respondents expressed the opinion that the development of new races or pathotypes, or the redistribution of species currently in the US was of equal concern to exotic introduction. Several nematologists speculated that warming temperatures will allow overwintering of *Meloidogyne incognita* at higher latitudes complicating management in soybean which has primarily been focused on soybean cyst nematode. Two nematologists mentioned that they are currently investigating nematodes that are new records in their states. One respondent wrote that the defunding of regulatory agencies will soon substantiate the "everything is everywhere" model of nematode distribution. Some respondents offered predictions of specific nematode species that they believed will increase in economic significance. Among their concerns was the potential interstate transport of *M. marylandi, M. graminis,* and other root-knot nematodes associated with sod production, walnut seedling shipments that might be infested by *M. partityla*, movement of *M. enterolobii* on horticultural stock, undetected races of *M. chitwoodi*, and the spread of *Heterodera avenae* throughout wheat producing states. One nematologist paradoxically stated that resistant cultivars need to be developed in order to detect resistance breaking pathotypes.

2. **If a new potentially damaging species is introduced, do you think we have the knowledge, infrastructure, and resources to limit its damage?**

Several related themes emerged from this intentionally open-ended question. Nine respondents identified the rapid and comprehensive actions by APHIS following the 2006 discovery of *Globodera pallida* in Idaho, as a model for addressing a potentially destructive nematode species. The early response was deemed critical for success. One nematologist commented that *G. pallida* in Idaho appears to be the best case scenario, a relatively confined infestation on an economically important crop, and questioned whether a similar response would be mounted for commodities of lesser economic significance. The initial discoverer of the cysts in the Idaho infestation was a trained nematologist with years of field experience. Fifteen nematologists said that states that lack a trained field nematologist will impair early detection and management efforts. One nematologist speculated that we are currently at a 40-year low in terms of scientists who work on plant-parasitic nematodes that feed on food and fiber plants in the US. Similarly, five respondents mentioned lack of training opportunities as a limiting resource. And while the National Plant Diagnostic Network has performed well in the monitoring of some high profile plant pathogens, its ability to address soil borne pathogens like nematodes was questioned by one respondent. A general frustration was expressed by nematologists over the lack of funds, the reduction in regulatory personnel, lack of effective chemicals for nematode management, limited success in incorporating genetic resistance into cultivars used by producers, the unfulfilled promise of GMOs, limited genetic basis for resistance (e.g. soybean/ soybean cyst nematode), poor performance of biological controls, and the lack of practical approaches for managing crops with multiple pest species (e.g. soybean/ soybean cyst nematode + southern root-knot nematode).

3. **In managing the existing pest nematode species in the U.S., would you say we are winning the battle, staying even, or losing the battle?**

Overall 18 nematologists thought we are losing the battle against nematode pest species, 19 said we are staying even, 2 said we are winning, and 3 felt that we cannot tell at this moment. The two respondents that felt we were winning supported their position by stating: 1. that the loss of chemical nematicides has forced us to broaden our management approaches in ways that are ultimately more sustainable, 2. we have paid greater attention to sanitation and clean nursery stock as a means to prevent nematode movement. The nearly 50% of respondents that felt we were losing repeated many of the reasons in question #2. Seven respondents explicitly stated that the alternatives to methyl bromide and other nematicides removed from the market are not as effective as those they replaced. The purpose of the alternatives to methyl bromide program was questioned on the basis that it is replacing one chemical nematicide with another, which ultimately will have to be replaced in the future.

Regarding alternative treatments one nematologist put it this way, "New seed treatment nematicides, while achieving a great deal of press, do not (in my experience) provide efficacy comparable to that of traditional compounds." Somewhat surprisingly several nematologists complained that genetic plant resistance is either too narrowly effective in that it only applies to a subset of species or genotypes, new nematode races overcome resistance relatively rapidly, resistant varieties do not possess desirable agronomic traits, or that the resistance genes have not been bred into cultivars that are suitable for their region. Despite these limitations a few nematologists maintain moderate optimism that existing management approaches will allow us to "hold our own" while waiting for genetically engineered control options.

VII. Recovery Plan Realities

Any recovery plan designed to mitigate the impact of new species or genotypes of cyst or root-knot nematodes must address the following realities.

1. Recent history clearly indicates that new species will be introduced into the U.S.
2. It is highly likely that new species or genotypes already exist in the U.S. presently undetected.
3. There will be a significant lag time between nematode introduction and detection.
4. Early detection of established infestations is critical for mitigation success.
5. Many states do not have personnel trained to act as nematode infestation "first responders."
6. Opportunities for training in nematology are decreasing.
7. USDA APHIS/PPQ has the incidence command structure to rapidly respond to new nematode detections. States do not.
8. Once a nematode species is widely established it is practically impossible to eradicate.
9. The management tools of 2013 are essentially the same as they were 50 years ago, minus the variety of chemical nematicides.
10. The current management tools have greater precision but require an increased understanding of the site-specific nematode problem.
11. Genetic resistance is available for some crops and some regions and effective against some nematode genotypes.
12. No one knows how climate change will affect future nematode management.

Dealing with the Realities.

Realities 1-6 in the list above concern the process of detection, survey, identification and the training of personnel engaged in those activities. Nematode management is addressed in points 7-12.

VIII. Detection, Surveys, Identification, and Recommendations

There is wide-spread agreement among nematologists regarding the value of early recognition of exotic nematode species. Port of entry detection, which involves cooperation between federal and state officials, is beyond the scope of this report. It is sufficient to state that nematodes will continue to cross U.S. borders and a percentage of those introduced will become established in U.S. soil. First responders to a pest infestation include producers, crop consultants, and extension agents. None are necessarily likely to have training in nematology.

Depending on the severity of the problem, soil or root samples may get shipped to laboratories with nematode diagnostic capability. Few diagnostic laboratories have the time or resources to identify all the plant-parasitic nematodes to the species level, so most reports focus on the genus level. This level of taxonomic resolution will not detect exotic species nor will it improve the reference database for U.S. nematode pest species distribution. There are several important reasons for refining nematode pest databases. With improved taxonomic and geographic resolution of nematode distributions, risk assessment models will increase in accuracy and simultaneously test model validation. Recent molecular examinations of nominal cyst and root-knot species have revealed significant intraspecific variation, even to the extent of supporting the existence of cryptic species. The prevalence of cryptic species, host-races, and resistance breaking genotypes suggest greater effort should be spent monitoring the occurrence of these entities. Molecular diagnostic methods are available for many plant parasitic nematode species, although validation and online access to diagnostic information including validation studies is scarce. SOPs for sampling and identification have been created for only a few high profile pests such as *Globodera rostochiensis*, *G. pallida*, and *Meloidogyne chitwoodi*. More SOPs emphasizing validated molecular diagnostic approaches are needed.

The Cooperative Agricultural Pest Survey program (CAPS) is the only annual survey program in the U.S. that samples nematodes. The 2012 CAPS Pest List developed through the Analytic Hierarchical Process (AHP) targeted two nematodes in the 50 ranked pests, *Ditylenchus angustus* the rice stem nematode, and a complex of *Meloidogyne* species identified as the citrus root-knot nematodes. Additional prioritized nematodes not on the AHP list included eight cyst species and two root-knot species, previously listed by the AHP process. The National Agricultural Pest Information System (NAPIS) tracks 25 nematode species, 18 of which are root-knot or cyst nematodes. Given the typically lengthy lag-time from initial infestation until the time when nematode population density becomes economically-significant, an aggressive monitoring and detection program should be the foundation for a rapid response to prevent the establishment and spread of new pest species.

Recommendations:

a. Expand the nematode survey component of the CAPS program.

b. Build reference data bases to facilitate rapid identification and geographic location of species.

c. Increase taxonomic resolution of ongoing surveys to accurately record endemic species, regional diversity, host-races, and resistance breaking genotypes.

d. Encourage and support international coordination of reference databases.

e. Encourage the development of more SOPs for nematode identification.

f. Increase Nematology training of diagnostic "first responders."

g. Invest in automation/large-scale diagnostic DNA sequencing at identification centers.

h. Increase resources for pest risk assessment models and establish linkages between modelers and nematologists.

i. Support the generation of biological, developmental, physiological, and environmental parameters for model development.

j. Integrate modelers into Nematology related Multistate Projects.

IX. Management Tools and Recommendations

The traditional approaches of crop rotation, sanitation, plant resistance, and chemical control continue to be the mainstay of nematode management. Generally the higher value of the crop, the more likely that chemical tactics is used in management strategies. Methyl bromide was the single most effective broad-spectrum pre-plant soil fumigant used in nematode control. Its phase-out has been prolonged by critical use exemptions (CUE) allowed by the Montreal Protocol in cases where there is a lack of available alternatives to avoid significant disruption to regulatory programs and commodity markets. In 2012 a majority of the CUEs were issued for vegetables (cucurbits, eggplant, peppers, sweet potato, and tomatoes), strawberries, ornamentals, grapes, nuts, and orchard replants. Largely the targeted pests were root-knot nematodes. Zasada et al., (2010) have described the obstacles in developing and implementing alternatives to methyl bromide. They envision a future in which current high-value crops still dependent on methyl bromide will be forced to adopt a multi-tactic management approach that may not be able to reduce annual crop losses by nematodes to below 10%. Compounding the situation is the diminishing number of "field-savvy nematologists to develop and implement alternative management strategies".

If we assume that the next exotic nematode introduction corresponds to the nematodes listed in the AHP and Prioritized Pest Lists, it is notable that all but one of the *Meloidogyne* species on the list infects trees. These include six species of *Meloidogyne* that infect citus, *M. coffeicola* the coffee root-knot nematode, *M. paranaensis*, the Parana coffee root knot nematodes, and *M. mali* the apple root-knot nematode. The detection of an infestation by any of these species will necessitate drastic control procedures that will undoubtedly involve destruction of all infected trees and repeated fumigation. Replant options for root-knot species of citrus and apple are hampered by the lack of root-knot nematode resistant root-stock. Resistant root-stock is available for coffee, and grafting or resistant rootstock has been successful in management of *M. incognita* and *M. paranaensis* in Brazil (Campos and Silva, 2008).

An Integrative Approach

Most field savvy nematologists are committed to an integrative approach for nematode management. They really have little choice given the reduction of broad spectrum nematicides. Implicit in the integrative approach is the understanding that no single tactic alone will provide adequate or long-term nematode control. Collectively, a multi-tactic approach in some cropping systems can suppress or manipulate nematode populations sufficiently to generate yields comparable to those achieved with fumigant nematicides. The approach, however, is highly dependent on a detailed understanding of nematode biology, including information about species identity, host range, survival capabilities, temperature optima, and longevity (Zasada et al., 2010).

Crop rotation has long been a cultural practice in nematode management. Constraints exist when economics dictate maintenance of year-round high-value crops or investment in a crop production system limits production versatility. The presence of cyst nematodes in a rotation schemes naturally lengthen periods of growing non-hosts due to the survival capabilities of eggs encased in the cyst. In some situations, such as the barley/potato rotation found in several western states, barley permits reproduction of *Heterodera. avenae*, the cereal cyst nematode, and potato supports *Meloidogyne chitwoodi*. Both are nematodes of economic and regulatory significance. Similarly, cropping systems in southern states that produce cotton and soybeans may be confronted with developing strategies for *Meloidogyne incognita* on cotton and soybeans, *H. glycines*, the soybean cyst nematode, and *Rotylenchulus reniformis*, the reniform nematode which reproduces on both crops. What would be extremely beneficial in this cropping system would be cultivars of soybeans that were genetically resistant to all three nematode species.

Genetic host resistance to nematodes is estimated to have prevented hundreds of millions of dollars in yield losses to nematodes. Host resistance in soybeans to the soybean cyst nematode has been crucial in achieving record yields in spite of the ubiquitous presence of the nematode. But resistance in soybeans and a number of other crops is steadily decreasing as "resistance-breaking" nematode genotypes increase. While thousands of SCN resistant cultivars are available to producers, virtually all of them use the same set of resistance genes derived from PI88788. The discovery and incorporation of new sources of host resistance into agronomically acceptable cultivars is a slow process. Marker assisted selection has accelerated the process in some crops, but achieving resistance to multiple nematode species or in cases where the genetics of the host-parasite reaction is complex, is still a difficult challenge. On a positive note, breeding programs in the Pacific Northwest have successfully incorporated multiple resistance genes to both cyst and lesion (*Pratylenchus*) nematodes in cereals. These programs were built upon decades of biological studies and strong international collaborations.

Organic amendments, seed treatments, and biofumigation have seen a resurgence of research interest in the wake of nematicide reduction. Amendments such as green manures, animal manures, composts or slurries have been tested for years. Conflicting evidence of efficacy have dampened enthusiasm among many nematologists, but the hard work of integrating these tactics into large-scale production systems is still in initial stages. Seed treatments with abamectin, *Bacillus firmus*, and harpin proteins have also divided researchers concerning their impact on nematode management, yet unrealistic expectations given the standard of fumigant nematicides could be tempered as these treatments are viewed as a component of a broader management strategy.

Currently the highest hopes and expectations of durable nematode control reside in biotechnological applications. Foremost among the newer approaches is RNA interference (RNAi). RNAi induced suppression of genes essential for nematode development, reproduction, and parasitism has been demonstrated for major pest species. Importantly this gene suppression extends across different nematode genera and includes migratory endoparasitic species as well as sedentary endoparasitic groups represented by cyst and root-knot nematodes. Numerous genes targeted for suppression are under investigation with leading candidates those involved in establishing and maintaining the feeding site within roots, developmental genes such as those involved with hatching or mating, and genes associated with mRNA metabolism. Researchers are already emphasizing the potential durable nature of this form of resistance and paralleling traditional plant breeders, seek to "stack" multiple gene targets. They also emphasize the many obstacles, both scientific and regulatory, that must be overcome before RNAi can become another tool for nematode management.

Recommendations:

a. Provide incentives for participants in Multistate/Regional Nematology projects to work jointly on specific integrated management approaches.
b. Just as nematode "first-responders" require training, field-savvy nematologists need to educate the next generation of nematologists to facilitate the implementation of integrated management.
c. Organize Gordon Research Conference style meetings bringing together the field-savvy nematologists with biotechnologists.
d. Establish a nationwide program of field nematology internships.
e. Support broad-based approaches nematode management, if only as a backup for potential failure of "silver-bullet" solutions.
f. Use the Society of Nematologists as organizing body to facilitate recommendations.

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https://www.ars.usda.gov/ARSUserFiles/np303/NPDRS/Recovery%20Plans/Cyst%20and%20Rootknot%20Nematode%20Diseases%20Final%20Recovery%20Plan.pdf

Meloidogyne chitwoodi (Columbia root-knot nematode) - Fact sheet

Background

The Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi*, was first described from the Pacific Northwest region of the United States in 1980. Records from Europe show that the nematode may have been present in the Netherlands as early as the 1930's. It is possible that this nematode has a wider distribution than is currently reported.

Distribution

- **Africa:** South Africa
- **Europe:** Belgium, The Netherlands
- **North America:** Mexico (Near Mexico City), USA (United States of America): California, Colorado, Idaho, New Mexico, Nevada, Oregon, Texas, Utah, Washington.
- **South America:** Argentina

Biology

The life cycle takes approximately 3 to 4 weeks under favourable conditions. It begins in the spring as soil temperatures rise and the second stage juveniles hatch from eggs either in the soil or attached to previously infested roots (or tubers). Larvae enter the roots (or tubers) of their host plants, where they become sedentary, feed and mature within the cortex. As they increase in size, they induce alteration in the cells resulting in the formation of galls from which the nematode derive their food.

The nematode undergoes 3 additional molts within the host. Worm-like males emerge from the root after the fourth molt. Females are pear-shaped, whitish when mature and remain within the root/tuber tissue, usually near the surface, where they deposit a gelatinous egg sac from the posterior end of their body. The egg sac may contain 200 to 1,000 eggs. Reproduction commonly occurs in the absence of the males through a process called mitotic parthenogenesis.

First-stage juveniles molt within the egg to become second-stage juveniles, which emerge from the eggs about 10 days later to repeat the process, infesting other parts of the roots or developing tubers. Generation time under favourable conditions in temperate regions where potatoes are grown is typically 3 to 4 weeks The nematodes overwinter as eggs, or sometimes juveniles, in infested roots, tubers, or soil.

Detection and identification

Symptoms on potato

Symptoms of CRKN (Columbia Root-knot nematode) infection in potato tubers are highly variable and may be symptomless. Pimple-like galls may or may not be produced on the tuber surface, depending on the cultivar. When galls are produced, they appear as small, raised lumps above the developing nematodes, giving the skin a rough appearance.

Galls may be grouped in a single area or scattered near the tuber eyes. Infestations are difficult to detect in freshly harvested tubers, but after a few months the egg sacs turn from translucent to brown and can be seen as brown spots in the cortex of cut tubers. Brown spots only become evident when the females begin egg production. Internally, brown spots are usually within 5 to 6 mm (millimetres) of the tuber surface. There are no symptoms on potato roots and above ground symptoms are generally lacking.

Particular care needs to be exercised when examining seed potato tubers for possible root-knot nematode infection. Seed potatoes are usually produced in areas where environmental conditions may be less than ideal for root-knot nematode development and thus symptom expression. Seed potato production areas tend to have shorter growing seasons resulting in 1 to 2 generations of CRKN (Columbia Root-knot nematode) per year, compared to 2 or more in commercial areas, consequently, symptoms and damage on seed tubers may be lacking.

Although external symptoms of *M. (Meloidogyne) chitwoodi* may be present, it is often difficult to detect any internal tuber damage because the females are immature and brown spots may not have developed. The best method to determine if a seed-lot is infested is to obtain soil samples from the field in question or to sample the tare soil.

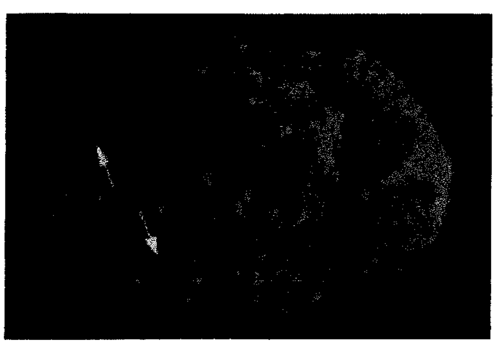

Figure 1

Figure 2

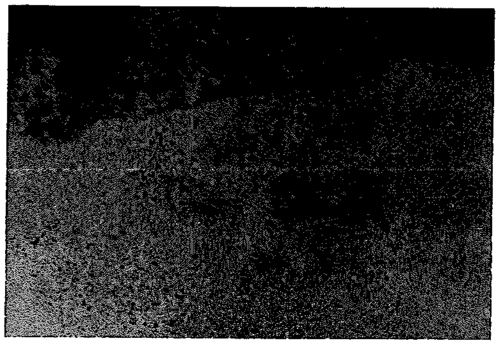

Figure 3

Figure 4

Figure 5

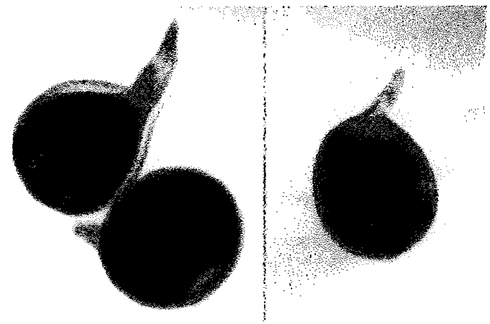

Figure 6

Photos: Agriculture and Agri-Food Canada, Research Branch, Ottawa

Related site

- University of Nebraska-Lincoln

Date modified:
2022-01-24

Disease Note.

Occurrence of *Meloidogyne chitwoodi* in Potato Fields in Colorado. J. N. Pinkerton, Department of Plant Pathology, and Weed Science, Colorado State University, Fort Collins 80523. G. A. McIntyre, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins 80523. Plant Dis. 71:192. Accepted for publication 20 November 1986. DOI: 10.1094/PD-71-0192D.

Meloidogyne chitwoodi (Golden et all, the predominant root-knot nematode species in potato (*Solanum tuberosum* L.) production areas of the northwestern United States, can severely reduce yield and tuber quality, making the crop unmarketable (2). In 1983, 2,150 ha were surveyed for phytonematodes in the San Luis Valley, Colorado. *M. chitwoodi* was detected in 16% of the fields sampled. This is the first report of *M. chitwoodi* in Colorado. Its distribution throughout the surveyed area in fields with different grower and cropping histories suggests that it is not a recent introduction. Densities of second-stage juveniles were low at harvest ï¿½ 20/500 cm3 of soil). Light tuber symptoms were observed in only one field. Cool soil temperatures in this 2,400-m elevation valley appear to limit *M. chitwoodi* to one generation per year (I). These data suggest that the impact of M. chitwoodi on fresh market potato production in the San Luis Valley may be negligible. This is supported by 1984 and 1985 shipping inspections involving over 48,000 individual shipments, only 0.0001% of which contained symptomatic tubers.

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SOIL MICROBIOLOGY

Bacterial Microbiome and Nematode Occurrence in Different Potato Agricultural Soils

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Abstract *Pratylenchus neglectus* and *Meloidogyne chitwoodi* are the main plant-parasitic nematodes in potato crops of the San Luis Valley, Colorado. Bacterial microbiome (16S rRNA copies per gram of soil) and nematode communities (nematodes per 200 g of soil) from five different potato farms were analyzed to determine negative and positive correlations between any bacterial genus and *P. neglectus* and *M. chitwoodi*. Farms showed differences in bacterial communities, percentage of bacterivorous and fungivorous nematodes, and numbers of *P. neglectus* and *M. chitwoodi*. The farm with the lowest population of *P. neglectus* and *M. chitwoodi* had higher abundances of the bacterial genera *Bacillus* spp., *Arthrobacter* spp., and *Lysobacter* spp., and the soil nematode community was composed of more than 30% of fungivorous nematodes. In contrast, the farm with higher numbers of *P. neglectus* and *M. chitwoodi* had a lower abundance of the abovementioned bacterial genera, higher abundance of *Burkholderia* spp., and less than 25% of fungivorous nematodes. The α-Proteobacteria *Rhodoplanes*, *Phenylobacterium*, and *Kaistobacter* positively correlated with *M. chitwoodi*, and the Bacteroidia and γ-Proteobacteria positively correlated with *P. neglectus*. Our results, based largely on co-occurrence analyses, suggest that the abundance of *Bacillus* spp., *Arthrobacter* spp., and *Lysobacter* spp. in Colorado potato soils is negatively correlated with *P. neglectus* and *M. chitwoodi* abundance. Further studies will isolate and identify bacterial strains of these genera, and evaluate their nematode-antagonistic activity.

Keywords *Pratylenchus neglectus* · *Meloidogyne chitwoodi* · Microbiome · *Bacillus* · *Arthrobacter* · *Lysobacter*

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Introduction

Currently, there is a growing interest in the study of soil and rhizosphere microbiome to understand the soil community composition and biodiversity, and the impact their interactions have on plants [1]. The soil ecosystem is a complex and diverse environment that contains millions of bacteria and fungi, nematodes, mites, earthworms, and arthropod species in a single gram of soil [2]. It has been demonstrated that the diversity of underground microorganisms significantly determines the aboveground biodiversity and ecosystem functioning [1, 2]. For the specific case of agricultural ecosystems, intensive agriculture is causing soil degradation, loss of biodiversity, reduction of soil-food trophic levels (predators), and decreasing functional groups with larger biomass (earthworms, enchytraeids, collembolans, mites) [3]. Hence, there is a need to understand soil microbial community, diversity and ecology, and beneficial or deleterious plant-microbe interactions in order to develop a more sustainable agriculture. The use of molecular biology techniques to study the soil and rhizosphere bacterial microbiome [4] and the use of soil free-living nematodes as soil health bioindicators can be used as tools to understand soil interactions within an agricultural system [5].

Genomic studies in recent years are allowing high-throughput analysis of cells, organisms, and populations, and are starting to reveal different types of mechanisms and

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interactions between nematodes and bacteria [4]. Bacteria can be a food source for nematodes, can be pathogens of nematodes, or can develop symbiotic interactions [6–10]. Genome sequencing has allowed the study of transcriptional profiles of bacteria and nematodes, as well as the identification and quantification of proteins in complex mixtures. As an example of these genomic studies, it has been demonstrated that root-knot nematodes (*Meloidogyne* spp.) acquired most of the parasitism genes that encode for enzyme production from bacteria by horizontal gene transfer (HGT) [11, 12]. Studies on the bacterial microbiome of the soybean cyst nematode (*Heterodera glycines*) reported 290 bacteria a
nematode. Thirty of these bacteria
as nematode antagonists (*Lysobac*
er isolates produce polymer-hyd
crease the rigidity of the cyst and
the nematode juveniles [13]. Furtl
strated that the bacterial microbio
wood nematode (*Bursaphelenchus xylophilus*) helps the nematode to degrade α-pinene (the main compound in the pine resin that inhibits reproduction and development of pinewood nematodes) and successfully parasitize the pine tree [14].

Free-living or beneficial nematodes composed 60–80% of the soil nematode community, and due to the key role they play in the soil food web and soil-ecological processes (nutrient cycling, mineralization, dispersal of microbes), they can be used as soil health bioindicators to understand biological mechanisms and interactions in soil [15–17]. Soil nematodes are categorized into a 1–5 colonizer-persister (*c-p*) scale, which range from *r*- to *K*-strategists. At the lower end of the *c-p* scale are located the "colonizer" nematodes that are considered enrichment opportunists and therefore indicate resource availability. In contrast, at the high end of the *c-p* scale are located the "persister" nematodes that indicate system stability, food web complexity, and connectance [5, 16, 18, 19]. Furthermore, Ferris et al. [16] developed indices for nematode diversity, genera richness, structure, and functional guilds and based on them classified the nematode community in a graph composed by four quadrants: quadrant A for soils enriched but unstructured, quadrant B for enriched and structured, quadrant C for resource limited and unstructured, and quadrant D for resource-depleted and unstructured [16, 18].

Within the nematode soil community, the two main plant-parasitic nematodes in San Luis Valley of Colorado potato crops are the Columbia root-knot nematode (*Meloidogyne chitwoodi*) and root-lesion nematode (*Pratylenchus neglectus*). *Meloidogyne chitwoodi* is a major pest in commercial potato production in the northwestern USA, and has additional alternative hosts such as tomato, barley, oat, wheat, and rye [20, 21]. *Pratylenchus neglectus* is the most common plant-parasitic nematode in Colorado crops, has a wide host range, and can be a problem in potato, grape, wheat, and corn crops of Colorado [21].

Therefore, understanding the interaction(s) of bacteria with *P. neglectus* and *M. chitwoodi* in the soil will allow a better understanding about their ecology, and will provide new insights into possible biocontrol agents. The objective of this work was to study the soil bacterial microbiome, and the soil nematode community present in potato crops of the San Luis Valley, Colorado, and evaluate if there were any specific correlations between bacterial genera and the two main plant-parasitic nematodes present in potato crops of Colorado: *M. chitwoodi* and *P. neglectus*.

levels of plant-parasitic nematodes (Agro Engineering Inc., *personal communication*). Soil conditions for farms sampled were Monte Vista (sandy soil; sand 88.8%, silt 6.2%, clay 5%; organic matter 0.9%; pH 7.4), Blanca (loamy sand soil; sand 82.5%, silt 10%, clay 7.5%; organic matter 0.4%; pH 8.0), Mosca I (loamy sand soil; sand 86%, silt 10%, clay 3.7%; organic matter 0.4%; pH 8.3), Mosca II (loamy sand soil; sand 81.3%, silt 8.7%, clay 10%; organic matter 0.5%; pH 8.6), and Sargent (loamy sand soil; sand 80%, silt 10%, clay 10%; organic matter 0.6%; pH 7.8).

Potato farms Monte Vista, Blanca, and Sargent were circular-shaped, while farms Mosca I and II were half-circular-shaped. Each circular crop was divided in quadrants, and in each quadrant two soil samples were taken for a total of four to eight soil samples per farm. Each soil sample consisted of a composite of rhizosphere soil from three randomly selected plants in the potato crop. Each plant was first removed using a spade, and then a single soil core (2″ dia. × 6″ deep) was collected and mixed by hand to create a pooled sample. All potato plants were approximately 60 days old at the time of sampling. Soil nematodes were extracted from each sample, taking 200 g of soil and using the modified gravity-sieving followed by the sucrose centrifugation-flotation method [22], and counted on an inverted microscope. Free-living and plant-parasitic nematodes were identified and counted, and the nematode community indices were estimated as described by Bongers and Ferris [5], Ferris and Bongers [23], and Neher and Darby [19].

Soil DNA Extraction and Amplification for Microbiome Analysis

DNA extraction from the soil (0.5 g) was conducted using a custom single-tube DNA extraction technique, which includes the initial lysis and cleanup steps of the PowerSoil DNA

Extraction Kit (MoBio) and MagNA Pure LC DNA Isolation Kit (Roche). Briefly, soils were weighted into garnet bead tubes (MoBio) and lysed in 750 μL of bead solution (MoBio, Cat. no. 12855-50-BS) and 60 μL C1 solution (MoBio, Cat. no. 1288-50-1) at 6000 rpm for 60 s using a MagNA Lyser (Roche). After lysis, the tube was cooled at 4C for 10 min, and then the supernatant (450 μL) was transferred to a new tube and combined with 250 μL of C2 solution (MoBio no. 1288-50-2). After a 10-min incubation at 4 °C, the sample was centrifuged at 10,000 rpm to 1 min. The supernatant was transferred to a new tube and mixed with 200 μL of C3 solution (MoBio, Cat. no. 1288-50-3) and incubated at 4 °C for 10 min. After a final centrifugation at 10,000 rpm for 60 s, 700 μL of the supernatant was transferred to a new tube and purified using a MagNA Pure LC DNA Isolation Kit (Roche) run on a MagNA Pure Compact robot (Roche). The final elution volume was 50 μL, and samples were stored at −20 °C until PCR analysis.

16S rRNA Quantitative PCR

Quantitative PCR (qPCR) amplification of the bacterial 16S ribosomal RNA (rRNA) genes (V1–V3 hypervariable region) was performed with the 27F and 388R primers [24, 25]. Each reaction contained 2 μL template DNA (diluted 1:20), 0.5 μM of each primer, and 1× Maxima SYBR Green Master Mix (Cat. no. K0242, Thermo Fisher Scientific). Amplification was performed as follows: (1) 95 °C for 8.5 min; (2) 95 °C × 15 s, 58 °C × 30 s, 72 °C × 60 s, repeated 35 times; and (3) 72 °C × 5 min. Genomic DNA isolated from *Pseudomonas putida* KT2440 was used as an external standard in order to calculate 16S rRNA copies per gram of soil FW extracted assuming a *P. putida* genome size of 3.174 fg and seven 16S rRNA copies per genome. qPCR efficiency was 93% and could detect as little as 100 *P. putida* genomes in a single PCR reaction.

Illumina 16S rRNA Library Preparation

The 515f-806r primers were used to amplify the v4 region of the bacterial 16S rRNA gene based on the methods outlined in Caporaso et al. [26]. PCR conditions were the following: initial denaturation for 2 min at 95 °C, 30 cycles of 20 s at 95 °C, annealing for 15 s at 55 °C, and extension of 50 s at 72 °C, and final extension of 15 min at 72 °C. The amplification product of the qPCR was confirmed as a single band of 400–500 bp in a 1% agarose gel. The DNA was cleaned by ethanol precipitation followed by a DNA quantification using Quant-iT™ dsDNA assay on a Qubit® fluorometer. All samples were pooled contributing exactly the same amount of DNA in the final library. Final DNA concentration in the library was 21.2 ng/μL. The final library was sent to the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign.

The raw Illumina sequence data was curated using the default sequencing pipeline contained within myPhyloDB v.1.2.0 [27] prior to statistical analyses. Briefly, sequence reads were (i) trimmed (bdiff = 0, pdiff = 0, qaverage = 25, minlength = 100, maxambig = 0, maxhomop = 10); (ii) aligned to the bacterial subset SILVA alignment available at the Mothur website (http://www.mothur.org); (iii) screened (optimize = minlength-end, criteria = 95) and filtered (vertical = T, trump = .) so that all sequences covered the same genetic space; (iv) pre-clustered (diff = 2) to remove potential sequencing noise and clustered (calc = onegap, coutends = F, method = average) into operational taxonomic units or OTUs [28]; (v) screened for chimeras with vsearch [29]; (vi) classified using the Greengenes reference database (gg_13_5_99) and the naïve Bayesian classifier [30] embedded in Mothur v.1.38 [31], after which all sequences identified as chloroplast or mitochondria (<1% of the total sequences) were removed; and finally (vii) assigned to unique phylotypes (i.e., taxonomic classifications). Data normalization was conducted with myPhyloDB using rarefaction (keep), sub-sample size = median (8633 reads), iterations = 100, and lambda = 0.1. Phylotype-specific abundances (16S rRNA copies per g soil) were calculated by multiplying each genus' relative abundance by the total community bacterial abundance (16S rRNA copies per g soil) obtained from the 27F-388R qPCR.

Statistical Analysis

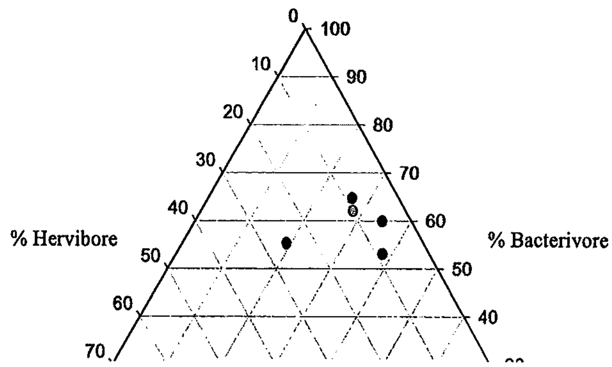
Statistical analysis of the nematode community was conducted using SAS 9.2 (SAS Institute Inc.) using the PROC GLIMMIX software. Nematode counts and nematode community indices were fixed effects in the model statement, and random effects include replications. Normal distribution was evaluated with the student panel graphs. Nematode counts were transformed to log-normal to satisfy the normality assumption, and later were back-transformed to the original scale and then presented in the graphs. Nematode variables were analyzed at an alpha 0.05.

Statistical analysis of the microbiome was conducted using the following R packages embedded within myPhyloDB v.1.2.0 [27]. Genus-specific total abundances tested for differences between farms were analyzed by one-way ANOVA, and all pairwise comparisons were corrected using Tukey's HSD. Genus-specific total abundance profiles were analyzed by principal component analysis (PCA) performed using the R package FactoMineR [32], and weighted correlation network analysis (WGCNA) was performed using the R package WGCNA [33].

Results

Nematode Community Assessment

There were statistical differences in the total number of nematodes in the farms ($P = 0.001$), number of bacterivores ($P = 0.001$), number of fungivores ($P = 0.001$), and number of plant-parasitic nematodes ($P = 0.001$). However, no statistical differences in nematode community variables were observed among the farms such as dominance ($P = 0.449$), diversity ($P = 0.528$), maturity index ($P = 0.714$), channel index



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pared to Mosca II, Sargent, and Monte Vista which have less than 25% of these nematodes ($P < 0.001$). The Monte Vista farm was the only farm where the percentage of herbivore nematodes (25%) was higher than the percentage of fungivorous nematodes (18%) (Fig. 2).

The composition of plant-parasitic nematodes showed that *P. neglectus* is present in all farms, while *M. chitwoodi* was absent in the Mosca I farm. There were differences in the number of *P. neglectus* ($P = 0.024$) and *M. chitwoodi* ($P = 0.001$) among farms. The Blanca farm has the lowest number of *P. neglectus* ($P = 0.031$) and *M. chitwoodi*

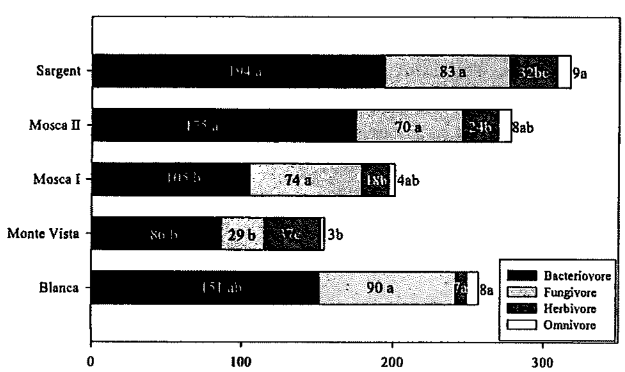

Fig. 1 Number of bacteriovore, fungivore, herbivore, and omnivore nematodes per 200 g of soil from SLV farms. Means followed by the same *letter* are not significantly different ($P \leq 0.05$)

Microbiome Community Assessment

In order to get an understanding of the possible relationship between the presence of plant-parasitic nematodes and soil microbial communities, we conducted a soil microbiome analysis associated with each farm, using qPCR and a sequencing approach. Data collected was used to perform a targeted and non-targeted approach to see which bacterial genera correlate negatively or positively to *P. neglectus* and *M. chitwoodi*.

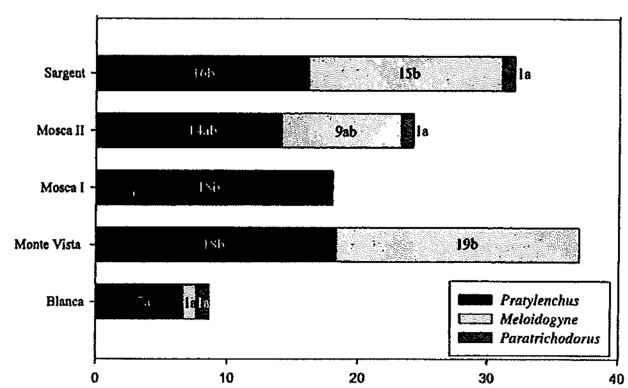

Fig. 3 Number of plant-parasitic nematodes per 200 g of soil in SLV farms. Means followed by the same *letter* are not significantly different ($P \leq 0.05$)

Summary of Comments on Bacterial_Microbiome_and_Nematode_Colorado potato farms_mchitwoodi2017.pdf

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Research Sampling Locations with positive finds occur in 4 different counties within this published report.
Sargents, CO= Saguache County
Mosca, CO= Alamosa County
Monte Vista, CO= Rio Grande County
Blanca, CO= Costillo County

Targeted Microbiome Analysis—ANOVA

Total abundance of bacteria (16S rRNA copies g^{-1} soil), assessed by qPCR, was highest at the Sargent farm compared to the other farms ($P = 0.024$) (Fig. 4). Based on a literature search, we identified 35 genera that have previously been identified as potential biocontrol agents of plant-parasitic nematodes [34–36]. Differences in the total abundance (genus-specific 16S rRNA copies g^{-1} soil) between each farm were then tested using a simple one-way ANOVA. A total of 25 of the 36 potential biocontrol bacteria genera were present at each farm, 9 of which differed significantly between farms (Table 1). The genera *Agrobacterium*, *Arthrobacter*, *Bacillus*, and *Pseudomonas* were highest ($P < 0.05$) at the Blanca farm compared to all the other farms. No differences were observed in the abundance of *Pasteuria* spp., which has been regarded as an effective biocontrol of plant-parasitic nematodes, among the SLV soils ($P = 0.768$).

Non-targeted Microbiome Analysis

Principal component analysis of the bacterial microbiome showed distinct groupings with Monte Vista differentiating from the rest of the farms along the 2nd principal component axis (Fig. 5). Nine of the top 10 genera contributing to this axis were positively correlated with the abundance of *Meloidogyne*, whereas only one was positively correlated with the abundance of *Pratylenchus* (Table 2).

Weighted gene correlation network analysis (WGCNA) identified 31 modules of co-occurring genera, and 198 genera were not assigned to a module (identified as gray) (Fig. 6). Nine of the modules (yellow (4), magenta (8), cyan (13), midnight blue (14), light cyan (15), dark turquoise (22), orange (24), steel blue (28), and violet (30)) were positively correlated ($P < 0.05$) with *Meloidogyne* abundance, and two modules (red (6) and violet (30)) were positively correlated ($P < 0.05$) with *Pratylenchus* (Table 3). These modules appear to be closely linked as they are all clustered on the right side of the network (Fig. 6). The largest module of this group was the yellow, which consisted of 25 genera, whereas the smallest was the steel blue, which consisted of 6 genera (Fig. 7).

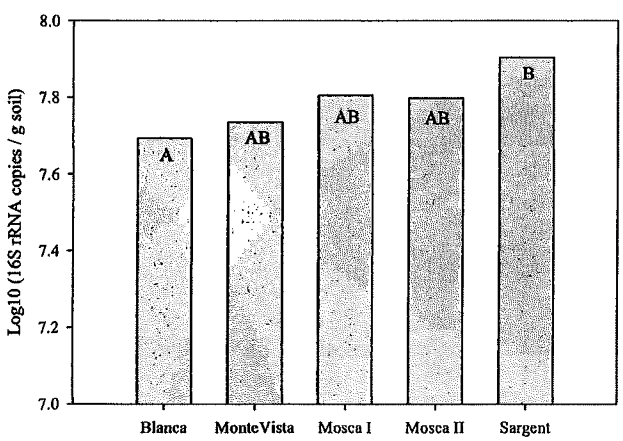

Fig. 4 Total abundance of bacterial 16S rRNA copies per gram of soil. Means followed by the same *letter* are not significantly different ($P \leq 0.05$)

Four of the modules (turquoise (1), light yellow (18), dark green (21), and dark gray (23)) were negatively correlated ($P < 0.05$) with *Meloidogyne* abundance, and one module (light yellow (18)) was negatively correlated ($P < 0.05$) with *Pratylenchus* (Table 4). The modules were also linked together and formed a cluster in the upper left of the network. The largest module of this group was the turquoise, which consisted of 97 genera, whereas the smallest was the dark grey, which consisted of 8 genera (Fig. 7). The module (dark green) with the highest negative correlation ($r = -0.663$) with *Meloidogyne* abundance contained the genus *Arthrobacter*, which was one of the a priori determined potential biocontrol agents of *Meloidogyne*.

Discussion

The nematode feeding group that predominates in the potato crops of SLV are the bacterivores (50–60%). This is common for agricultural soils where the continued chemical and physical disturbance reduces the flow through the fungal channel, favoring bacteria and bacterivorous nematodes with short-generation times, small body size, and rapid dispersal [38, 39]. Using nematodes as soil health bio-indicators to infer the condition of the soil food web of SLV farms [15, 16, 18, 19], farms Blanca and Mosca I fit in quadrant A of the nematode faunal profile proposed by Ferris et al. [16], in which food web was disturbed, there was a low C/N ratio, and the decomposition channel was mainly bacterial. In contrast, farms Mosca II, Sargent, and Monte Vista fit in quadrant D, where the food web was degraded, the C/N ratio was high, and the decomposition channel was fungal. A disturbed and degraded soil food web is common for conventional agricultural soils under continued disturbance [16]. Fungivorous nematodes affect food web status. In farms Blanca and Mosca I, where the food web was disturbed (based on nematode faunal profile), fungivorous nematodes composed 36% of the total nematode community compared to Mosca II, Sargent, and Monte Vista where the food web is degraded; these nematodes composed less than 25% of the total nematode community. Fungivorous nematodes spread fungi (including nematode biocontrol agents) on their bodies or on the gut within the soil, and grazing of nematodes on microbial populations stimulates microbial growth [39].

In all the farms, the main plant-parasitic nematode we found was *P. neglectus*, which agrees with Pokharel [21] who suggest that *Pratylenchus* is the most prevalent plant-parasitic nematode genus in Colorado. *M. chitwoodi* was only found in Sargent, Mosca II, and Monte Vista farms in equal

Table 1 ANOVA results comparing genus-specific total abundances (16S rRNA copies/g soil) of potential nematode biocontrol agents between farms in the San Luis Valley

Mode of action	Genus	Farm					*P* value	Ref.[1]
		Blanca	Monte Vista	Mosca I	Mosca II	Sargent		
Parasitic	*Pasteuria*	2.865	2.902	2.729	2.886	3.019	0.768	a
Opportunistic parasitic	*Brevibacillus*	3.112	3.185	2.914	3.198	3.343	0.312	a
	Bacillus	5.331a	4.893b	4.781bc	4.330c	4.880bc	<0.001	a,b
	Pseudomonas	4.888a	4.204b	4.948ab	4.537ab	4.447ab	0.046	a
Rhizobacteria	*Actinomyces*	–	–	–	–	–	–	a
	Agrobacterium	4.363a	3.787ab	3.740ab	3.462ab	3.568b	0.038	a
	Arthrobacter	6.292a	5.597c	6.131ab	5.892bc	5.791bc	<0.001	a,b
	Alcaligenes[d,e]	3.439	3.280	2.892	3.061	3.353	0.530	a
	Aureobacterium	–	–	–	–	–	–	a
	Azotobacter	–	–	–	–	–	–	a
	Beijerinckia[d,f]	3.722b	3.492b	4.379a	4.623a	4.513a	0.001	a
	Burkholderia	3.724b	4.733a	3.141b	3.441b	3.681b	<0.001	a
	Brevundimonas	3.249b	2.821b	4.325a	2.876b	3.160b	<0.001	b
	Chromobacterium	–	–	–	–	–	–	a
	Clavibacter	–	–	–	–	–	–	a
	Clostridium	3.703	3.814	3.629	3.718	3.876	0.600	a
	Comamonas[d,g]	5.653	5.752	5.640	5.788	5.917	0.093	a
	Corynebacterium	2.952	2.824	2.622	2.894	3.200	0.509	a
	Curtobacterium	–	–	–	–	–	–	a
	Desulfovibrio	–	–	–	–	–	–	a
	Enterobacter[d,h]	3.530	3.413	2.834	3.248	3.541	0.785	a
	Flavobacterium	4.256	4.682	4.357	4.111	4.509	0.376	a
	Gluconobacter	–	–	–	–	–	–	a
	Hydrogenophaga	2.700	2.854	2.905	3.325	3.081	0.228	a
	Klebsiella	–	–	–	–	–	–	a
	Lysinibacillus	–	–	–	–	–	–	b
	Lysobacter	4.865a	4.053b	5.182a	5.116a	4.892ab	0.002	c
	Methylobacterium	3.236a	3.821a	3.117a	3.164a	3.539a	0.036	a
	Mycoplana	4.958	4.699	4.755	4.511	5.014	0.221	b
	Phyllobacterium	3.369	3.362	3.751	4.173	3.550	0.288	a
	Rhizobium	4.391a	4.173ab	3.244c	4.471a	3.926bc	0.005	a
	Serratia	–	–	–	–	–	–	a,b
	Sphingobacterium	3.536	3.387	3.613	3.376	3.602	0.751	a
	Stenotrophomonas	4.331	3.985	3.907	4.030	4.172	0.706	a,c
	Streptomyces	5.320	5.233	5.146	5.043	5.542	0.149	b
	Variovorax	3.767	3.190	3.722	3.360	3.085	0.254	a

Means with different lowercase letters are significantly different (Tukey HSD, $P < 0.05$). All data were log10 transformed

[a] Reference: [37]

[b] Reference: [35]

[c] Reference: [34]

[d] A unique phylotype at the genus level was not identified. The phylotype used to perform the ANOVA analysis includes all parent taxonomic level (i.e., family) sequences that could not be assigned to a genus

[e] Parent taxonomic level: Alcaligenaceae

[f] Parent taxonomic level: Beijerinckiaceae

[g] Parent taxonomic level: Comamonadaceae

[h] Parent taxonomic level: Enterobacteriaceae

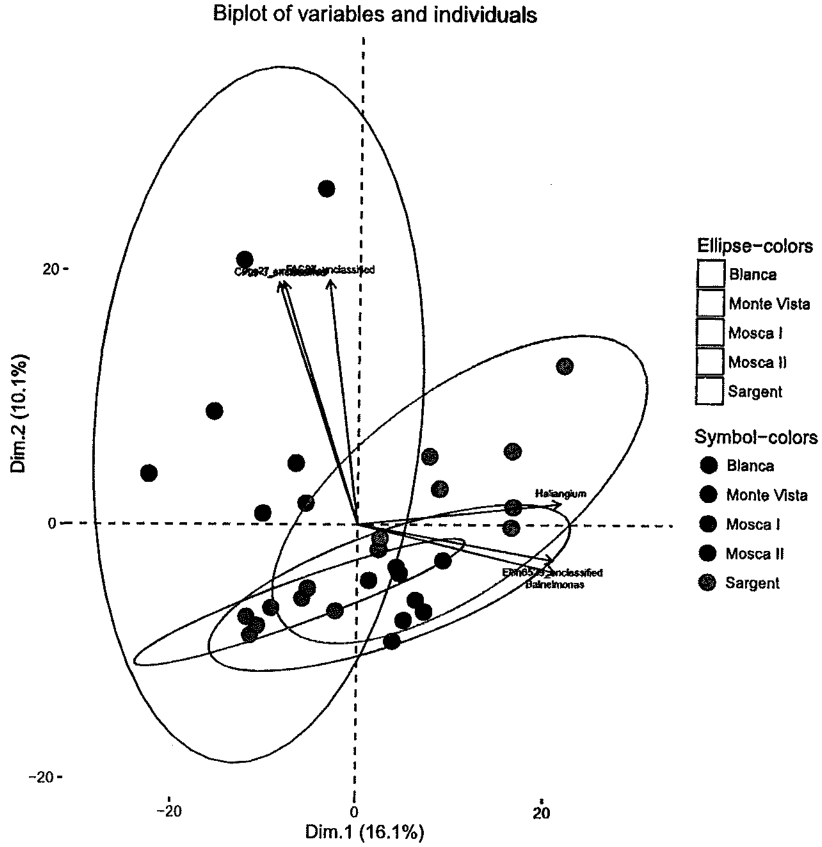

Fig. 5 Principal component analysis (PCA) analysis of the farms in SLV. Additional biplot details can be found in Table 2

proportions than *P. neglectus*. This nematode has been reported as a pest in potato crops of Colorado [20, 40].

Blanca farm has the lowest plant-parasitic nematode population, and the highest presence of the bacteria *Bacillus* spp. and *Arthrobacter* spp. of all the farms. The presence of these bacteria may explain the lower populations of *M. chitwoodi* and *P. neglectus*, because both genera have been reported as nematode antagonists. *Bacillus megaterium*, *B. thuringiensis*,

Table 2 Regression analysis relating the abundance of *Meloidogyne* or *Pratylenchus* abundance and the genus-specific abundance of the top 10 contributors to the 2nd PCA axis (see Fig. 5)

Phylum	Class	Order	Family	Genus	PCA[a] Contrib.	ANcOVA			
						Pratylenchus		*Meloidogyne*	
						Slope	*P* value	Slope	*P* value
Proteobacteria	α-Proteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	0.999	–	0.287	11,155	0.009
Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	Unclassified[b]	0.985	–	0.921	3915	<0.001
Proteobacteria	α-Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Unclassified[b]	0.969	–	0.598	6085	0.008
Acidobacteria	Solibacteres	Solibacterales	Unclassified[b]	–	0.963	–	0.881	7928	0.002
Proteobacteria	α-Proteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	0.958	–	0.301	4698	0.028
Actinobacteria	Actinbacteria	Actinomycetales	Frankiaceae	Unclassified[b]	0.940	–	0.226	1618	0.055
Proteobacteria	α-Proteobacteria	Ellin329	Unclassified[b]	–	0.901	–	0.366	7809	0.003
Proteobacteria	β-Proteobacteria	SC-I-84	Unclassified[b]	–	0.883	–	0.407	1353	0.009
Proteobacteria	α-Proteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	0.878	–	0.749	-	0.130
Verrucomicrobia	Pedosphaerae	Pedospaerales	Ellin515	Unclassified[b]	0.856	531	0.042	930	0.002

[a] Variable contribution to axis ($PC_i^2/\Sigma PC_i^2 * 100$, where PC_i is the axis score for each phylotype)

[b] Phylotype includes all parent taxonomic level sequences that could not be assigned to a unique taxon for the designated taxonomic level (i.e., unclassified)

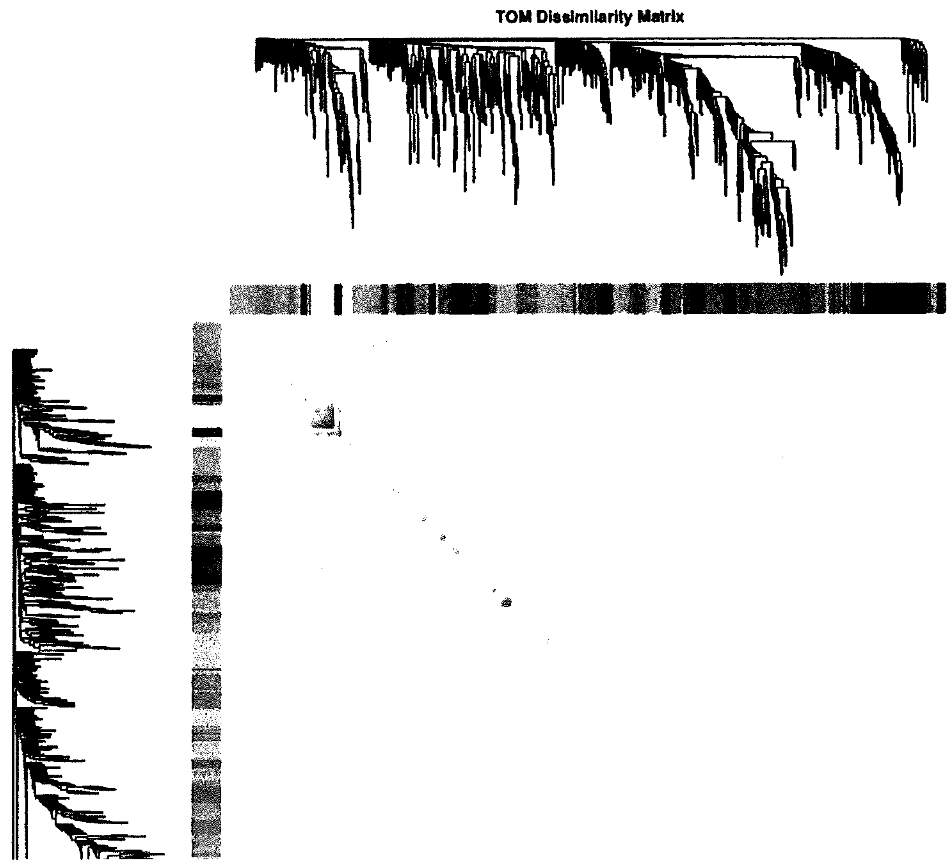

Fig. 6 Weighted correlation network analysis (WGCNA) of soil bacteria communities from five farms ($n = 3$–8) in the San Luis Valley. Bacterial phylotypes (genera) were clustered by total abundance (16S rRNA copies/g soil) as shown by the dendrogram and correlation heat map. Clusters of co-occurring phylotypes or modules are indicated by the color bars. The intensity of red coloring (heat map) indicates the strength of the correlation between pairs of phylotypes

B. idriensis, and *B. altitudinis* have been reported as efficient biocontrol agents for *M. incognita* by producing nematode-toxic volatiles in the soil [35, 41]. A commercial strain of *B. firmus* produces a biosurfactant compound toxic to *M. incognita*, *Radopholus similis*, *Ditylenchus dipsaci*, and *Rotylenchulus reniformis*, and reduced *M. incognita* and *R. reniformis* in tomato and cotton field crops, respectively [36, 42–44]. *Arthrobacter globiformis*, *A. humicola*, *A. mysorens*, *A. scleromae*, *A. tumbae*, and *A. nicotianae* produce VOCs that are toxic to *M. incognita* [45, 46]. The S-methyl thiobutyrate was the VOC from *A. nicotianae* that showed high nematicidal activity against *Caenorhabditis elegans* and *M. incognita*, even stronger nematicidal activity than the commercial standard dimethyl disulfide [46]. Results obtained by Gu et al. [47] show that VOCs from bacteria usually have more than one kind of nematicidal compounds. The mixture of nematicidal compounds produced by soil bacteria is more effective to control nematodes than a synthetic nematicide composed by one single compound [47]. The VOC identification with nematicidal activities can provide a basic chemical structure for novel nematicidal compounds. Therefore, the mix of VOCs and metabolites produced by species of the genus *Bacillus* and *Arthrobacter* present in the Blanca farm may have an antagonistic effect against *M. chitwoodi* and *P. neglectus*.

In contrast, farm Monte Vista has the highest plant-parasitic nematode populations and the highest population of *Burkholderia* spp. of all the farms. This positive correlation has been previously reported between both *Pratylenchus* sp. and *Helicotylenchus* sp. and *B. tropica*, and between *Helicotylenchus* sp. and *Burkholderia cepacia* in sugar cane crops [48]. Typically, most of the species of *Burkholderia* spp. have an intimate association with plant roots, and have been reported as biocontrol agents of soil-borne pathogens, nitrogen fixers, and nematode antagonists [48–50]. However, the species within this genus of bacteria present in Monte Vista farm are positively correlated with *M. chitwoodi* and *P. neglectus*.

Table 3 WGCNA modules positively correlated with *Meloidogyne* or *Pratylenchus* abundance and the three top phylotypes associated with each module

Module	*Pratylenchus*		*Meloidogyne*		Taxonomy					
	r	*P*	*r*	*P*	Phyla	Class	Order	Family	Genus	
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
					Proteobacteria	α-Proteobacteria	Rhodospirillales	Rhodospirillaceae	*Azospirillum*	0.897
					FCPU426	Unclassified[a]	–	–	–	0.856
Midnight blue (14)	0.350	0.057	0.362	0.049	Armatimonadetes	Chithonomonadetes	Unclassified[a]	–	–	0.890
					Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Winogradskyella*	0.875
					Proteobacteria	γ-Proteobacteria	34P16	Unclassified[a]	–	0.846
Light cyan (15)	–	0.262	0.558	0.001	Acidobacteria	Solibacteres	Solibacterales	Unclassified[a]	–	0.903
					Proteobacteria	α-Proteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	0.865
					Proteobacteria	α-Proteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	0.863
Dark turquoise (22)	–	0.909	0.374	0.041	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	*Garciella*	0.912
					Chlamydiae	Chlamydia	Chlamydiales	Rhabdochlamydiaceae	*Rhabdochlamydia*	0.860
					Acidobacteria	iii1–8	Unclassified[a]	–	–	0.822
Orange (24)	–	0.824	0.501	0.004	Chloroflexi	TK10	B07_WMSP1	FFCH4570	Unclassified[a]	0.900
					Proteobacteria	β-Proteobacteria	SC-I-84	Unclassified[a]	–	0.850
					Chloroflexi	TK10	B07_WMSP1	Unclassified[a]	–	0.846
Steel blue (28)	–	0.976	0.499	0.004	Planctomycetes	C6	Unclassified[a]	–	–	0.939
					Dlusimicrobia	Unclassified[a]	–	–	–	0.904
					Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae	*Dyella*	0.817
Violet (30)	0.391	0.032	0.455	0.011	WS2	Kazan-3B-09	Unclassified[a]	–	–	0.925
					Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unclassified[a]	0.846
					Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae	*Luteibacter*	0.843

[a] Phylotype includes all parent taxonomic level sequences that could not be assigned to a unique taxon within the designated taxonomic level (i.e., unclassified)

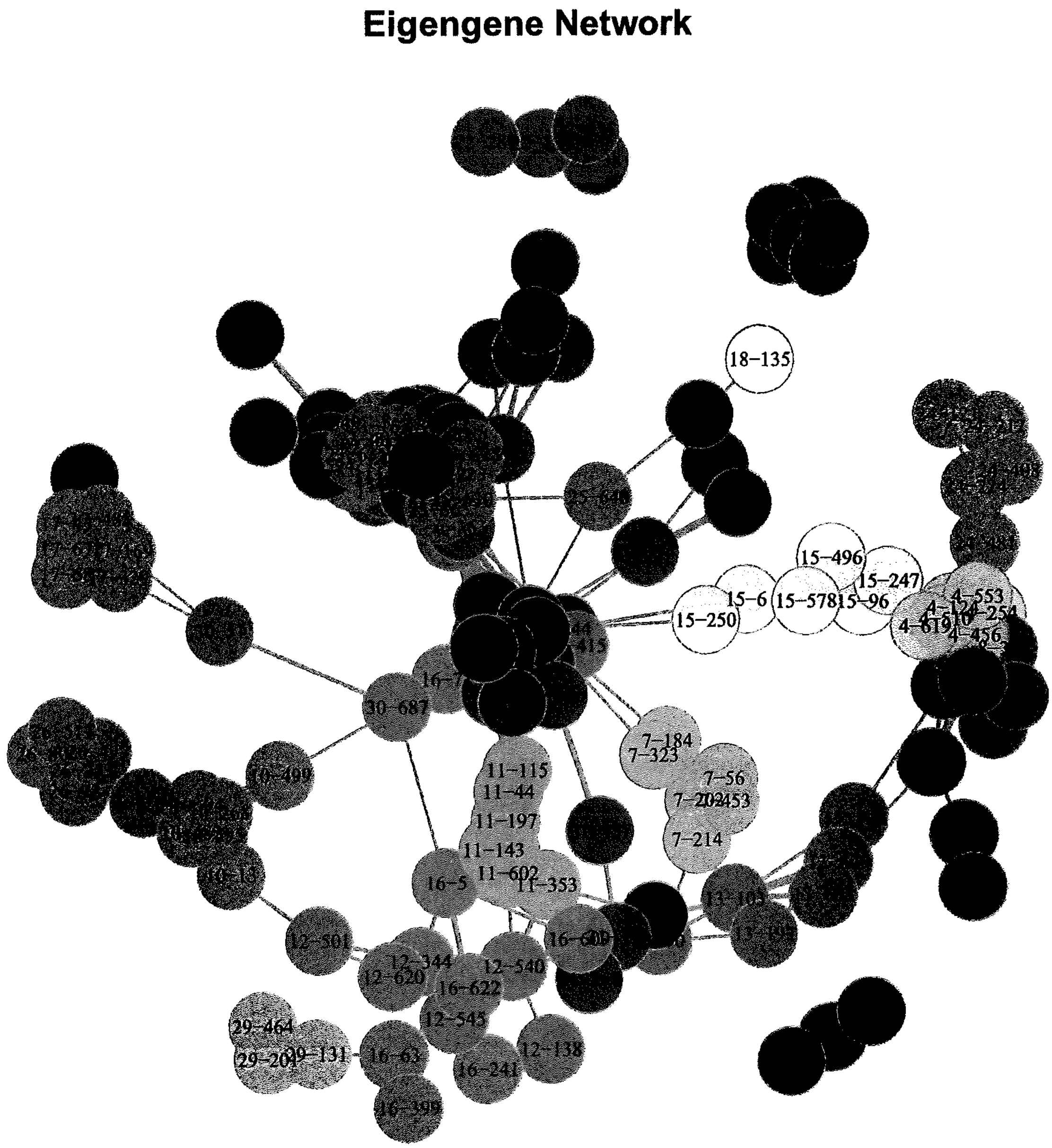

Fig. 7 Neural network depicting the weighted correlation network analysis (WGCNA) of soil bacteria communities from five farms (n = 3–8) in the San Luis Valley. Only the top six phylotypes for each module (*color*) are shown. The first number of each node's label refers to the assigned module, and the second number is a unique identifier for each phylotype (see Table S1). Connection strength is represented by edge width (edges <0.05 omitted)

Furthermore, α-Proteobacteria *Rhodoplanes*, *Phenylobacterium*, and *Kaistobacter* are all positively correlated with *M. chitwoodi*; however, there are no previous reports of such correlations in the literature. *Rhodoplanes* are photosynthetic bacteria with denitrification properties. The ecological significance of this genus remains unclear [51]. *Phenylobacterium* is an inhabitant of the upper aerobic part of the soil, and is a chloridazon-degrading bacterium. Chloridazon is the active ingredient of an herbicide used for broadleaf weeds [52]. Our results suggest that these genera are favoring *M. chitwoodi* directly or indirectly. Symbiotic associations are common between plant-parasitic nematodes and bacteria. The bacterial endosymbiont '*Candidatus* Paenicardinium endonii' has been found in *H. glycines* in reproductive organs of the females and hypodermal chords of males [9]. *Wolbachia*-like bacteria have been reported in the reproductive tract of female adults of *R. similis* [8]. These two endosymbionts could be playing an important role in reproduction and nematode development similar to the α-Proteobacteria *Wolbachia* spp. due to filarial nematodes, where the bacteria manipulate the reproduction of the nematode (i.e., killing or feminization of genetic males, induction of

Table 4 WGCNA modules negatively correlated with *Meloidogyne* or *Pratylenchus* abundance and the three top phylotypes associated with each module

Module	*Pratylenchus*		*Meloidogyne*		Taxonomy					
	r	*P*	*r*	*P*	Phyla	Class	Order	Family	Genus	kME
Turquoise (1)	–	0.141	−0.472	0.007	Acidobacteria	Acidobacteria-6	CCU21	Unclassified[a]	–	0.964
					Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Unclassified[a]	0.953
					Acidobacteria	iii1–8	DS-18	Unclassified[a]	-	0.935
Light yellow (18)	−0.386	0.035	−0.391	0.032	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	*Parapedobacter*	0.838
					Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	0.82
					Proteobacteria	δ-Proteobacteria	Myxococcales	Myxococcaceae	*Corallococcus*	0.807
Dark green (21)	–	0.234	−0.663	<0.001	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Arthrobacter*	0.959
					Firmicutes	Bacilli	Bacillales	Planococcaceae	*Planomicrobium*	0.838
					Proteobacteria	δ-Proteobacteria	Myxococcales	Cystobacteraceae	*Cystobacter*	0.825
Dark grey (23)	–	0.626	−0.575	<0.001	Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteraceae	*Rubrobacter*	0.938
					Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	*Chloronema*	0.933
					Chloroflexi	Anaerolineae	A31	S47	Unclassified[a]	0.908

[a] Phylotype includes all parent taxonomic level sequences that could not be assigned to a unique taxon within the designated taxonomic level (i.e., unclassified)

parthenogenetic reproduction, and induction of reproductive incompatibility) [53]. Furthermore, bacteria associated with the pinewood nematode (*B. xylophilus*) allow the nematode to successfully parasitize the pine tree by degrading α-pinene, which is the main compound in the pine resin that inhibits reproduction and development of pinewood nematodes [14].

Bacteria from the class γ-Proteobacteria (*Luteibacter* spp.) and Bacteroidia (unclassified genus) correlated positively with *M. chitwoodi* and *P. neglectus*, thus suggesting possible symbiosis/mutualism between these bacteria and the two main plant-parasitic nematodes in potato crops of Colorado. Bacteria from these classes have been documented as nematode endosymbionts. The γ-Proteobacteria *Xenorhabdus* and *Photorhabdus* have symbiotic associations with entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis*, respectively [54]. The infective juveniles of EPNs carry the bacteria and search for their insect hosts. Once the nematode penetrates its host, it releases the bacteria into the insect hemolymph. The bacteria will kill the insect, and degrade its contents to make nutrients available to the nematode. Finally, bacteria re-associate with new generations of nematode-infective juveniles [54]. Furthermore, *H. glycines* endosymbiont 'Candidatus *Paenicardinium endonii*' is related to the class Bacteroidia [9].

Lysobacter is present in higher proportions in the Blanca farm (lowest plant-parasitic nematodes), and in lower proportions in the Monte Vista farm (highest plant-parasitic nematodes). This suggests a possible antagonistic effect of strains of *Lysobacter* present in the Blanca farm and absent in Monte Vista. *Lysobacter* spp. are soil inhabitants that produce a wide variety of lytic enzymes and antimicrobial compounds with biocontrol potential against nematodes [55]. *Lysobacter antibioticus* inhibits hatch and survival of second-stage juveniles of *M. incognita* under greenhouse conditions [45], and *L. enzymogenes* has toxins and enzymes that are active against, *Meloidogyne javanica*, *Pratylenchus penetrans*, and *Heterodera schachtii* [56].

The genus *Beijerinckia* is present in higher populations in farms Mosca I, Mosca II, and Sargent, and in lower proportions in Blanca and Monte Vista, which suggests that it does not have any beneficial or deleterious effect against plant-parasitic nematodes. *Beijerinckia* are characterized as free-living, aerobic, chemoheterotrophic bacteria with the ability to fix nitrogen [57]. There is a report on *Beijerinckia* as a nematode antagonist; however, its mode of action against nematodes is not clear [37]. The strains present in *Beijerinckia* do not have any effect on plant-parasitic nematodes in Colorado.

In summary, the lower number of plant-parasitic nematodes in the Blanca farm may be partially explained by the higher abundance of *Bacillus* spp., *Arthrobacter* spp., and *Lysobacter* spp. These three genera have been reported as antagonists to plant-parasitic nematodes by the production of VOCs, metabolites, toxins, and enzymes. Furthermore, the presence of fungivorous nematodes (36% of the total nematode community) may be contributing indirectly to the lower populations of *M. chitwoodi* and *P. neglectus* by spreading antagonistic fungi within the soil profile; however, we did not quantify the fungal communities of these soils. Due to the correlative nature of the work reported in this manuscript, additional studies will be required to determine if the identified genera (e.g., *Bacillus*, *Arthrobacter*, and *Lysobacter*) truly act as plant-parasitic nematode antagonists in potato soils. Therefore, we are conducting further studies with isolates of these genera from the Blanca farm soil to evaluate their effectiveness as biocontrol potential to *M. chitwoodi* and *P. neglectus*.

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Review

Top Ten Most Important U.S.-Regulated and Emerging Plant-Parasitic Nematodes

Mihail Kantor, Zafar Handoo, Camelia Kantor and Lynn Carta

Special Issue
Recent Advance in the Identification and Diagnostics of Plant-Parasitic Nematodes

Edited by
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Review

Top Ten Most Important U.S.-Regulated and Emerging Plant-Parasitic Nematodes

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Abstract: Plant-parasitic nematodes (PPNs) are important pests that cause an estimated ten billion dollars of crop loss each year in the United States and over 100 billion dollars globally. The Animal and Plant Health Inspection Service (APHIS) within the U.S. Department of Agriculture maintains and updates the U.S. Regulated Plant Pest list. Currently, the number of PPNs regulated by APHIS includes more than 60 different species. This review focuses on the top ten most economically important regulated and emerging plant-parasitic nematodes and summarizes the diagnostics of morphological and some molecular features for distinguishing them. These ten major previously described nematode species are associated with various economically important crops from around the world. This review also includes their current distribution in the U.S. and a brief historical background and updated systematic position of these species. The species included in this review include three PPNs considered by the U.S. Department of Agriculture as invasive invertebrates *Globodera pallida, Globodera rostochiensis,* and *Heterodera glycines;* four regulated PPNs, namely *Bursaphelenchus xylophilus, Meloidogyne fallax, Ditylenchus dipsaci,* and *Pratylenchus fallax;* and the three emerging PPNs *Meloidogyne chitwoodi, Meloidogyne enterolobii,* and *Litylenchus crenatae mccannii.*

Keywords: APHIS; *Bursaphelenchus xylophilus; Ditylenchus dipsaci; Globodera pallida; Globodera rostochiensis; Litylenchus crenatae mccannii; Meloidogyne chitwoodi; Meloidogyne enterolobii; Meloidogyne fallax; Pratylenchus fallax;* regulated and emerging plant-parasitic nematodes

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1. Introduction

In the United States, the Department of Agriculture regulates and oversees pests and diseases, especially through the Animal and Plant Health Inspection Service (APHIS) agency. APHIS protects the United States' agricultural interests as well as monitors existing agricultural pests and diseases. APHIS maintains and regularly updates the U.S. Regulated Plant Pest [1]. The table with Regulated Plant Pests provided by APHIS includes a list of more than 60 PPN species [1].

The National Invasive Species Information Center (NISIC) was established in 2005 at the USDA's National Agricultural Library (NAL) to meet the information needs of users [2]. NISIC included in their list of terrestrial invertebrates three PPN species, namely *Globodera pallida, Globodera rostochiensis* and *Heretodera glycines* [2]. *G. pallida* and *G. rostochiensis* are perhaps the most important species of PPNs and are subject to strict regulatory restrictions in the United States and other countries [3]

Globally, PPNs are considered important pests and are responsible for important crop losses, with an estimated annual loss of USD 173 billion [4]. Potato cyst nematodes (PCN) have a global distribution and economic impact on potato crops [5,6]. Together, *Globodera pallida* and *G. rostochiensis* are responsible for potato crop losses of approximately 9% worldwide [7,8].

Heterodera glycines or soybean cyst nematode (SCN) is another plant-parasitic nematode listed under the NISIC list. The SCN continues to be the most devastating pest of soybean throughout U.S. and Canadian soybean-producing areas [9]. A recent census conducted in 2020 by Tylka and Marett [9] revealed a steady expansion of the distribution of SCN throughout the U.S. and Canada. The SCN was first reported in the U.S. in 1954 from North Carolina, almost 40 years after it was first identified in Japan in 1915 [10].

Bursaphelenchus xylophilus, or the pine wood nematode, is included in the APHIS U.S. Regulated Plant Pest Table [1] and is the causal agent of pine wilt disease. The pine wood nematode is native to North America and is one of the most damaging pest problems to forests around the world [11]. Many countries outside of the U.S. have labeled *Bursaphelenchus xylophilus* as a quarantine nematode because of its potential for destruction of their native conifers [11].

Meloidgyne fallax, or false Columbia root-knot nematode, is another nematode included in the APHIS U.S. Regulated Plant Pest Table [1]. It has a wide host range, which includes several major horticultural and agricultural crops. In potato, this nematode could lead to total yield losses due to quality defects caused to the tubers [12,13]. In the U.S., the false Columbia root-knot nematode was first reported in 2013 from a golf course in San Francisco, California [14]. After conducting a nematode survey in golf courses from several counties in California, APHIS did not find this nematode, listing this species as not present in the U.S. [15].

Meloidogyne [redacted] is another root-knot nematode species considered as an important pathogen of economically important crops, especially potatoes. It is not listed in the APHIS U.S. Regulated Plant Pest Table, but it is on the lists of prohibited organisms in many other countries such as Canada, Mexico, the EU and in several countries from South America and Asia [13]. In the Pacific Northwest of the U.S, this species is considered as one of the major pests of potatoes [16].

Meloidogyne enterolobii, or the guava root-knot nematode, is a species with many host plants, including cultivated crops and weeds, that can cause significant damage [13,17]. In the U.S., this species was first reported from Florida in 2004 [18]. Currently, this species is not under any in force quarantine requirements. This root-knot nematode species is considered by many nematologists as an emerging pest and should be regulated to prevent its spread.

Litylenchus crenatae mccannii is a newly recognized nematode subspecies that causes beech leaf disease (BLD) in *Fagus grandifolia* [19]. This species is considered an important foliar pathogen that parasitizes beech trees in U.S. [20]. Since the first report of BLD in 2012 from Ohio, the disease has spread across the Northeastern U.S. and has been reported from nine different states [21].

Pratylenchus fallax is a nematode species that is also included in the APHIS U.S. Regulated Plant Pest Table [1]. In the U.S., it was first reported in 1980 [22]. More recently, this nematode was reported on soybean from Wisconsin, thus providing its first molecular characterization in the U.S. [23]. It was also reported in 1997 on turfgrass in Canada (Ontario) [24].

Ditylenchus dipsaci, or the stem and bulb nematode, has a worldwide distribution and is considered a quarantine pest by many countries around the world. This species is also listed in the APHIS U.S. Regulated Plant Pest Table [1], and it has a wide host range, which includes more than 1200 species of cultivated and wild plants [25]. It has a wide U.S. distribution and has been reported from more than 20 different states (CABI-ISC, 2021) [26].

1.1. Bursaphelenchus xylophilus (Steiner & Buhrer, 1934) Nickle, 1970

- *Common name*: pine-wood nematode
- *Type plant host*: *Pinus palustris* Mill. or longleaf Louisiana pine
- *Type locality*: Bogalusa, LA, USA

Measurements: (see Table 1).

Molecular characterization: PCR-based identification methods of the soybean cyst nematode have been developed based on ribosomal DNA of ITS,28 S and *COI* regions [65–68]. Additionally, PCR species-specific primers for *H. glycines* were developed by several researchers [66,68].

Pathogenicity: The soybean cyst nematode has a significant negative impact on soybean-producing areas of the U.S. and Canada [9,69,70]. In the U.S. alone, this pathogen caused estimated yield losses totaling nearly USD 32 billion from 1996 through 2016, which is more than USD 1.5 billion annually [9,71]. From 2003 to 2005 alone, this nematode caused 2–3 million tons of yield loss annually [66]. In Japan, yield losses have been estimated to be between 10 and 79% [66]. The widespread distribution and the resilience of this pest could lead to great yield losses [9,72]

Diagnosis (modified after Thorne [30], Hirschmann [63], Subbotin [66], and Handoo and Subbotin [67]).

This species can be differentiated from *H. trifolii* based on the length of the juveniles, which are smaller when compared to *H. trifolii* (439.6 vs. 496.6 µm). The dorso-esophageal opening is 4.0 µm behind the stylet vs. 7.3 µm in *H. trifolii.* The shape of the knobs are somewhat rounded instead of forward-pointing as in *H. trifolii.* From *H. medicaginis*, it differs by having a shorter J2 stylet (21–23 vs. 25 µm) and a shorter tail (39–51 vs. 52 µm). It differs from *H. schachtii* by having a shorter average stylet length in J2 (21–23 vs. 25–26 µm), in the shape of the J2 knobs (slightly convex vs. moderately or strong concave), and by having a longer average fenestra length (34–58 vs. 35–38 µm). From *H. daeverti*, it differs by having a shorter average stylet in J2 (21–23 vs. 25–26 µm) and shorter tail (39–51 vs. 54–57 µm) and hyaline tail terminus (22–30 vs. 30–33 µm).

1.6. *Meloidogyne chitwoodi Golden, O'Bannon, Santo & Finley, 1980*

- *Common name*: Columbia root-knot nematode
- *Type plant host*: Solanum tuberosum L. potato
- *Type locality*: Quincy, Washington, USA

Measurements: (modified after Golden et al. [73]).

Female: n = 60; the average females length is 591 ± 60 (430–740) µm; a = 1.4 ± 0.2 (1–1.8); body width 422 ± 42 (344–518) µm, stylet length 11.9 ± 0.3 (11.2–12.5) µm; stylet knobs width 3.8 ± 0.3 (3.4–4.3) µm; DGO = 4.2 ± 0.6 (3.4–5.5) µm; anterior end to median bulb valve distance 63 ± 7 (52–80) µm; anterior end to excretory pore distance 18 ± 5 (10–27) µm; vulva slit 27 ± 3 (19–32) µm; vulva to anus distance 18 ± 2 (13–22) µm.

Male: n = 30; length 1068 ± 100 (887–1268) µm; a = 36 ± 4 (28–46); b = 7.2 ± 1 (6–9); c = 162± 20 (140–226); body width 30 ± 3.9 (22–37) µm; diameter of labial region 18.3 ± 0.2 (18.1–18.5); DGO = 3.0 ± 0.4 (2.2–3.4) µm; anterior end to median bulb valve distance 71 ± 5 (61–77) µm; spicule length 27 ± 1.2 (26–29) µm; gubernaculam length 7.7 ± 0.6 (6.5–8.2) µm; tail length 6.8 ± 0.9 (4.7–9.0) µm.

Juvenile: n = 60; length 390 ± 16 (336–417) µm; a = 27.5 ± 1.2 (24.5–29.8); b = 3.6 ± 0.2 (3.3–3.8); c = 8.9 ± 0.4 (7.9–9.6); body width 14.2 ± 0.6 (12.5–15.5) µm; height of labial region 2.3 ± 0.2 (1.7–2.6) µm; diameter of labial region 5 ± 0.2 (4.7–5.2) µm; stylet length 9.9 ± 0.3 (9.0–10.3) µm; DGO = 3.2 ± 0.2 (2.6–3.9) µm; anterior end to median bulb valve distance 51 ± 3 (43–56) µm; tail length 43 ± 1.8 (39–47) µm; hyaline tail region 11 ± 1 (8.6–13.8) µm.

Description (modified after Subbotin [13], Golden et al. [73], and Eisenback and Triantaphyllou [74]).

Female: The females are mostly found in the galls, and the egg masses protrude from the root tissue. Body pearly white, pear-shaped, with slight posterior protuberance visible occasionally, and with distinct neck situated anteriorly on a median plane with terminal vulva. Vulva appears sometimes to be located on a slight posterior protuberance. Several small vesicles or vesicle-like structures usually present within median bulb and clustered around lumen anterior to valve plates of median bulb. Labial region with distinct but weak labial framework, offset from neck but variable in exact shape, bearing a labial cap and usually one labial annulus. Excretory pore clearly visible and commonly located at a distance equal to about 1.5 stylet lengths from anterior end. Stylet length between 11 and 12.5 (12) µm but strong, having a dorsal curvature and rounded knobs sloping posteriorly. The distance of the DEGO to the base of the stylet between 3.5 and 5.5 (4.2) µm. The female stylet of this species is morphologically unique and very useful for species identification. The morphology of the stylet knobs is characteristic for the species; they are small, irregular in outline, indented medially, and taper onto the shaft. Perineal pattern is round to oval, with distinctive striae around and above anal area being broken, curved, twisted, or curled. Dorsal arch variable, ranging from low and round to high and sometimes squarish, like *M. incognita*. The striae in the dorsal area are more twisted when compared with those from the ventral area. Striae further away from the perineal area are smoother. Sometimes punctations visible in a small area between the anus and first inner striae (tail terminus) or the striae are absent. Vulva sunken in an area variable in shape and devoid of striae. Lateral striae may bend toward the vulva.

Male: Slender, vermiform, tapering slightly at both extremities. The shape of the male head is characteristic for the species. Labial disc large with median lips are fused into a labial cap. However, the labial disc slightly higher than median lips and post-labial annulus lacking annulations, and the posterior edges are irregular in outline. In labial region, two large, not well-delimited lateral lips that may have irregular outlines are marked by additional short grooves. Cuticular annuli distinct, more prominent a short distance from either end. The stylet is long and thin. The stylet opening is approximately 3 µm from the tip. Four lines present in the lateral field, the center band smaller than outer two. Areolation evident with SEM but difficult to observe under light microscope. Testis one or two. Spicules arcuate, under SEM can be seen to have dentate tips ventrally. Phasmids located at or anterior to cloacal aperture. Tail short, rounded.

Juvenile: Vermiform, small, tapering at both extremities but mostly posteriorly. The shape of the head of juveniles is not diagnostic for the species. Labial region not offset, with weak framework, bearing a labial disc and a large post-labial annulus lacking striations. The labial disk and medial lips are fused to form the head cap. The head cap appears anchor-shaped. The stylet of *M. chitwoodi* juveniles is useful for species identification. The anterior margins of the stylet knobs are indented and round to irregular and appear stippled. Cuticular annulation on most of body is very fine. Lateral field with four lines, areolated. Cephalids indistinct or not seen. Phasmids small, difficult to see, located in anterior one-third of the tail. Rectum not inflated. Tail ends in a broad, blunt tip, with the blunt hyaline region remaining almost the same diameter for its length and with little or no taper. The hyaline tail terminus is short and clearly delineated.

U.S. Distribution: see Figure 7.

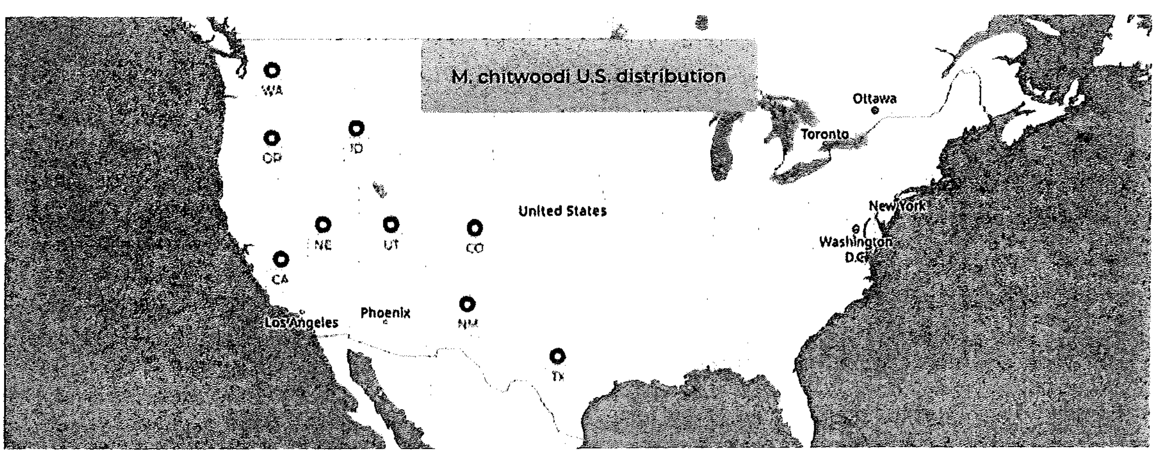

Figure 7. Continental U.S. distribution map of *Meloidogyne chitwoodi* (adapted from CABI-ISC) [26].

Molecular characterization: Molecular approaches based on PCR, PCR-RFLP of ITS region, ribosomal DNA of the intergenic spacer (IGS) region, and mitochondrial DNA and ribosomal RNA regions were successfully developed to distinguish this species from other closely related species. A simple ITS PCR-RFLP differentiation method was developed by Zijlstra [75,76] for *M. chitwoodi*, *M. fallax*, *M. hapla*, *M. incognita*, and *M. javanica* [13]. Later on (2000), Zijlstra [77] used a highly sensitive PCR method with species-specific SCAR primers. In 2002, Wishart et al. [78] designed a sensitive PCR method based on species-specific primers from ribosomal IGS regions [15]. MtDNA and rRNA genes for *M. chitwoodi* were sequenced by several authors [13]. LAMP assays for *M.chitwoodi* were designed recently by Zhang and Gleason [79].

Pathogenicity: This species is a major economic potato pest in Pacific Northwest U.S. [73,74]. It is predicted to cause annual losses of approximately USD 40 million if not treated [13]. The major damage that this nematode causes to potato tubers is a blemish that lowers the tuber marketability [80].

Diagnosis: Morphologically, this species is close to *M. hapla*, but it can be distinguished by differences in the perineal pattern, which has broken, curved, twisted, or curled striae around and above the anal area; by the sunken vulva; and by the presence of vesicles or vesicle-like structures in the median bulb of the females and by the tail shape in juveniles [73]. *M. fallax* is another close species to *M. chitwodi*, but the latter can be differentiated by having a shorter stylet length (16–19 vs. 19–21 µm) in males and a shorter J2 tail length (39–51 vs. 46–57 µm) and hyaline region (8–14 vs. 12–16 µm) [13,15]. Molecularly, *Meloidogyne chitwoodi* can be distinguished from *M. fallax* and other species by the ITS rRNA and *COI* gene sequence.

1.7. *Meloidogyne enterolobii Yang & Eisenback, 1983*

- *Common name*: Guava root-knot nematode
- *Type plant host*: *Enterolobium contortisiliquum* (Vell.) Morong, pacara earpod tree.
- *Type locality*: Hainan Island, Hainan Province, China.
- *First U.S. report*: 2001, FL.

Measurements: (modified after Yang & Eisenback, 1983 [81]).

Female: n = 20; the average females body length is 735 ± 92.83 (541.3–926.3) µm; a = 1.25 ± 0.23 (0.97–1.94); body width 606.8 ± 120.52 (375.7–809.7) µm, neck length 218.4 ± 74.16 (114.3–466.8); stylet length 15.1 ± 1.35 (13.2–18.0) µm; stylet knobs width 4.9 ± 0.39 (4.1–5.6) µm; stylet knobs height 2.4 ± 0.26 (1.9–3.1) µm; DGO = 4.9 ± 0.78 (3.7–6.2) µm; excretory pore to head end 62.9 ± 10.5 (42.3–80.6) µm; interphasmidial distance 30.7 ± 4.78 (22.2–42.0) µm; vulva length 28.7 ± 1.76 (25.3–32.4) µm; vulva to anus distance 22.2 ± 1.76 (19.7–26.6) µm; number of body annules from head end to excretory pore 36 ± 6.73 (24–48).

Male: n = 20; body length 1599.8 ± 159.91 (1348.6–1913.3 µm; a = 37.9 ± 3.15 (34.1–45.5); c = 131.6 ± 24.15 (72.0–173.4); body width 42.3 ± 3.56 (37.0–48.3) µm; stylet length 23.4 ± 0.96 (21.2–25.5) µm; stylet knob height 3.3 ± 0.33 (2.6–3.9) µm; stylet knob width 5.4 ± 0.3 (4.5–5.8) µm; DGO= 4.7 ± 0.41 (3.7–5.3) µm; excretory pore to head end 178.2 ± 11.16 (159.7–206.2) µm; spicule length 30.4 ± 1.16 (27.3–32.1) µm; gubernaculum length 6.2 ± 0.96 (4.8–8.0) µm; tail length 12.5 ± 2.24 (8.6–20.2) µm, testis length 810.1 ± 24.15 (597.0–1055.0).

Juvenile: n = 30; body length 436.6 ± 16.61 (405.0–472.9) µm; a = 28.6 ± 1.88 (24.0–32.5); c = 7.8 ±0.65 (6.8–10.1); body length/head end to posterior end of metacorpus 6.5 ± 0.18 (6.2–6.9) µm; body width 15.3 ± 0.89 (13.9–17.8) µm; excretory pore to head end distance 91.7 ± 3.34(84.0–98.6) µm; stylet length 11.7 ± 0.45 (10.8–13.0) µm; stylet knobs width 2.9 ± 0.25 (2.4–3.4) µm; stylet knobs height 1.6 ± 0.13 (1.3–1.8) µm; DGO = 3.4 ± 0.33 (2.8–4.3) µm; tail length 56.4 ± 4.48 (41.5–63.4) µm.

Description (modified after Yang & Eisenback, 1983 [81] and Subbotin et al. [13]).

Female: Body white, pear-shaped to globular, variable in size, with prominent neck variable in size, without posterior protuberance. Labial region not distinctly offset from neck. Labial disc and median lips fuse to form head cap. Hexaradiate labial framework distinct but weak; vestibule and vestibule extension prominent. Cephalids and hemizonid

THESIS

CONTROL OF *MELOIDOGYNE CHITWOODI* (COLUMBIA ROOT-KNOT NEMATODE) BY MICROBIAL SOIL INOCULANTS IN POTATOES (*SOLANUM TUBEROSUM*)

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CONTROL OF *MELOIDOGYNE CHITWOODI* (COLUMBIA ROOT-KNOT NEMATODES) IN COMMERCIALLY GROWN RUSSET NORKOTAH POTATOES WITH BIOFIT N, A MICROBIAL SOIL INOCULANT, IN THE SAN LUIS VALLEY, COLORADO

Introduction

Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden, O'Bannon, Santo, & Finley, 1980) is a major pest in commercial potato production in the northwestern, United States of America. *M. chitwoodi* infestation is widespread throughout the potato growing regions of California, Colorado, Idaho, Oregon, Utah and Washington (Nyczepir, O'Bannon, Santo, & Finley, 1982; Pinkerton & McIntyre, 1987; Santo, O'Bannon, Finley, & Golden, 1980). *M. chitwoodi* have also been detected in Argentina, Mexico, South Africa, and Turkey (Elling, 2013). In 2014, Colorado planted 24,373 ha of potatoes with 21,943 ha planted in the San Luis Valley of Colorado. The total Colorado potato crop value in 2014 has been calculated to be worth $214,802,000 (USDA NASS Mountain Regional Office, 2015). In the U.S. it is estimated that *M. chitwoodi* have the potential to inflict potato crop losses up to $40 million per year if not controlled. Potato farmers in the U.S. spend approximately $20 million per year in nematode soil fumigants and chemical controls; with some nematicide chemicals costing more than $741 per hectare (Suszkiw, 2009). Worldwide, yearly crop losses due to plant parasitic nematodes have been estimated at $173 billion; with estimated yearly U.S. losses of $13 billion (Elling, 2013).

M. chitwoodi reproduce on potato roots and tubers, which can allow for its spread to uninfected fields and distant potato farming areas by the transfer of infested soil and infested seed potato tubers. *M. chitwoodi* females are found in the outer 5.25 mm vascular ring of the tuber. Potato tuber damage consist of nematode-induced blemishes that lower tuber market value or make the crop unsellable when tuber damage is 10% or greater. In the fresh eating potato tuber export market and in all seed potato tubers there is a zero tolerance for infested tubers by *M. chitwoodi*; which enhances the economic impact of this pest. This is due to *M. chitwoodi* being designated as a quarantine pest by regulatory agencies in many countries (Elling, 2013). *M. chitwoodi* also has a very large host range, which can make it difficult to control by crop rotation alone. *M. chitwoodi* can feed and reproduce on approximately 3,000 plant species to include vegetables, legumes, cereals, grasses, bushes, tree fruits and ornamentals. *M. chitwoodi* also reproduce at high rates on oats (*Avena sativa*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*); which are common rotational crops in commercial potato production (Mojtahedi, Santo, & Wilson, 1988).

M. chitwoodi eggs and second-stage juveniles can survive subfreezing soil temperatures and overwinter in crop fields in the northwestern U.S. *M. chitwoodi* have a low temperature developmental threshold of 5°C, and an optimal developmental temperature of 20°C to 25°C. Below 5°C *M. chitwoodi* do not develop and go into a dormant state; where they can survive the winter until next season's crop is planted. The overwintered viable female eggs, once they hatch and the overwintered second-stage juvenile female *M. chitwoodi* population infest roots, develop into adult females within the roots and produce eggs masses at 600°C to 800°C degree-days after planting. The second-stage juveniles from the second-generation hatch from their eggs 950°C to 1,100°C degree-days after planting and then infest both roots and young tubers during the tuber bulking stage between 988°C to 1,166°C degree-days after planting. The third generation of *M. chitwoodi* females are then able to re-infest roots and tubers at 1,500°C to 1,600°C degree-days after planting (Table 2.1 and Figure 2.1) (Pinkerton, Santo, & Mojtahedi, 1991).

https://api.mountainscholar.org/server/api/core/bitstreams/0f00ce2b-6409-402c-b188-2efc696e8056/content

11 Root-knot Nematodes

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Abstract

The root-knot nematodes (RKN) are highly adapted plant parasites and have a very specialized mode of life marked by extreme sexual dimorphism, the females being massively swollen and sedentary whereas the males, when present, retain their mobility and are vermiform. The chapter provides an introduction to their general morphology, life cycle, plant-host relationships, classification, major species, and the various management strategies. There is a key to the genera of the Meloidogyninae, although only *Meloidogyne* is discussed. The importance of accurate identification and diagnostics for control/management strategies of RKN species is emphasised, morphological and morphometric criteria, in combination with biochemical and molecular diagnostics and other specialized techniques, being paramount. Ten major species of economic importance, including *M. chitwoodi*, *M. enterolobii*, *M. ethiopica*, *M. exigua*, *M. incognita* and *M. javanica*, are dealt with in more detail with information on morphology, molecular diagnostics, host range and distribution.

11.1. Introduction

Root-knot nematodes were first discovered by Berkeley (1855) on the root of cucumber growing in an English glasshouse (Figs 11.1, 11.2). Initially regarded more as a curiosity, their importance has gradually been recognized. Today, along with the cyst nematodes and pratylenchids, they are among the most economically important plant-parasitic nematodes in the world. Root-knot nematodes belong to the genus *Meloidogyne* Göldi, 1887, a genus originally created for a species found attacking coffee roots in Brazil (Göldi, 1887). Due mainly to poor descriptions and confusion with cyst nematodes, much uncertainty existed as to the status of root-knot nematodes until Chitwood (1949) established the genus on a firm footing, establishing, as first reviser, five species: *M. exigua* Göldi, 1887, the type species known for attacking coffee roots, together with *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949. A full account of the early history of the genus is given by Hunt and Handoo (2009).

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morphology. However, some species, such as *M. exigua*, *M. hapla* and *M. kikuyensis*, produce characteristic galls that may be useful in species identification.

Plant-growth regulators have been implicated in the development of giant cells and galls. Auxins (promoters of cell growth) have been identified in higher concentrations in root-knot infected tissue than in non-galled tissue. Cytokinins (promoters of cell division) may also increase in *Meloidogyne*-infected plants. Application of these plant-growth regulators to resistant plants reverses the resistant response and makes plants susceptible. Another plant-growth regulator, ethylene, has been associated with gall formation. In a few interactions, such as that occurring with parasitism of coffee trees by *M. coffeicola*, root knots are formed in young plants, but not in older ones (up to 5 years old). Accordingly, root knots are usually present in *Meloidogyne* infections, but exceptions do occur! For more details about host-parasite relationships, see Taylor and Sasser (1978), Sasser and Carter (1982), Hussey (1985), Endo, (1987), Eisenback and Triantaphyllou (1991), Di Vito *et al.* (1996, 2004), Ferraz and Brown (2002), Mitkowski and Abawi (2003), Gheysen and Jones (2006), Karssen and Moens (2006), Khan (2008), Manzanilla-López and Starr (2009). The current host races and host range for some major agricultural crops are given in Subbotin *et al.* (2021).

11.5. Management

Meloidogyne species constitute a major group of plant-pathogenic nematodes affecting crop production throughout the world, and, therefore, preventing the introduction and spread of these species is a vital component of management strategies. The extensive host range, worldwide distribution and involvement with fungi and bacteria in disease complexes place these nematodes high on the list of major plant pathogens affecting the world's food supply (Carter and Sasser, 1982). Collectively, various root-knot nematode species attack nearly every crop that is grown, thus reducing not only the yield but also the quality of underground crops, such as potatoes, carrots and peanuts. All methods for the control of plant pathogens, including parasitic nematodes, have been categorized under one or more principles in Table 1.1 of Moens *et al.* (2009), and all of the various methods for the control of nematodes fit within one of these principles. The basic objective in any pest management is to increase both quantity and quality of crop yield, and increasing the yield quality is sometimes the most important objective for *Meloidogyne*. Targeting species as quarantine organisms reduces the risk of spread through international trade. *Meloidogyne chitwoodi* is a serious pest of economically important crops, such as potatoes and carrots, and is a prohibited pest in many countries. In future, with many more new species being described, additional species are likely to be of regulatory concern. For more details about the management of nematodes, the following references may be consulted (Canto-Saenz, 1985; Fassuliotis, 1985; Imbriani, 1985; Jatala, 1985; Johnson, 1985a, b; Mendoza and Jatala, 1985; Raymundo, 1985; Sasser and Carter, 1985; Schmitt, 1985; Bird, 1986; Heald, 1986; Morgan-Jones and Rodriguez-Kabana, 1986; Pinochet, 1986; Thomason and Caswell, 1987; Bridge, 1996; Atkins *et al.*, 2003; Karssen and Moens, 2006; Coyne *et al.*, 2009; Starr and Mercer, 2009; Nyczepir and Thomas, 2009; Williamson and Roberts, 2009; Mahfouz *et al.*, 2010). *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (Sikora *et al.*, 2018) also provides a good account of nematode management. In addition, another recent book, *Nematode Diseases of Crops and their Sustainable Management* (Khan and Quintanilla, 2023), includes several chapters detailing sustainable management strategies for root-knot and other nematodes on various crops (Shokoohia *et al.*, 2023).

The use of resistant plants to combat *Meloidogyne* species and the associated molecular studies on resistance are given in detail by Cook and Starr (2006). The nematode population reduction may permit growing of susceptible crops more often in the rotation scheme. Resistant cultivars inhibit root-knot nematode reproduction, and cultivars resistant to these species are known in both woody and non-woody plants as well as in perennial and annual plants of various families. Their resistance is monogenic or polygenic. As mentioned by Karssen and Moens (2006), they are sometimes resistant to a single *Meloidogyne* species (e.g., coffee and *M. exigua*), while other cultivars show resistance to several species (tomato and *M. arenaria*, *M. incognita* and *M. javanica*). The *Mi-1* gene, which confers resistance in tomato to root-knot nematodes, is inactivated at soil temperatures above 28°C. Resistance-'breaking' biotypes or *Mi*-virulent isolates of root-knot nematodes have been reported in many areas of the world. Heat-stable resistance genes have been found in tomatoes. Several cultural and physical control methods have been used with some degree of success, but these methods are often of local or regional relevance (see Gaur and Perry, 1991) and are only of use in regions where solar energy is available for long periods of time. The era of nematicides lasted from the 1950s to the 1980s, and their effectiveness is often cited as a reason why, for many years, other alternative management systems did not receive greater attention (Moens et al., 2009). Starting in the 1970s, the use of fumigants was initially greatly restricted and then forbidden entirely, and suspension of some of the granular nematicides followed. There is now little prospect for new and effective nematicides being released in the future.

The principles and main components of effective control programmers and integrated pest control have been discussed in detail (Taylor and Sasser, 1978; Johnson and Fassuliotis, 1984; IFAS, 1989; Netscher and Sikora, 1990). We agree with the following main aspects that were considered important by Netscher and Sikora (1990):

1. Prevention of infestations by controlling nematode spread must be top priority.
2. Only root-knot nematode-free transplants should be used as planting material.
3. In view of the high multiplication rate of root-knot nematodes and difficulty in determining occurrence of low population densities, previously infected land should always be considered infested, even if the presence of *Meloidogyne* cannot be demonstrated by soil analysis.
4. Efforts should be made when planting vegetable crop rotations to select and develop pest management approaches that prevent the build-up of high nematode densities.
5. Integrated pest management should combine rotations with non-host crops, resistant, tolerant and susceptible cultivars as well as judicious use of nematicides, based on proper soil sampling estimations of damage threshold levels.
6. An integrated approach will control economically important nematodes, reduce pesticide costs and prevent unnecessary environmental contamination.
7. Proper selection of a combination of resistant, moderately resistant and tolerant vegetable crops can increase the number of vegetables in a short rotation cropping system.
8. Resistant cultivars should be used in rotation with susceptible cultivars and with other control techniques to prevent the development of resistance-breaking pathotypes.
9. All non-host crops and resistant cultivars should be challenged by local populations to determine true host status.
10. In regions where irrigated rice (or flooding) constitutes one of the components of the farming system, inundation can give good control.
11. Destruction of roots after harvest, soil drying and cultivation as a 'clean' fallow will significantly reduce population densities.
12. Time of planting may be effective in the cooler upland tropics and in the winter season in the subtropics.
13. Organic amendments or the use of non-host cover crops and green manures can reduce nematode densities.

11.6. Pathotypes

In his article on *The concept of race in phytonematology*, Dropkin (1988) refers the term 'pathotype' to a population whose members can reproduce on a host with genetic resistance to other parasite populations of the same species. The term does not imply partial isolation or uniform genetics, while 'race' designates a population with distinctive characters, either in morphology or in physiology, or both. A race is partially isolated from other intraspecific groups by geography or genetics. Races of phytonematodes are descriptively defined by their ability to reproduce on certain members of a set of differential hosts.

Meloidogyne spp. differ in their ability to attack certain host plants. Such pathotypes have been found to attack resistant peach rootstocks (Young and Sherman, 1977), and, because of the time required to develop and test new rootstock cultivars, they are a serious concern in rootstock breeding programmes for trees and

<u>These data were updated</u>

Data after that date are estimated using average temperature for the previous 7 days.

Columbia Root Knot Nematode Degree-Day Calendar

Use planting date and the weather station located nearest to your field to estimate Columbia Root Knot Nematode Degree-Day accumulated on: **7/3/2024**

800 degree-day threshold is needed to time nematicide (such as vydate C-LV) application.

[Location of weather stations: **Center-1:** Lat: 37.7067 Lon: -106.1440 ; **Center-2:** Lat: 37.8288 Lon: -106.0380 ; **La Jara:** Lat: 37.2443 Lon: -105.9722 ; **San Acacio:** Lat: 37.1417 Lon: -105.6110]

Planting Date		**4/15/2024**	**4/16/2024**	**4/17/2024**	**4/18/2024**	**4/19/2024**	**4/20/2024**
Center-1		1086.8	1077.6	1068.1	1058.6	1048.5	1038.0
Center-2		863.8	858.6	852.8	846.8	840.7	834.3
La Jara		893.9	888.2	882.2	875.9	[illegible]	861.5
San Acacio		832.7	827.2	822.1	816.6	[illegible]	803.8
Planting Date	**4/21/2024**	**4/22/2024**	**4/23/2024**	**4/24/2024**	**4/25/2024**	**4/26/2024**	**4/27/2024**
Center-1	1027.5	1016.6	1005.4	993.1	980.4	967.7	956.2
Center-2	[illegible]	820.7	813.3	805.2	796.7	[illegible]	780.1
La Jara	[illegible]	846.5	838.5	829.9	820.6	[illegible]	803.1
San Acacio	[illegible]	790.6	782.9	774.8	766.0	[illegible]	750.0
Planting Date	**4/28/2024**	**4/29/2024**	**4/30/2024**	**5/1/2024**	**5/2/2024**	**5/3/2024**	**5/4/2024**
Center-1	946.2	936.5	926.0	914.5	902.3	889.8	877.8
Center-2	[illegible]	766.4	759.2	751.3	743.1	[illegible]	726.3
La Jara	[illegible]	787.7	780.1	771.2	762.5	[illegible]	745.6
San Acacio	[illegible]	738.4	733.2	726.6	719.4	[illegible]	704.5
Planting Date	**5/5/2024**	**5/6/2024**	**5/7/2024**	**5/8/2024**	**5/9/2024**	**5/10/2024**	**5/11/2024**
Center-1	866.1	855.1	844.6	834.9	824.8	815.9	811.6
Center-2	[illegible]	709.8	702.9	695.8	688.7	[illegible]	677.3
La Jara	[illegible]	730.3	722.5	715.8	708.5	[illegible]	699.4
San Acacio	[illegible]	690.2	683.0	676.7	670.1	[illegible]	659.4
Planting Date	**5/12/2024**	**5/13/2024**	**5/14/2024**	**5/15/2024**	**5/16/2024**	**5/17/2024**	**5/18/2024**
Center-1	807.8	803.4	796.3	788.7	780.4	770.8	759.8
Center-2	675.5	669.6	664.6	658.8	652.8	645.8	637.3
La Jara	696.7	692.1	686.2	679.4	672.8	[illegible]	653.6
San Acacio	655.1	651.2	646.1	640.0	633.8	627.5	619.8
Planting Date	**5/19/2024**	**5/20/2024**	**5/21/2024**	**5/22/2024**	**5/23/2024**	**5/24/2024**	**5/25/2024**
Center-1	747.4	734.0	720.7	708.2	696.5	684.1	671.2
Center-2	627.4	616.8	606.1	595.8	585.8	575.7	565.1
La Jara	642.6	630.2	618.7	608.3	598.7	587.9	577.1
San Acacio	610.9	600.9	591.2	581.8	572.9	563.3	553.3

(Weather data source: https://coagmet.colostate.edu/rawdata_form.php)

Projected 800 degree day date for Columbia Root Knot Nematode management

(Estimated based on average degree days for past seven days)

Planting Date		4/15/2024	4/16/2024	4/17/2024	4/18/2024	4/19/2024	4/20/2024
Center-1		N/A	N/A	N/A	N/A	N/A	N/A
Center-2		N/A	N/A	N/A	N/A	N/A	N/A
La Jara		N/A	N/A	N/A	N/A	N/A	N/A
San Acacio		N/A	N/A	N/A	N/A	N/A	7/3/2024
Planting Date	**4/21/2024**	**4/22/2024**	**4/23/2024**	**4/24/2024**	**4/25/2024**	**4/26/2024**	**4/27/2024**
Center-1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Center-2	N/A	N/A	N/A	7/3/2024	7/3/2024	7/4/2024	7/4/2024
La Jara	N/A	N/A	N/A	N/A	N/A	N/A	7/3/2024
San Acacio	7/3/2024	7/4/2024	7/4/2024	7/5/2024	7/5/2024	7/6/2024	7/6/2024
Planting Date	**4/28/2024**	**4/29/2024**	**4/30/2024**	**5/1/2024**	**5/2/2024**	**5/3/2024**	**5/4/2024**
Center-1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Center-2	7/5/2024	7/5/2024	7/6/2024	7/6/2024	7/7/2024	7/7/2024	7/8/2024
La Jara	7/3/2024	7/4/2024	7/4/2024	7/5/2024	7/5/2024	7/6/2024	7/6/2024
San Acacio	7/7/2024	7/7/2024	7/7/2024	7/8/2024	7/8/2024	7/9/2024	7/9/2024
Planting Date	**5/5/2024**	**5/6/2024**	**5/7/2024**	**5/8/2024**	**5/9/2024**	**5/10/2024**	**5/11/2024**
Center-1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Center-2	7/8/2024	7/9/2024	7/9/2024	7/10/2024	7/10/2024	7/10/2024	7/11/2024
La Jara	7/7/2024	7/7/2024	7/8/2024	7/8/2024	7/9/2024	7/9/2024	7/9/2024
San Acacio	7/9/2024	7/10/2024	7/10/2024	7/11/2024	7/11/2024	7/11/2024	7/12/2024
Planting Date	**5/12/2024**	**5/13/2024**	**5/14/2024**	**5/15/2024**	**5/16/2024**	**5/17/2024**	**5/18/2024**
Center-1	7/3/2024	7/3/2024	7/3/2024	7/4/2024	7/4/2024	7/5/2024	7/5/2024
Center-2	7/11/2024	7/11/2024	7/11/2024	7/12/2024	7/12/2024	7/13/2024	7/13/2024
La Jara	7/10/2024	7/10/2024	7/10/2024	7/11/2024	7/11/2024	7/12/2024	7/12/2024
San Acacio	7/12/2024	7/12/2024	7/13/2024	7/13/2024	7/13/2024	7/14/2024	7/14/2024
Planting Date	**5/19/2024**	**5/20/2024**	**5/21/2024**	**5/22/2024**	**5/23/2024**	**5/24/2024**	**5/25/2024**
Center-1	7/6/2024	7/7/2024	7/8/2024	7/8/2024	7/9/2024	7/10/2024	7/11/2024
Center-2	7/14/2024	7/14/2024	7/15/2024	7/16/2024	7/16/2024	7/17/2024	7/18/2024
La Jara	7/13/2024	7/14/2024	7/14/2024	7/15/2024	7/16/2024	7/16/2024	7/17/2024
San Acacio	7/15/2024	7/15/2024	7/16/2024	7/17/2024	7/17/2024	7/18/2024	7/18/2024

Degree Day accumulation for past seven days

Date	Center-1	Center-2	La Jara	San Acacio
6/26/2024	18.52	16.83	17.61	16.71
6/27/2024	17.51	16.66	15.28	16.52
6/28/2024	16.41	14.92	14.81	15.61
6/29/2024	16.81	15.12	16.56	16.26
6/30/2024	17.62	15.98	16.80	16.44
7/1/2024	17.17	16.12	15.03	15.77
7/2/2024	17.89	16.10	16.16	15.56
Average	**17**	**16**	**16**	**16**

Summary of Comments on SLV_Nematode-Weather-Data_July-03-2024_4different County Locations for CRKN model.pdf

Page: 1

Number: 1 Author: aplan Subject: Sticky Note Date: 3/16/2026 9:54:09 AM
https://www.coloradopotato.org/wp-content/uploads/2024/07/Nematode-Weather-Data_July-03-2024.pdf

Number: 2 Author: aplan Subject: Sticky Note Date: 3/16/2026 9:54:05 AM
The 4 different sites that are listed (Center-1, Center-2, La Jara, and San Acacio), are in 4 different counties within the San Luis Valley.

[1] [2]

SOIL DEGREE DAY ACCUMULATION - RUSSET NORKOTAH, SLV RESEARCH CENTER - 2024

For root-knot nematode control, Vydate applications should begin at 800 Degree-days (DD_{5C}) or 1,440 DD_{41F}. To estimate that date, add the number of "Total DD" on the day you planted to 800 (or 1,440). Where that value occurs in the "Total DD" column estimates the date on which 800 DD_{5C} or 1,440 DD_{41F} will occur in that field.

Incidence and Distinguishing Characteristics of *Meloidogyne chitwoodi* and *M. hapla* in Potato from the Northwestern United States[1]

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Abstract: From September 1980 to June 1981, a survey was conducted in the major potato growing regions of northern California, Idaho, Nevada, Oregon, and Washington to determine the distribution of *Meloidogyne chitwoodi* and other *Meloidogyne* spp. *Meloidogyne chitwoodi* and *M. hapla* were the only root-knot nematode species detected parasitizing potato in all the states surveyed. *Meloidogyne chitwoodi* occurred alone in 83% of the samples and *M. hapla* in 11%, with 6% of all samples containing both species. The greater incidence of *M. chitwoodi*, as compared to *M. hapla*, may be due to the cool growing season encountered in 1980 (which favored *M. chitwoodi* but not *M. hapla*) and to the increased acreage of small grains (which are good hosts for *M. chitwoodi* but not *M. hapla*) planted in rotation with potato. Differentiation between these two species can be determined by a differential host test, perineal patterns of mature females, and shape of the tail tip and of the tail hypodermal terminus of L_2 juveniles.
Key words: Meloidogyne chitwoodi, M. hapla, potato.

Journal of Nematology 14(3):347-353. 1982.

Potato (*Solanum tuberosum* L.) is an important agricultural commodity of the Pacific Northwest. In 1980, four of the five leading states in potato production were from northwestern United States, with Idaho and Washington ranked first and second, respectively (1). Root-knot nematodes (*Meloidogyne* spp.) are a severe problem in potato production because they reduce the quality of the potato tuber (9).

Until recently, the northern (*Meloidogyne hapla* Chitwood), southern (*M. incognita* [Kofoid and White] Chitwood), and Thames (*M. thamesi* Chitwood) root-knot nematodes were the only species recognized as parasitizing potatoes in the Pacific Northwest (7). *Meloidogyne hapla* was reported widespread in Idaho, Oregon, and Washington; *M. incognita* in limited regions of Oregon and Washington; and *M. thamesi* in limited regions of Washington (3,7,10). In 1978, a previously unreported root-knot nematode, the Columbia root-knot nematode (*M. chitwoodi* Golden et al.) was found in potato tubers from Washington and Idaho (5,10). Examination of tubers from several production areas revealed that this nematode was present along the Columbia River Basin of Washington, the upper Snake River regions of Idaho, and isolated areas of northeastern Oregon and northwestern Washington (10). This survey indicated that *M. chitwoodi* was more widespread than initially presumed. In September 1980, a more extensive survey of the major potato growing regions of the Pacific and Northwestern United States was conducted to determine more precisely the incidence of *M. chitwoodi* and other *Meloidogyne* spp. This paper reports results of that study.

MATERIALS AND METHODS

Potato tubers and/or soil samples containing *Meloidogyne* spp. were collected from the major potato growing regions of northern California, Idaho, Nevada, Oregon, and Washington. From September 1980 to June 1981, tuber samples were obtained from processing facilities as they were being placed in or removed from storage. When regulatory inspectors detected root-knot nematode infection or symptoms on tubers, three or four of the tubers from that infected lot were removed, location to nearest town recorded, and the sample mailed to the nematology laboratory at Prosser, Washington. During the survey, a limited number of soil samples were obtained from a private consultant.

Root-knot nematode populations were increased and maintained in a greenhouse by removing 15–20 egg masses from an in-

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Appreciation is extended to William L. Boge and Marj Lauer for technical assistance and to L. R. Faulkner for providing original slides of *M. incognita* from Washington.

fected tuber and placing them on the roots of two tomato seedlings *Lycopersicon esculentum* Mill. 'Columbian'. Each tomato plant was planted in a separate 10-cm-d plastic pot containing methyl bromide fumigated sandy loam soil. Infested soil samples were placed in pots and planted directly with a tomato seedling.

Eggs were extracted from the tomato roots after 55 days using the method of Hussey and Barker (6) to prepare inocula for a differential host test. Inoculations were made by pipetting 2,000 eggs per pot into depressions made in the soil around each host's hypocotyl. Plants used in our standard differential host test included pepper (*Capsicum frutescens* L. 'California Wonder'), watermelon (*Citrullus vulgaris* Shrad 'Charleston Grey'), and wheat (*Triticum aestrum* L. 'Nugaines'). The North Carolina differential host test (11) was used whenever a population did not appear to conform to our standard differential test (Table 1). Treatments consisted of inoculating two seedlings of each cultivar, and the pots were randomly arranged on greenhouse benches. Fiberglass dividers (112 × 36 cm) separated individual populations on the same bench in order to prevent cross contamination during watering. Ambient greenhouse temperatures ranged from 24 to 26 C. Plants were watered daily and fertilized every 2 wk.

After 55 days, the plants were harvested and the roots washed in tap water and stained with phloxine B (150 mg/l for 15 min.) (2). Root galls and egg masses on each root system were counted, and plants were rated as a host or nonhost. The following rating system was used: 0 = no galls or egg masses, 1 = 1–2 galls or egg masses, 2 = 3–10 galls or egg masses, 3 = 11–30 galls or egg masses, 4 = 31–100 galls or egg masses, and 5 = > 100 galls or egg masses (11). A root system rating of 0–2 was considered a non to poor host. After the root rating was determined, eight mature females and their egg masses were removed from each root system. Morphological criteria, in conjunction with the differential host test, were used to identify the root-knot population to species. The morphological criteria were the length, tail tip shape, and the shape of the tail hypodermal terminus from 20 freshly hatched second-stage juveniles (L_2), perineal patterns, stylet length, and presence/absence of median bulb vesicles of mature females. Juveniles were obtained by incubating egg masses in water for 24 h at 25 C. The survey was terminated at the end of June 1981.

RESULTS

Meloidogyne chitwoodi and *M. hapla* were the only species of root-knot nematode detected in our survey. Both species were found in major potato producing regions of the states sampled, occurring either along a major waterway used for irrigation or in isolated areas in northern California, southern Oregon, and northwestern Washington (Fig. 1). *Meloidogyne chitwoodi* occurred alone in 83% of the samples, *M. hapla* alone in 11%, and 6% of the samples contained both species (Table 2). *Meloidogyne chitwoodi* usually occurred as a single species in the samples, while *M. hapla* was primarily present mixed with *M. chitwoodi,*

Table 1. Differential host test for *Meloidogyne* spp.*

Meloidogyne species	Differential host†						
	Wheat	Pepper	Water-melon	Tobacco	Cotton	Peanut	Tomato
M. chitwoodi	+	–	–	–	–	–	+
M. hapla	–	+	–	+	–	+	+
M. incognita‡	+	+	+	–	–	–	+
M. arenaria‡	+	+	+	+	–	+	+
M. javanica	+	–	+	+	–	–	+

*+ = host; – = nonhost. See reference # 11.

†Wheat, 'Nugaines'; pepper, 'California Wonder'; watermelon, 'Charleston Grey'; tobacco, 'NC 95'; cotton, 'Deltapine 16'; peanut, 'Florrunner'; tomato, 'Rutgers.'

‡Refers to race one of *Meloidogyne* spp. See reference # 11 for race differentiation within a species.

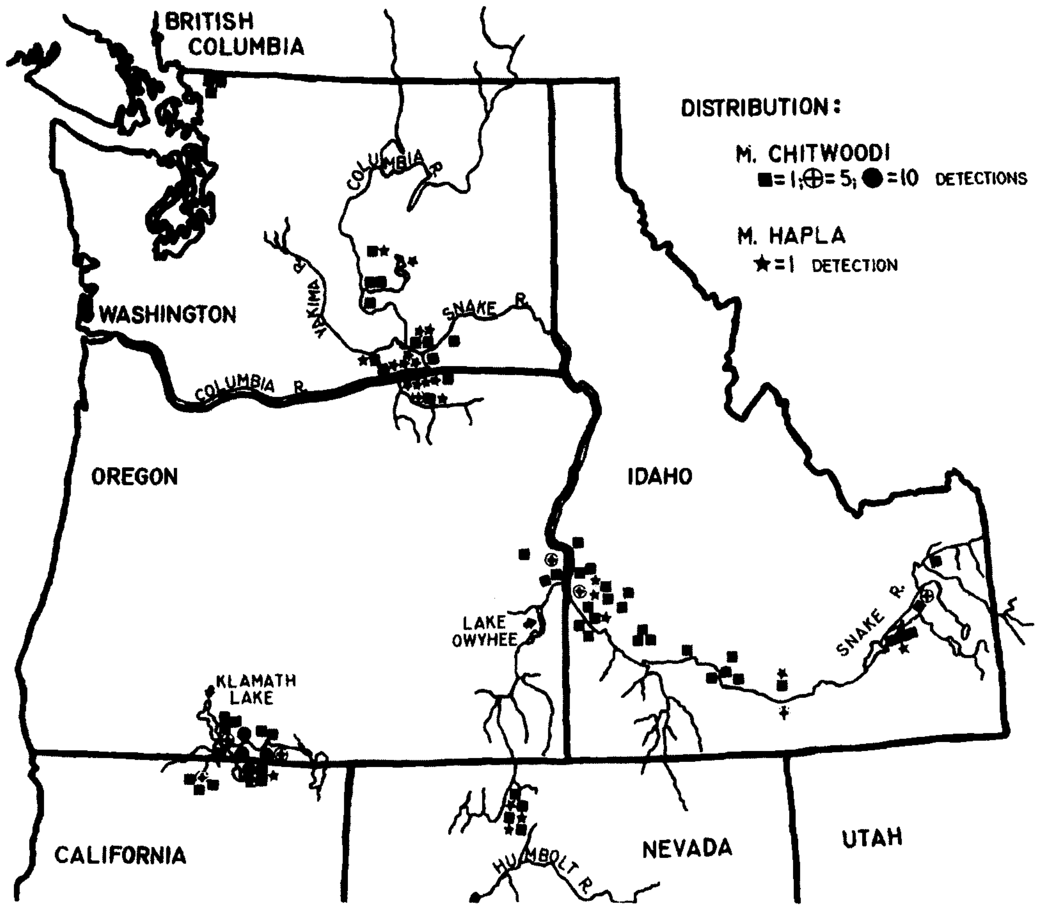


Fig. 1. Distribution of *Meloidogyne chitwoodi* and *M. hapla* on potato in five northwestern states surveyed, September 1980 to June 1981.

except in a few samples received from Washington and northeastern Oregon. *Meloidogyne chitwoodi* was the predominant root-knot nematode found in all states sampled except Nevada.

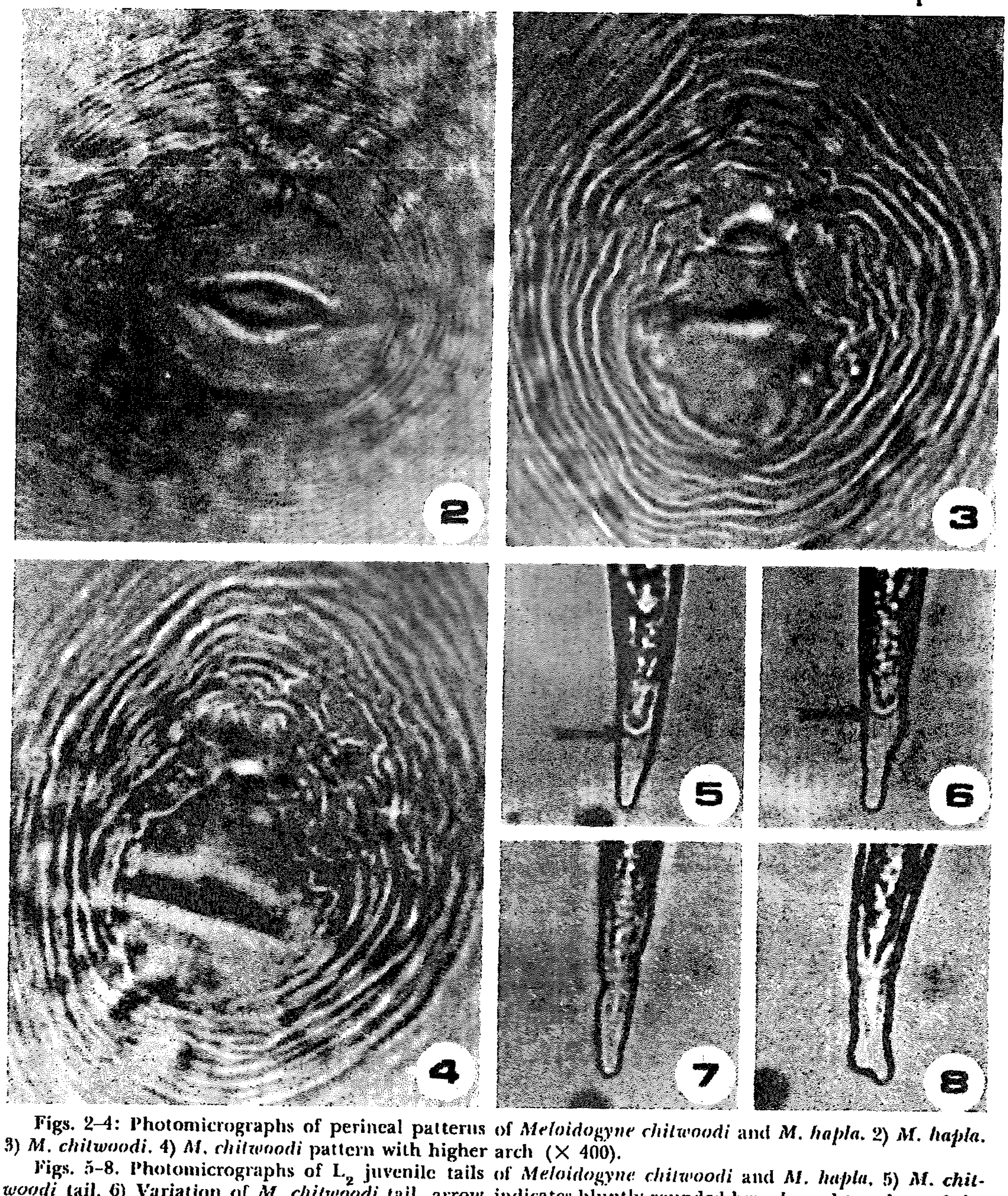
'California Wonder' pepper and 'Nugaines' wheat are excellent differential hosts for *M. chitwoodi* and *M. hapla*. Questionable results for species identification occurred in only one sample which was received from northern California. However, after repeating the original differential host test in conjunction with the North Carolina test and using morphological characteristics, it was confirmed as *M. chitwoodi*.

Morphological characteristics were used

Table 2. Occurrence of *Meloidogyne chitwoodi* (Mc) and *M. hapla* (Mh) alone or together in potato tubers and soil samples collected from the northwestern United States, September 1980 to June 1981.

State	Number and type of samples received			Number of samples infested with		
	Tuber	Soil	Total	Mc	Mh	Mc + Mh
Washington	41	3	44	25	17	2
Idaho	40	0	40	36	0	4
Oregon	72	4	76	71	3	2
California	23	0	23	22	0	1
Nevada	0	4	4	1	0	3
Total	176	11	187	155	20	12

to identify each root-knot nematode population to species. The most reliable characters differentiating *M. chitwoodi* from *M. hapla* included the adult females' perineal pattern and L_2 juvenile tail tip shape and shape of the posterior end of the hypodermis in the tail. Perineal patterns of these two species are distinctly different. Patterns of *M. hapla* (Fig. 2) were usually delicate without distinct lateral lines, the striae were smooth and parallel, and the arch was flattened to more or less rounded. Punctations were present around the tail region, whereas wing formation and number varied. Perineal patterns of *M. chitwoodi* (Fig. 3) had arches that were usually oval with thick, twisted striae in and around the anal region, and an anus and vulva set in a depression



Figs. 2–4: Photomicrographs of perineal patterns of *Meloidogyne chitwoodi* and *M. hapla*. 2) *M. hapla*. 3) *M. chitwoodi*. 4) *M. chitwoodi* pattern with higher arch (× 400).

Figs. 5–8. Photomicrographs of L_2 juvenile tails of *Meloidogyne chitwoodi* and *M. hapla*. 5) *M. chitwoodi* tail. 6) Variation of *M. chitwoodi* tail, arrow indicates bluntly rounded hypodermal terminus of the tail. 7) Tapered tail of *M. hapla*. 8) Toe-shaped tail of *M. hapla* (note tapered hypodermal terminus of *M. hapla* tails) (× 640).

(sunken). More variation occurred within patterns of *M. chitwoodi* populations than within *M. hapla*. Some patterns of *M. chitwoodi* had higher arches (Fig. 4), yet the striae were typical of *M. chitwoodi*.

Tail tip shape and the shape of the tail hypodermal terminus of freshly hatched heat-relaxed L_2 juveniles were distinctly different in the two species. The slightly tapered and bluntly rounded tail tip of *M. chitwoodi* L_2 juveniles (Fig. 5) was previously described and illustrated as being characteristic of this species (5). However, some variations between and within populations were noted. The tail tip of some L_2 juveniles were not always bluntly rounded, but sometimes deformed, exhibiting a slightly clavate tail tip (Fig. 6). However, the perineal pattern of the female from which these L_2 juveniles were obtained was typical for *M. chitwoodi*, as was the parasitism in the differential host test. One tail characteristic that was consistent among all *M. chitwoodi* L_2 juveniles was the shape of the tail hypodermal terminus, which was bluntly rounded even when variations occurred in the tail tip (Figs. 5, 6; see arrows).

Two tail tip shapes were encountered between and within populations of *M. hapla*. The tail tip was either uniformly tapered to a bluntly rounded tip (Fig. 7) or 'toe-shaped' (Fig. 8). The shape of the tail hypodermal terminus was more tapered (Figs. 7, 8) than in *M. chitwoodi*.

DISCUSSION

Meloidogyne chitwoodi and *M. hapla* were the only root-knot nematode species found in the major potato growing regions of the Northwest. Although *M. incognita* had previously been reported in Washington, it was not detected in this survey. Slides (courtesy L. R. Faulkner) containing perineal patterns of nematodes identified as *M. incognita* in 1962 have been reexamined and are now identified as *M. chitwoodi*. Failure to detect other root-knot species was not surprising since *M. chitwoodi* and *M. hapla* have a relatively high tolerance for cooler soil temperatures compared to the other three most common root-knot species—*M. arenaria*, *M. incognita*, and *M. javanica*—in the United States (9,11). In four of the five states surveyed, the daily annual average temperature from 1969 to 1979 taken in seven locations was 10.5 C; the daily maximum and minimum temperatures were 17.2 and 3.6 C, respectively (Table 3). This would result in relatively low soil temperatures. These relatively low soil temperatures help explain why these two species predominate in the Northwest. *Meloidogyne chitwoodi* is more of a problem than *M. hapla* on potato in years when spring temperatures are unusually cool. Santo and O'Bannon (9) have shown that *M. chitwoodi* reproduces more on potato at lower temperatures than *M. hapla*. Therefore, *M. chitwoodi* would tend to have more generations than *M. hapla* during the growing season, resulting in earlier tuber infection and a greater reduction in tuber quality. The low temperatures in the spring of 1980 could account for the higher incidence of *M. chitwoodi* found in potato tubers in this survey. Nematode analysis of soil samples from Idaho, Nevada, Oregon, and Washington (Santo, unpublished) show that *M. hapla* occurs more frequently than indicated by this survey where the majority of the samples were potato tubers.

Crop rotation plays an important role in determining which of these two root-knot nematode species predominates in a particular field. In the Pacific Northwest, alfalfa, wheat, other small grains and cereals, peppermint, and sugarbeet are the principle crops rotated with potato. Wheat,

Table 3. Daily annual average temperature at seven locations in Northwestern United States, 1969–79.

State and station	Temperature (C) Maximum	Minimum	Average
Idaho			
Boise	17.1	4.2	10.7
Pocatello	13.7	0.9	7.9
Oregon			
Medford	19.2	4.8	12.0
Pendleton	17.3	5.3	11.3
Nevada			
Winnemucca	19.2	0.1	9.7
Washington			
Quincy	16.6	2.7	9.7
Walla Walla	17.4	6.9	12.2
Mean	17.2	3.6	10.5

other small grains, and cereals are good hosts for *M. chitwoodi* but poor to non hosts for *M. hapla* (10). Alfalfa was the primary crop rotated with potato in most Northwestern states (particularly Idaho and Washington), but since it is a good host for *M. hapla* it is not often used in the rotation. However, alfalfa is a poor to non host to *M. chitwoodi* (10). Peppermint is a poor host for *M. chitwoodi* but a good host for *M. hapla,* whereas sugarbeet is a good host for both nematodes (3,8). Knowing the cropping history of a field infested with *Meloidogyne* spp. could help determine which of these two species is predominant. Six percent of all samples contained both species, and whenever this occurred one species always predominated. The previous cropping sequence was probably the determining factor.

Results from this survey suggest that dissemination of *M. chitwoodi* and *M. hapla* in the potato growing regions of the Northwest occurred principally by two means; reused irrigation water (canal and river water), and infected seed potato. Irrigation water has been shown to be an excellent source for disseminating *Meloidogyne* L_2 juveniles (4). It has been estimated that the Yakima Valley and Columbia Basin of Washington receive approximately 0.144×10^6 to 15.362×10^6 plant parasitic nematodes per hectare per year in irrigation water alone. Nematode contaminated irrigation water would explain the distribution of *M. chitwoodi* and *M. hapla* along the major waterways used in irrigating potato fields in Idaho, Oregon, and Washington. Root-knot infected seed potato would account for the occurrence of both nematodes in areas removed from major waterways, such as northern California, southern Oregon, and northwestern Washington (Fig. 1). Three tuber samples infected with *M. chitwoodi* came from potato fields grown for seed, two in northwesten Washington and one in eastern Idaho.

Since only *M. chitwoodi* and *M. hapla* were detected in the survey, the perineal patterns and characteristics of L_2 juvenile tails were the easiest and most reliable criteria used to differentiate between these two species. Vesicle-like structures in the median bulb of *M. chitwoodi* females were less reliable because their number varied from 1 to 15. Other characteristics examined included total length of L_2 juveniles; position of the excretory pore in females, which is posterior to the stylet knobs for both species; the DGO, which is reported as being 4.3 μ and 5–6 μ for *M. chitwoodi* and *M. hapla* females, respectively; and female stylet lengths which are 12 μ and 13μ for *M. chitwoodi* and *M. hapla,* respectively. Though these morphometric criteria are essentially similar in *M. chitwoodi* and *M. hapla,* they would be useful in separating these two species from many other *Meloidogyne* spp.

The differential host test was a useful tool in determining which species were present. However, since races of *Meloidogyne* spp. are known to occur, the host test should not be the sole basis for species identification. Taylor and Sasser (11) emphasize that accuracy in identifying root-knot nematodes to species is increased as more criteria are used.

Meloidogyne chitwoodi and *M. hapla* were two root-knot nematode species found in major potato growing regions of northwestern United States in 1980-81. Knowledge of which species is present in a field will enable the grower to implement the proper rotation sequence in addition to other control practices, thereby suppressing root-knot nematode soil populations enough to increase potato tuber quality.

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Disease Note.

The Columbia Root-Knot Nematode, *Meloidogyne chitwoodi*, Discovered in the State of Utah. G. D. Griffin, USDA-ARS, Forage and Range Research Laboratory, Utah State University, Logan 84322. S. V. Thomson, Department of Biology, Utah State University, Logan 84322. Plant Dis. 72:363. Accepted for publication 11 December 1987. DOI: 10.1094/PD-72-0363A.

The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al) is an important pathogen of potato and may cause economic losses unless adequately controlled. In the spring of 1983, a population (P3) of *M. chitwoodi* was found in Iron County, Utah. P3 is more virulent on potato than two populations (P I and P2) from Idaho. Greenhouse tests at 24 C resulted in 24, 32, and 64% infected and galled tubers from PI, P2, and P3, respectively. Because of the shorter growing season and cooler weather conditions in Utah, P3 causes minor galling of potato tubers. P3 is capable of parasitizing and reproducing on alfalfa; population ratios (final population/initial population) on Ranger, Lahontan, and Nevada Synthetic XX at 22ï¿½ 4 C were 7.4, 5.8, and 2.7, respectively. This is the first report of *M. chitwoodi* in Utah and the second report of a population reproducing on alfalfa.

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Disease Note

First Report of the Columbia Root-knot Nematode (*Meloidogyne chitwoodi*) in Virginia. J. D. Eisenback, E. L. Stromberg, and M. S. McCoy, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg 24061. Plant Disease 70:801, 1986. Accepted for publication 16 April 1986. DOI: 10.1094/PD-70-801a.

Meloidogyne chitwoodi Golden, O'Bannon, Santo & Finley was isolated from winter barley (*Hordeum vulgare* L.) roots and soil from Westmoreland County in eastern Virginia on 4 December 1985. The previous crop was corn (*Zea mays* L.), a known host for this nematode. The barley plants were stunted and chlorotic and the leaves were colonized heavily by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker, the causal agent of leaf blotch. The rootlets had very small galls (1–2 mm) containing female root-knot nematodes. Species identification was based on perineal patterns, head shapes of males, and stylets of females, males, and second-stage juveniles. To our knowledge, this is the first record of this nematode occurring in the eastern United States. The previously known distribution of *M. chitwoodi* includes Washington, Oregon, California, Idaho, Utah, Nevada, and Colorado in the United States and Estado de Mï¿½xico in Mï¿½xico.

Reference: A. M. Golden *et al.* J. Nematol. 12:319, 1980.

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Disease Notes

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First Report of Columbia Root Knot Nematode (*Meloidogyne chitwoodi*) in Potato in Texas

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Abstract

Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden et al. (1) was identified from potatoes, *Solanum tuberosum* L., collected from Dallam County, Texas in October 2000. Seed potatoes are the most likely source for this introduction. This nematode is currently found infecting potatoes grown in California, Colorado, Idaho, New Mexico, Nevada, Oregon, Utah, and Washington. Some countries prohibit import of both seed and table stock potatoes originating in states known to harbor *M. chitwoodi*. Lesions on the potatoes had discrete brown coloration with white central spots in the outer 1 cm of the tuber flesh. Female nematode densities averaged 3 per square centimeter of a potato section beneath the lesions. Nematodes were morphologically identified as *M. chitwoodi* based on the perineal pattern of mature females and the tail shape of juveniles per Golden et al. (1). Using polymerase chain reaction-RFLP of the rDNA ITS1 region and the mtDNA COII-16S rRNA region (2), individual juveniles were identified as *M. chitwoodi* based on their

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infecting potatoes in Texas. The distribution of this nematode in potato fields throughout central United States should be determined.

References: (1) A. N. Golden et al. J. Nematol. 12:319, 1980. (2) T. O. Powers and T. S. Harris. J. Nematol. 25:1, 1993.

Disease Notes

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First Report of Columbia Root-Knot Nematode (*Meloidogyne chitwoodi*) in Potato in New Mexico

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Abstract

Following a report of Columbia root-knot nematode in potatoes (*Solanum tuberosum* L.) imported by Mexico from the United States in spring 2000, six fields in San Juan County, NM, were surveyed in August 2000. Soil samples from two fields in which the exported potatoes had been produced contained second-stage juveniles that were tentatively identified as Columbia root-knot nematode. During the 2000 potato harvest, state inspectors detected tubers from four additional fields that exhibited symptoms of Columbia root-knot nematode, including warty exteriors and discrete small brown lesions that were apparent to a depth of 1 cm below the tuber surface. *Meloidogyne chitwoodi* Golden et al. (1) was confirmed from a subsample of tubers sent to the USDA Nematology Laboratory in Beltsville, MD, in October 2000. Identification was based on morphological examination of the nematodes recovered from tubers. To our knowledge, this is the first

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recovered from soil samples collected at 26 locations throughout San Juan County in 1988 and 1989, nor had symptomatic tubers or plants been observed in this area previously. Columbia root-knot nematode most likely represents a recent introduction into northwestern New Mexico. Additional information regarding distribution of this nematode within the region is needed.

References: (1) A. M. Golden et al. J. Nematol. 12:319–327, 1980.

Chapter 5
Plant-Parasitic Nematode Problems in Organic Agriculture

Shabeg S. Briar, David Wichman and Gadi V.P. Reddy

Abstract Crop protection approaches differ widely among organic growers both globally and regionally, yet organic farming faces the same plant-parasitic nematode (PPN) issues as conventional farming. Due to the restrictions on use of synthetic chemical inputs and the limited number of options for nematode management in organic fields, organic producers are often at greater risk to nematode problems than their conventional counterparts. While worldwide estimates of crop losses of about 12 % annually of food and fiber due to nematode damage are reported in the literature, such information for organic farming systems is scarce. Comparative studies of organic and conventional farming systems and surveys conducted in organic farms in distinct regions show that the genera of nematodes attacking organic crops are similar to that in conventional fields, including species of root-knot (*Meloidogyne* spp.), cyst (*Heterodera* and *Globodera* spp.), and root lesion (*Pratylenchus* spp.) nematodes, among others. For PPN management, organic farmers employ practices such as crop rotation, use of cover crops or resistant crop cultivars, and soil amendments. In many instances, however, these methods may not be sufficient for PPN management. Although resistant cultivars of some crops are available for root-knot and cyst nematodes, they are resistant to only a few races or species of nematodes and new races develop over time. Biological control, using microbial pathogens, endophytes, or antagonists may help control PPNs in organic production of some crops but have had limited commercial success. In contrast, use of soil amendments has provided some level of suppression of PPNs under field conditions. Increased populations of predatory nematodes or other beneficial species grazing microbial films and stimulating soil nutrient mineralization have been observed in organic systems, indicating an improvement in the soil health. Further studies are needed to estimate yield losses caused by the

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Sustainable Development and Biodiversity 9,
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5.3.1.2 Animal Manures

Composted animal manure is one of the most popular organic amendments for soils. Poultry or livestock manure has been tested for nematode management (Nahar et al. 2006; Akhtar and Alam 1993; Akhtar and Malik 2000; Rodríguez-Kábana and Ivey 1986; Trivedi and Barker 1986). Numerous studies have found positive correlations between the addition of compost and suppression of PPNs including the economically important species such as root-knot and root lesion nematodes (e.g., Marull et al. 1997; LaMondia et al. 1999; McSorley and Gallaher 1994, 1995, 1996; Everts et al. 2006; Kaplan et al. 1992). The degree of nematode suppression, however, is variable depending upon factors such as the type of manure, application rate, and natural microflora in it (McSorley 2011a).

5.3.2 Crop Rotation and Other Cultural Practices

As described previously in this chapter, nematodes do not move long distance on their own and by reducing their population below the damaging levels may result in increased crop yield. Planting non-host crop in the rotation would remove food source for the PPNs and consequently decline in their population below the damaging levels (Rodríguez-Kábana and Ivey 1986; LaMondia 1999). However, the effectiveness of rotation in suppressing PPN population depends upon the type of the nematode specie/s present in the field, host range, and the duration of time pest nematode can survive in the field in the absence of the host (Halbrendt and LaMondia 2004). In general, for specialized host-specific plant-parasitic nematode (such as root-knot and cyst nematodes species) selection of non-host crop is relatively less difficult as compared to the nematode with a wider host range (such as root lesion nematode) (LaMondia 1999). Nevertheless, accurate identification of plant-parasitic nematode/s prevalent in the field would help in selecting a non-host crop and planning a long-term rotation with a focus on nematode management in organic farming.

Other cultural practices to prevent colonization and establishment of plant-parasitic nematodes such as sanitation, nematode-free vegetative-propagating materials, adjustment of planting time, and removal of host weeds are recommended for both organic and conventional agriculture. However, they are even more important for organic farming, because curative measures such as synthetic fumigant nematicides applications are restricted (Letourneau and van Bruggen 2006). Prophylactic measure such as nematode-free planting material, cleaning equipment, and quarantine measures would help in minimizing the chances of nematode entry into the field and further spread. Adjustment of planting date (early or late) to coincide with the conditions when the temperature is too low or too high for nematode infection and development has been shown to be effective method for nematode management in vegetable cropping systems (Bridge 1996). Soil solarization using transparent polyethylene sheets to trap solar heat is usually considered