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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