**2016 Annual Report, Multistate Project NE-1040**

**Title: Plant-Parasitic Nematode Management as a Component of Sustainable Soil Health Programs in Horticultural and Field Crop Production Systems**

**Period Covered: 10-01-2016 to 09-30-2016**

**Date of this report: 20 September 2017**

**Annual Meeting Dates: 06-07 October 2016**

**Accomplishments**

**Objective 1:** Develop effective and economically-viable cultural management tactics for plant-parasitic nematodes based on host resistance, nematode antagonistic rotation or cover crops, soil amendments and biological agents.

Cover crops such as Brassicacae (oil seed radish and mustard), oats and legumes are often used for reducing soil erosion, retaining soil nutrients, building organic matter, and/or managing plant-parasitic nematodes in Michigan agriculture. Our studies on cover crop use in field cropping systems showed little effect on root-lesion (*Pratylenchus* spp.), most wide spread nematode in Michigan agriculture. A similar study was conducted in carrot production systems at two locations where oats, ‘Defender’ radish, ‘Dwarf Essex’ rape, a mixture of oats and Defender radish were grown in fall 2014 preceding summer 2015 carrot production. A fallow served as a control. Nematode soil population densities were assessed during cover crop growth, before planting carrots, during carrot production, and at carrot harvest. At site 1, root-lesion and stunt (*Tylenchorhynchus* spp.) nematodes were present at low population densities (less than 25 nematodes/100 cm3 soil), but were not affected (ANOVA, *P* > 0.05) by cover crops. At site 2, root-lesion nematode population densities were increased (ANOVA, *P* < 0.05) by Defender radish compared to other cover crops during cover crop growth and carrot production. The low population densities of plant-parasitic nematode were not related to marketable carrot yield at either site. It is also possible that yield losses observed at sites may be due to other pathogens and/or physicochemical soil factors. However, the results show growing plant-parasitic nematode-susceptible cover crops can be detrimental for nematode management in the cropping systems.

In Michigan a 2016 carrot/root-lesion field trial was conducted to identify biological and chemical controls as a replacement for Vydate. Compared to the non-treated control, Nimitz, Melocon and Majestine increased the number of marketable carrots 38%, 37% and 38% respectively; whereas, Vydate resulted in a 47% increase in marketable carrots. An analysis of 60 Michigan soybean cyst nematode populations in regards to sources of SCN resistance indicated that 95% of the populations were aggressive, with 2%, 32%, and 6% identified as Type 1, Type 2 and Type 1.2, respectively. An econometric analysis showed that comparative projected sugar beet net profits per hectare were $420, -$568, $1,141 and $1,318 when the population density of beet cyst nematode (BCN) was below the action threshold with a BCN susceptible cultivar, above the action threshold with a BCN susceptible cultivar, above the action threshold with a BCN tolerant cultivar and above the action threshold with a tolerant cultivar and a seed treatment, respectively. Following fall soil fumigation, tart cherry trees planted the next spring in planting holes treated with starter compost had greater trunk cross section areas (TCSA), compared to trees planted with or without surface applied compost or straw mulch, or no treatment. The TCSA measurements were made at the end of the second growing season after planting. After two years of tree growth, Tart cherry trees planted in fumigated soil following two years of pearl millet/Essex rape or oats/peas/mustard had significamtly greater TCSA than those planted following red clover or fallow soil.

In 2016, a total of 90 private and public soybean cultivars were assayed for their resistance to SCN HG Type 7 (race 3) in the greenhouse (MN). A number of germplasm lines, most of which were in MG 000-II, were tested or retested for their resistance to multiple SCN populations, and a few lines were identified to be resistant to SCN race 1 (HG Type 2-) and/or race 14 (HG Type 1-). The germplasm line PI567516C was selected for breeding SCN-resistant soybean cultivars. Advanced breeding lines from this source of resistance were evaluated for their resistance to SCN populations in the greenhouse, and a few of them are in regional yield trials. Greenhouse and field experiments were initiated in 2015 and 2016 in MN to study the impact of pennycress and camelina as winter oil seed cover crops on the SCN in the corn-soybean production systems. The primary data from greenhouse study showed that SCN reproduced well on pennycress, while camelina is non-host of SCN.

In Connecticut we have previously had success in managing plant parasitic nematodes using rotation crops but in many cases multiple years or cycles of rotation may be required. Experiments are under way to utilize a series of lesion nematode-suppressive rotation crops in tilled or no-till systems to try to achieve multiple cycles of suppression within a single year.

**Rotation 1st crop** **Rotation 2nd crop** **Rotation 3rd crop**.

Oats and clover no till Soybeans no till Rye and vetch no till

Barley no till Buckwheat no till Winter wheat no till

Pacific Gold brassica tilled Sudangrass tilled Millet forage radish tilled

Black oats no till Millet and Rudbeckia no till Dwarf Essex brassica tilled

Field plots (6ft by 25 ft with 12 ft borders) and 3-ft diameter microplots were planted with each of the above rotation schemes (6 reps and 15 reps for each experiment). Soils were sampled for pre-plant *Pratylenchus* nematode densities. The first crop was planted March 30, 2016 and subsequently mowed on 24 June at which time Pacific Gold residues were tilled in. Soils were sampled for nematodes on 1 July and plots seeded with the second crop. The sudangrass was tilled in on 13 September and cover crops drilled in the same day. Nematode densities followed similar trends in both experiments after the first set of spring rotation crops. Lesion nematode numbers were lowest after Pacific Gold and black oat crops and higher after oats and clover or barley. Our sampling results demonstrated that using the Abawi soybean assay was more effective for recovering lesion nematodes than nematode extraction from soil.

Effect of rotation crop on lesion nematode populations, 2016 – ========================================================

**Rotation 1st crop** **Pf/Pi Field plot Pf/Pi Microplots**

Oats and clover no till 0.77 10.3 BC

Barley no till 0.92 19.9 C

Pacific Gold brassica tilled 0.66 2.9 AB

Black oats no till 0.72 2.7 A

P = 0.14 0.01

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Trap crops are being developed for nonchemical control of cyst nematodes. A solanaceous weed, *Solanum* *sisymbriifolium* (sticky nightshade or Litchi tomato) is being evaluated to control potato cyst nematodes *Globodera pallida*. Because of the difficulties in working with this regulated pathogen, we conducted experiments with the closely related tobacco cyst nematode *G. tabacum* as a model system. Experiments were conducted to evaluate *S.* *sisymbriifolium* for ability to stimulate hatch of *G. tabacum* in comparison to a susceptible or resistant host plant, for ability of the nematode to reproduce and increase, and for efficacy against the nematode as a trap crop under field conditions in comparison to plant resistance*.*

Also in CT, twenty cysts per cell were exposed to full strength or 1 to 10 dilutions of root diffusates of tobacco, sticky nightshade or distilled water and hatch counted weekly for 8 weeks. Cysts were crushed after 8 weeks to determine the unhatched J2 in eggs remaining and the percent of juveniles that hatched. Hatch of nematodes was significantly higher for *S.* *sisymbriifolium* than from tobacco or water (Table 2). We inoculated tobacco or *S.* *sisymbriifolium* with *G. tabacum* and stained roots over time. Juveniles infected both plants but development to adult females with eggs only occurred in tobacco. Juveniles developed to males in Litchi tomato but no adult females were observed. We are currently evaluating the effects of *S.* *sisymbriifolium* as a trap crop in field microplots compared to resistant or susceptible tobacco crops. Tobacco cyst nematode- resistant tobacco lines are also being evaluated for host status and hatch stimulation of *G. pallida* in Idaho.

Table 2. Hatch of *Globodera tabacum* after 8 weeks exposure to root diffusates of *S.* *sisymbriifolium* or *N. tabacum* in comparison to water.

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Diffusate source Dilution Hatched juveniles % Hatch

*S.* *sisymbriifolium* 1:1 779 c 35.8 b

*S.* *sisymbriifolium* 1:10 308 bc 16.7 b

*N. tabacum*  1:1 171 ab 5.0 a

*N. tabacum* 1:10 121 a 5.5 a

Distilled water control114 a 5.1 a

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The influence of broadleaf cigar wrapper tobacco (*Nicotiana tabacum*), eastern black nightshade (*Solanum ptychanthum*), and Litchi tomato (*Solanum sisymbriifolium*) on egg hatch and subsequent development of the tobacco cyst nematode, *Globodera tabacum*, was investigated in CT. Our previous research indicated that eastern black nightshade was a more efficient host of *G. tabacum* than *N. tabacum,* as *S. ptychanthum* stimulated more hatch and resulted in greater *G. tabacum* reproduction than did *N. tabacum.* Root diffusates were prepared from 2 g of root of four-week-old plants soaked in 100 ml of distilled water for 2.5 hours, filtered and frozen. *S. ptychanthum* root diffusates stimulated juvenile hatching from eggs in cysts over 4 weeks more than root diffusates of *S. sisymbriifolium* or *N. tabacum* (Table 3). Tobacco increased hatch by four times compared to water alone. *S. sisymbriifolium* stimulated twice and *S. ptychanthum* three times the hatch of that for *N.* *tabacum*. *G. tabacum* juveniles were observed in stained roots of both *N.* *tabacum* and *S. sisymbriifolium* and development to adult females occurred within four weeks in tobacco but not *S. sisymbriifolium*. Cysts were extracted from roots and soil in pots that had been planted to *N.* *tabacum* or *S. sisymbriifolium* for 12 weeks and cysts crushed to count encysted juveniles. Final population densities were 324 *G. tabacum* J2 per 100 cm3 soil after *N. tabacum* and 4.5 *G. tabacum* J2 per 100 cm3 soil for *S. sisymbriifolium*. Litchi tomato, *Solanum sisymbriifolium*, stimulates cyst nematode hatch better than *N. tabacum* but unlike eastern black nightshade, does not allow significant reproduction in roots, indicating that it may be an effective trap crop for management of *G. tabacum* and possibly other cyst nematodes. Our results suggest that *G. tabacum* may be useful as a substitute model for the quarantined pathogen *Globodera pallida* for trap cropping with *S. sisymbriifolium* under field conditions.

Table 3. Hatch of *Globodera tabacum* after 4 weeks exposure to root diffusates of *S.* *sisymbriifolium, S. ptychanthum* or *N. tabacum* in comparison to water.

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Diffusate source Dilution Hatched J2/cyst

*S. ptychanthum* 1:1 158

*S. ptychanthum* 1:10 62

*S. ptychanthum* 1:100 15

*S.* *sisymbriifolium* 1:1 87

*S.* *sisymbriifolium* 1:10 68

*S.* *sisymbriifolium* 1:100 22

*N. tabacum* 1:1 50

*N. tabacum* 1:10 21

*N. tabacum* 1:100 9

Distilled water control6

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We evaluated the effects of growing *Globodera tabacum* resistant trap crops on nematode populations under field conditions in microplots. Sixty-five 1-m diameter plots infested with a range of *G. tabacum* populations were planted to susceptible 8212 shade tobacco, resistant B2 broadleaf tobacco, or *Solanum sisymbriifolium*. Nematode densities in soil were determined before planting and at the end of the season each year. Our microplot results from 2014 showed a final to initial population (Pf/Pi) ratio of 1.88 for cyst-nematode susceptible tobacco, 0.99 for B2 nematode-resistant tobacco, and 0.30 for Litchi tomato (P=-.000001). Typically, resistant tobacco results in a 0.6 ratio. We may be selecting for resistance-breaking *G. tabacum* nematodes as some microplots had the expected Pf/Pi ratios of 0.34 and others were over 2.0 with resistant plants.

Field microplot results for *S. sisymbriifolium* trap cropping in 2015 were even more effective; Pf/Pi ratios were 2.89 for cyst-nematode susceptible tobacco (almost a 3–fold increase), 0.38 for cyst nematode-resistant tobacco (typical of results seen over 10 years), and 0.14 for Litchi tomato (over 85% control, similar to fumigation) (P=-.000001). Over two years, our results demonstrate that Litchi tomato reduced cyst populations by 70 to 85% and were more effective than specific single-gene host plant resistance and comparable to what we might expect for soil fumigation. These field data correlate well with our previous laboratory and greenhouse work demonstrating that cyst nematode hatch is greater for *S. sisymbriifolium* than for resistant or susceptible host plants and that no reproduction occurs. Data from 2016 microplot experiments combining fungal biological controls with the different trap crop plants are currently being collected and analyzed.

Biological control fungi were evaluated for influence on tobacco cyst nematode hatch. *Plectosphaerella cucurerina*, *Trichoderma harzianum*, *Purpurieocillium lilacinum* and an *Arthrobotrys* sp. were directly exposed to cysts on water agar plates for 2 weeks and removed. There were no differences in the ratios of viable or nonviable eggs or total number of eggs in individually crushed cysts. Other cysts were exposed to hatching factors in root diffusates. All cysts exposed to fungi hatched more juveniles more quickly than the untreated cysts (Table 4). Batches of 25 cysts per pot were exposed to biocontrol fungi grown on oat seed and placed in soil subsequently planted to tobacco. After 8 weeks, cysts were removed and the numbers of viable or nonviable eggs in cysts were counted. Only *Purpurieocillium lilacinum* was different than the untreated control, and resulted significantly fewer (P=0.002) viable (21 vs. 100) and greater numbers of non-viable eggs per cyst (223 vs 69) (P=0.001). Our results demonstrating increased hatch in the presence of these fungi led to further experiments combining biocontrol with a *S. sisymbriifolium* trap crop as a possible means of increasing efficacy.

Table 4. Effect of biocontrol fungi exposure on *Globodera tabacum* hatch in root diffusates of *S. ptychanthum* and viablilty of eggs remaining in cysts.

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Treatment Hatch wk 1 Hatch wk 3 Remaining viable

*Arthrobotrys* sp.756 b 894 b 102

*Plectosphaerella cucurerina* 482 ab 683 ab 81

*Purpurieocillium lilacinum* 689 b 868 b 69

*Trichoderma harzianum* 622 b 761 ab 75

Control 181 a 541 a 108

P= 0.001 0.05 ns

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A field trial was conducted at the University of Hawaii Poamoho Experiment Station. Three pre-plant soil treatments 1) cover cropping with black oat (*Avena strigosa*) followed by no-till (NT) practice; 2) soil solarization (Sol), and 3) conventional till followed by bare ground (BG) fallow were installed for 3 months. ‘Hawaii Supersweet #10’ corn (*Zea mays*) was then planted. The trial will be periodically monitored for: 1) abundance of free-living nematodes as soil health indicators; 2) soil water properties (gravimetric soil moisture, water potential, and water infiltration rate); 3) relative abundance of entomopathogenic nematodes and fungi (using White trap larva baiting method and field cage method); 4) corn growth (corn height, chlorophyll content, and yields).

Azadirachtin in a 1.2% EC formulation was tested again this year but at 5x the labeled rate for turfgrass insects in MA. Azadirachtin was not phytotoxic at this rate, and there was no difference in nematode populations or turf quality between the treated and untreated plots. Seven golf greens from three different golf courses were chosen to evaluate the extent that *Hoplolaimus* and *Tylenchorhynchus* nematodes were infected by the bacterium *Pasteuria*. Several of these greens were evaluated similarly about 12 years ago. Comparisons and evaluations are still in progress. Several commercial nematicides, relatively new to the market, will be trialed in 2017, and evaluation of the effect of *Pasteuria* on turfgrass nematodes will continue.

**Objective 2:** Evaluate cultural management procedures for plant-parasitic nematodes in relation to their impacts on the sustainability of soil health: with special research to the utility of nematode community structure as an indicator of overall soil quality and their roles in plant nutrient cycling.

Michigan cover crop use in field cropping systems showed soil type-specific soil health outcomes. In the carrot production soils described above, nematode community analysis was done to gage the effects of cover crops on soil health conditions. At both sites, there were few short-term impacts of cover cropping on soil ecology based on the nematode community. At site 1, enrichment and structure indices were affected (ANOVA, *P* < 0.05) by cover crop treatments, but only at carrot harvest. Enrichment index was greater after oats-radish mixture or Dwarf Essex rape than oats alone or fallow control. Structure index was greater after radish alone or Dwarf Essex rape than oats alone. At site 2, bacterivore densities were increased by oats or radish cover crops compared to control, but only during carrot production. Overall, the variable effects of cover crops and other agronomic practices such as tillage and soil nutrient amendment use on soil health strongly point to location-specific application than a one-size-fits-all approach to get the best outcome.

In Tennessee a meta-analysis project was developed to assess the influence of agricultural intensification and urbanization on nematode genus and trophic diversity compared to forest and prairie ecosystems through analysis of published literature. Meta-analysis was conducted to compare the diversity and abundance of nematode communities according to trophic and colonizer-persister (CP) groups among urban, agricultural, and forest ecosystems. A total of 539 relevant articles were found by using a sequence of different search terms. Our results indicate that nematode genus diversity in omnivores, predators, plant feeders, fungivores, and bacterivores per 100g of soil is higher in forest ecosystems compared to agricultural and urban ecosystems. Similarly, nematode genus diversity in CP 2, CP 3, CP 4, and CP 5 categories is higher in forest ecosystems compared to agricultural and urban ecosystems. In contrast, total nematode abundance was significantly higher in agricultural ecosystems than in forest and urban ecosystems because of higher abundance of lower trophic and CP groups, indicating disturbance of the soil food web. Agricultural intensification and urbanization apparently negatively impact nematode community diversity that is critical for the maintenance of soil ecosystem services and resilience.

Four Michigan tart cherry enterprises each identified a highly productive and one with a history of poor productively for a soil health analysis. Soil samples were taken from each of the eight orchards an analyzed for fourteen soil health indicators. Three of them, water-stable aggregates, nitrogen mineralization and active carbon were positively correlated with tart cherry productivity.

Field plots of long-term corn-soybean crop sequences were established in 1982 in Minnesota, USA: (i) five-year rotation of each crop such that both crops are in years 1, 2, 3, 4, and 5 of monoculture every year; (ii) annual rotation of each crop with both crops planted each year; (iii) continuous monoculture of each crop. Samples of bulk soil, rhizosphere soil, rhizoplane soil, crop roots, and SCN cysts were collected in 2014-2016 to study crop sequences effect on fungal and bacterial communities associated with SCN with cultural methods as well as metagenomic analysis. Preliminary analysis of the data showed that crop, sequence of crops, and samples (soil, roots, and cysts) affected fungal community.

Persistence of enteric pathogens in manure-amended soils in northeast U.S. produce-growing environments was investigated in year 2 of a 3-year study. All plots were planted to spinach in late September to mimic Vermont farmer practice. Soil samples were collected 0, 1, 3, 7, 14, 28, 56, 128, 256 days after inoculation. There was negligible *E. coli* survival by 80 days. Decline of *E coli* was faster at surfaces of untilled plots than at 10 cm depth in tilled soils. Survival was not affected by soil type differences in the two field sites. Poultry manure compost was added to the worst-case scenario treatment #3 (manure + *E. coli*) to see if it was possible to revive *E. coli* applied the previous fall. No appreciable rebound of *E. coli* occurred, indicating that over-wintering in Vermont was sufficient to insure no carryover of *E. coli* applications the prior fall. These data will be used to inform the Food Safety and Modernization Act. In 2015, we observed that wet field soils and those amended with poultry-manure based compost were conducive to *E. coli* survival in soil. Unsaturated soils and dairy-manure based compost favored indigenous soil microbes and suppressed *E. coli* survival. Soil microbe dynamics were measured using functional ecoenzyme assays of basic polymers in soil before and after irrigation/rain events. Two experiments were performed, one in a hoophouse and one in a field experiment. Treatments included poultry and dairy manure-based compost to manipulate soil microbial community composition. All compost amendment rates were adjusted to deliver 100 lb N/acre, calculated for growing spinach. Overall, both sites exhibited an increased β-glucosidase activity under dry conditions that declined upon wetting. This expression was independent of poultry or dairy treatment. The expression of other ecoenzymes varied by experiment sites that differed in land use history. The high tunnel would not experience seasonal rainfall and was relatively free of weeds while the field site was converted pasture with heavy weed pressure. The primary difference between experiments was the expression of amino peptidases, represented by leucine amino peptidase. The field site express greater amounts under dry conditions while the hoophouse was opposite. This difference may be explained by nitrogen mineralization that could result from field versus hoophouse conditions. Although unanticipated, we measured a strong urease activity response to changes in soil moisture.

**Objective 3:** Provide educational materials and programs on cultural management of plant-parasitic nematodes based on host resistance, nematode antagonistic rotation or cover crops, soil amendments and biological agents.

We continue to engage stakeholders and grower communities about nematodes using popular outlets (<http://msue.anr.msu.edu/news/plant_parasitic_and_beneficial_nematode_distribution>). Despite a substantial body of knowledge pointing to the need for location-specific than a one-size-fits-all approach to get the best soil health outcome using agronomic practices, significant hurdles remain.

**Impacts:**

Several of the studies reported here will increase our knowledge of long-term agricultural practices on soil biological activities and crop productivities, which will help develop long-term effective strategies for management of plant-parasitic nematodes in the soybean-corn production system in the Midwest. Identification and development of new SCN resistance is critical for successful management of SCN in long-term.

The identification and use of biological controls and rotation crops that reduce plant parasitic nematode populations will assist in the development of effective nonchemical management.

Differences in reaction to Pasteuria isolates and resistance genes in pepper may be used to differentiate races of Meloidogyne hapla.

*Globodera tabacum* may be useful as a substitute model for the quarantined pathogen *Globodera pallida* for trap cropping with *S. sisymbriifolium* under field conditions.

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