RESEARCH PROGRAM - FINAL PERFORMANCE REPORT
PLEASE COMPLETE AND EMAIL REPORT TO:
MDARD-CRAFTBEV@MICHIGAN.GOV

The completed Final Performance Report will be posted to the AMS website.

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of the project within the grant agreement.

PROJECT INFORMATION

Project Title	*Finding Solutions to Manage Plant-Parasitic pests of Michigan Hopyards*			
Recipient Organization Name:	Michigan State University			
Period of Performance:	**Start Date:**	4/1/2020	**End Date:**	12/31/2021
Recipient's Project Contact				
Name:	Marisol Quintanilla-Tornel			
Phone:	517-881-3740			
Email:	marisol@msu.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Provide enough information for the reader to understand the importance or context of the project. This section may draw from the background and justification contained in the approved project proposal.

Worldwide, plant-parasitic nematodes are estimated to cause a loss of $157 billion annually (Singh et al., 2015). In a 1987 study conducted by the Society of Nematologists Crop Loss Assessment Committee, Michigan and South Carolina were the most consistent in reporting yield loss due to plant-parasitic nematodes for the widest range of crops. Despite this, yield loss on hop from plant-parasitic nematodes within the United States remains widely unstudied and undetermined. The lack of United States literature on plant-parasitic nematodes of hop highlights the importance for studies on this specialty crop. Michigan Hop growers often suffer from intense viral and fungal infections, insect and mite pest threats, and likely, plant-parasitic nematodes. These various pressures have been negatively impacting the Michigan craft beverage industry and severely prohibiting growers from receiving yields similar to the state of Washington. For these reasons, growers need to know how to best control plant-

parasitic nematodes. We believe growers should know to what extent plant-parasitic nematodes are influencing yield loss and how best to control these harmful pests. Using a multi-disciplinary approach, our research team scratches the surface of plant-parasitic nematode hop research (partnered with important viral and yield data) to uncover what nematodes should be managed, and potential ways of how to manage them.

ACTIVITIES PERFORMED

Objectives:
1) To establish a greenhouse trial to observe the above and below ground symptoms of 3 plant-parasitic nematodes on bine growth, leaf size, root weight, cone number and cone size. Nematodes tested will include: hop cyst, root lesion, root-knot, and dagger.
(2) To use molecular techniques to document the presence of dagger nematode species and subspecies within Michigan Hopyards.
(3) To establish a multi-year field trial to determine best potential methods of control for plant-parasitic nematodes impacting Michigan hopyards.

Objective 1: Greenhouse trial
Due to challenges listed below, there was a delay in establishing the greenhouse trial comparing damage of northern root-knot nematode (*Meloidogyne hapla*), root lesion nematode (*Pratylenchus penetrans),* hop cyst nematode (*Heterodera humuli*), and dagger (*Xiphinema americanum*). We found that hop cyst nematode infestations of young rhizomes reduces bine height by 40.4% at relatively low concentrations (10 cysts/100cm3 soil).

Objective 2: Molecular identification
To identify and document the *Xiphinema* species present in the field trial, we sequenced the mitochondrial marker cytochrome c oxidase subunit 1 (coxI) from *Xiphinema* spp. within our collected field samples. We successfully identified dagger nematodes as *Xiphinema americanum* and root lesion nematodes present in the study as *Pratylenchus penetrans*. This information will aid in establishing future trials to understand how these species interact with, parasitize, and damage hop plants.

Objective 3: Field Trial Year 1
A complete randomized block design field trial was established in Kent Co., Michigan on an 8-year-old Centennial hop yard (i) to determine the seasonal changes of plant-parasitic nematode populations and (ii) to evaluate the efficacies of several manure-based composts and one nematicide at nematode reduction. Each plot consisted of three rows wide by five plants long. Within each row, three of the fifteen plants were randomly selected to represent the plot using a random number generator. Plots were applied with either: poultry manure (pelletized, Herbrucks), poultry manure (Morgan Composting), Layer Ash Blend (Morgan Composting), Velum (Bayer), or not treated. Each treatment had five replicates, and rates were scaled down to plot size and applied in early summer. Soil sample cores were taken from the 75 sampling three points in the season: Pre-treatment, 30-days post-treatment, and at harvest. The Cobb sucrose floatation method was used for extraction of nematodes within the soil, and a scored petri dish was used for counting extracted nematodes. Nematodes were identified to genera. At harvest, one plant was randomly selected from each plot and harvested from. Harvest weight from the five plot replicates were averaged and scaled up to represent tons per acre. Once harvest data was collected, an ANOVA was used to quantify differences between initial and post-treated plant-parasitic nematode populations, and to quantify yield by treatment.

To complement these analyses, we additionally evaluated the extent of virus and viroid infections within the hop plants in the study. Globally, viruses of concern in hops include nematode-transmitted species such as *Arabis mosaic virus,* and *Tomato ringspot virus*, as well as *Apple mosaic virus* (Genus *Ilarvirus*), *Carlaviruses* (*Hop latent virus, American hop latent virus*, *Hop mosaic virus*); co-infections are frequent. We used next-generation sequencing to deep-sequence the experimental hop material to thoroughly assess the composition of the plant-associated virome and to characterize the genomic signature of any viruses detected. We prepared two libraries with bulked leaf samples from each treatment, along with a series of controls. We used ultra-centrifugation and filtration to concentrate virion-associated nucleic acids (VANA approach; Candresse et al., 2014) and extracted total RNA. After quality control, samples were submitted to MSU RTS for library preparation and Illumina sequencing (2 x 150 bp paired end). We used CLC Genomics WorkBench to QC reads, trim adaptors, and assemble contigs. Reads were also mapped to reference genomes of known hop viruses and viroids.

Results – Yield and Nematode Reduction

All treated H. lupulus plants yielded higher hop cone weights (Figure 1) than untreated control plants. However, only the pelletized poultry manure treatment ('Poultry2') significantly increased hop bine yield ($P<0.1$; Tukey HSD).

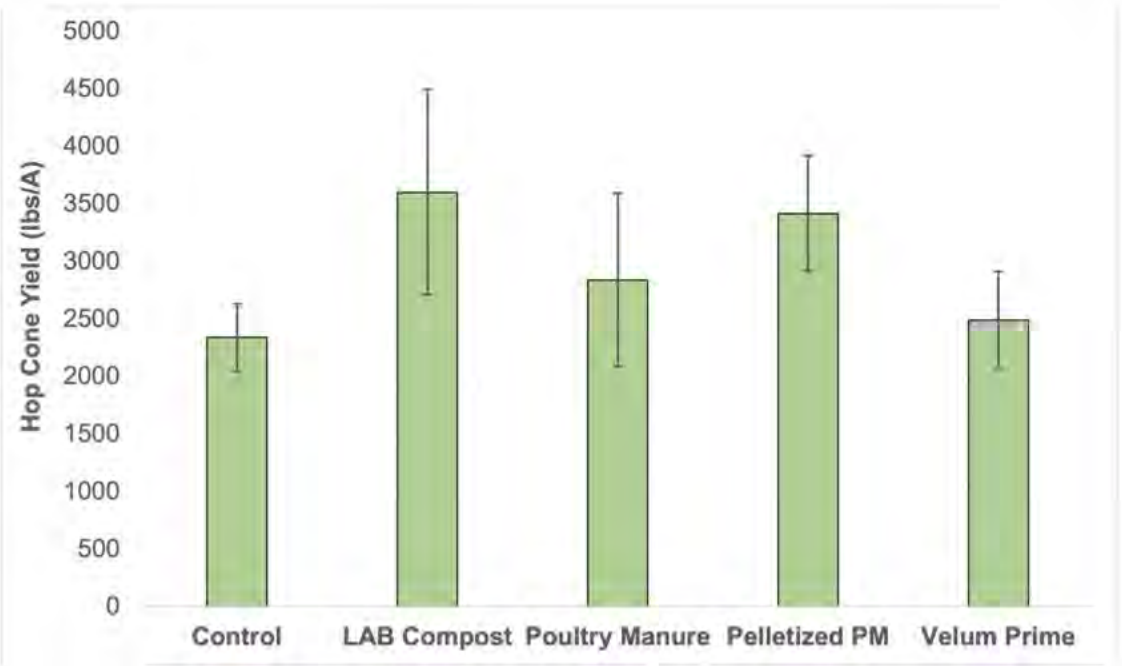


Fig. 1. Hop cone yield on average by treatment of the 2020 field trial in Kent Co. Standard Error bars represent standard error of the mean.

When total plant-parasitic nematodes were grouped together, there were no significant differences between treated and untreated plots between initial and final dates. However, there is a notable trend of total plant-parasitic nematodes increasing over time within control plots. Layer Ash Blend significantly reduced total plant-parasitic nematode populations from initial to midway

(P<0.1, Tukey HSD). When plant-parasitic nematodes were separated by genera, all treatments significantly reduced root lesion nematodes (P<0.1; Tukey HSD), and no treatments reduced Stunt nematode populations.

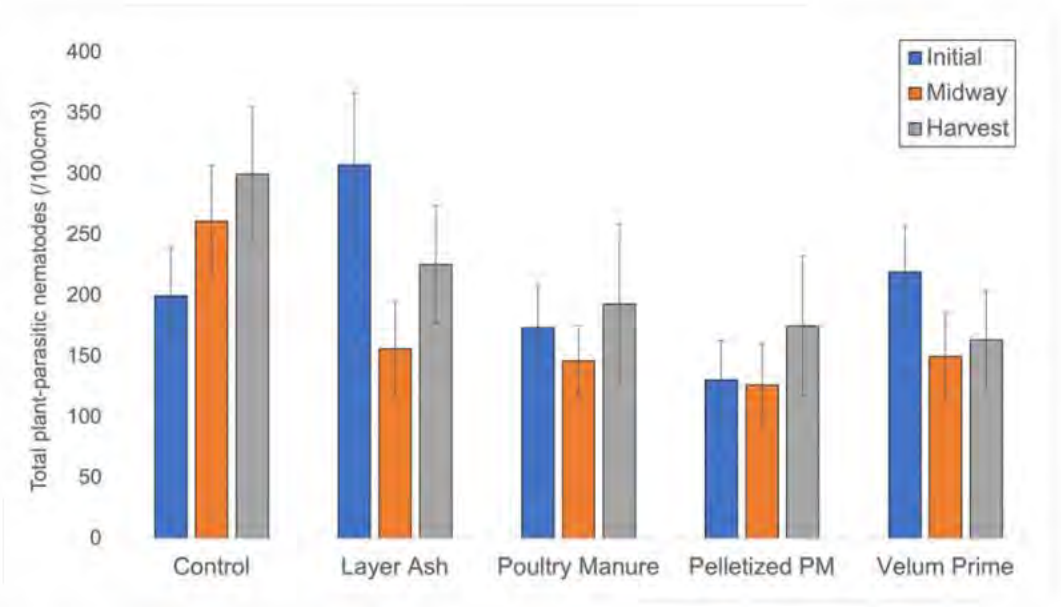


Fig. 2. Total average plant-parasitic nematode populations in treated and untreated plots thorough out the growing season. Standard error bars indicate standard error of the mean.

In conclusion, Pelletized poultry manure (Herbruck's Poultry Farm) significantly reduced root lesion nematode populations and increased scaled hop cone yield. Layer Ash Blend (Morgan Composting) significantly reduced total plant-parasitic nematode populations from initial to midway sampling. Trial was repeated the following year, in 2021, to account for nutrient bonuses in compost/manure added plots and see if reduction trends were consistent.

Results – Virus presence and interactions

Our deep-sequencing analysis found no evidence of the nematode-transmitted virus ArMV within the treatment plants. The severe pathogen Hop stunt viroid was also not detected. However, two common hop-infecting carlaviruses (HpLV and HpMV) were widespread in the study field (Figure 3). AmHpLV, ApMV, and Hop latent viroid were present as well but at lower levels.

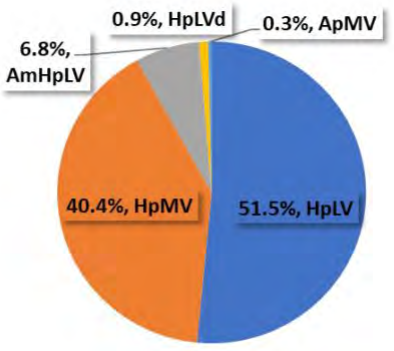


Figure 3. Study-wide species distribution of reads mapped to virus and viral reference genomes.

Virus and viroid loads were variable throughout the field, with no statistically significant difference evident among nematode treatments (Figure 4). However, there was an intriguing

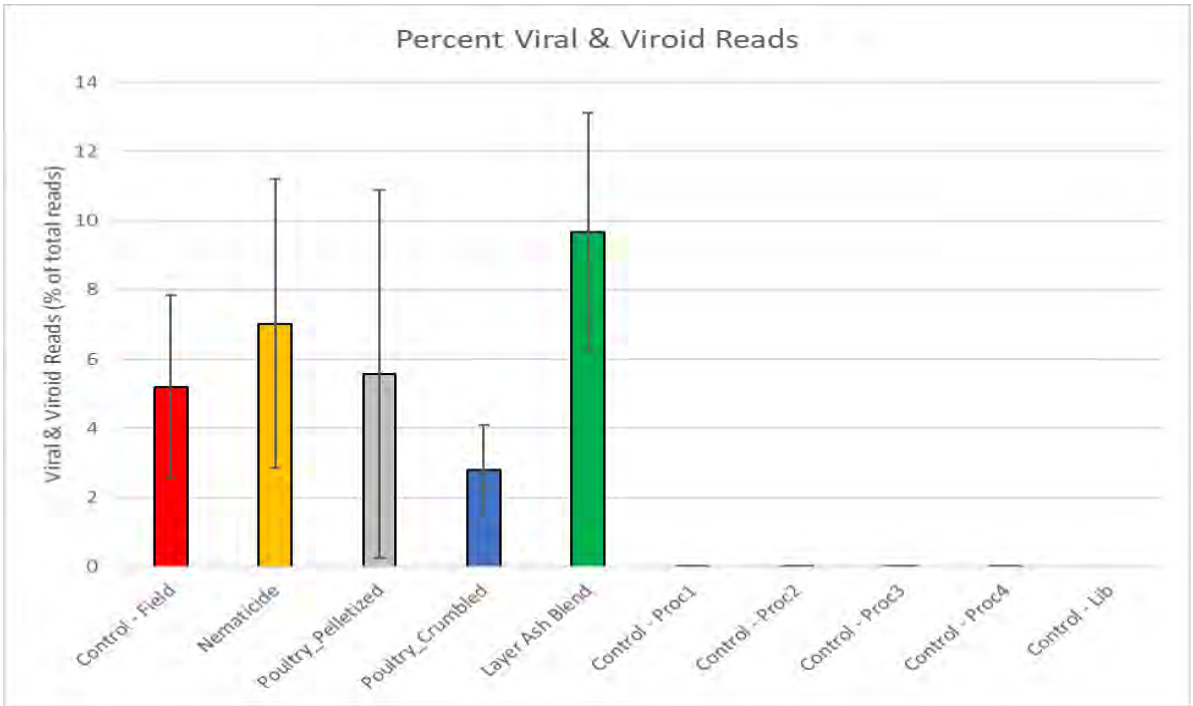


Figure 4. Viral and viroid reads (as percentage of total reads) by nematode treatment. Mean and standard error (N = 2 libraries per treatment). The process and library controls show minimal contamination.

non-significant tendency for viral load values to be higher in the ash treatment. Ash treatments may be high in P, which in some circumstances can benefit viral infections. This finding suggests that indirect nutrient-mediated effects of nematode-control treatments on viral disease may merit follow-up attention.

OBJECTIVES

Provide the approved project's objectives from your approved proposal/grant agreement.

#	Objective	Completed?	
		Yes	No*
1	To establish a greenhouse trial to observe the above and below ground symptoms of 3 plant-parasitic nematodes on bine growth, leaf size, root weight, cone number and cone size. Nematodes tested will include: hop cyst, root lesion, root-knot, and dagger.	Yes	
2	To use molecular techniques to document the presence of dagger nematode species and subspecies within Michigan Hopyards.	Yes	

3	To establish a multi-year field trial to determine best potential methods of control for plant-parasitic nematodes impacting Michigan hopyards.	Yes	
4			

**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

ACCOMPLISHMENTS

List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	We were able to set up two separate trials to investigate if hop cyst, root lesion, and root-knot nematodes significantly hindered plant growth.	Objective 1. To establish a greenhouse trial to observe the above and below ground symptoms of 3 plant-parasitic nematodes on bine growth, leaf size, root weight, cone number and cone size. Nematodes tested will include: hop cyst, root lesion, root-knot, and dagger
2	Molecular identification of *Xiphinema* (dagger) nematodes was successful and identified nematodes to the species *X. americanum.*	Objective 2. To use molecular techniques to document the presence of dagger nematode species and subspecies within Michigan Hopyards.
3	Year one of this trial was completed in 2020. The nematicide Velum Prime, and three types of composted manure were elevated for their impacts on plant-parasitic nematode populations and beneficial nematode populations, viral testing was preformed, and plot associated yields were collected. Our findings suggest that one compost, Pelletized Poultry Manure (Herbruck's Poultry Farm) showed potential for root lesion nematode management.	Objective 3. To establish a multi-year field trial to determine best potential methods of control for plant-parasitic nematodes impacting Michigan hopyards.
4	Deep-sequencing of virus and viroid infections in hop plants within this study was completed. Virus and viroid infection was widespread but did not significantly vary with nematode-treatment. Two carlaviruses (HpMV and HpLV) were dominant.The nematode-transmitted virus of concern (ArMV) was not detected. There was a non-significant suggestion that ash treatment	Objective 3. As part of Obj. 3 above, we evaluated the extent of nematode-transmitted and other viruses.

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	might have potential to increase viral load, perhaps due to higher P levels.	

CHALLENGES AND DEVELOPMENTS

Provide any challenges to the completion of your project or any positive developments outside of the project's original intent that you experienced during this project. Also, provide the corrective actions you took to address these issues. If you did not attain an approved objectives, outcome(s), and/or indicator(s), provide an explanation in the Corrective Actions column.

#	Challenge or Development	Corrective Action or Project Change
1	During 2020, the COVID-19 pandemic hindered many aspects of research for labs at Michigan State Universities. This did lead to delays for the greenhouse trial, however ultimately this trial was able to be completed.	Greenhouse trial was delayed, but successfully conducted.
2	Field trial – Viral testing	An acute shortage of reagents for virus work developed due to the enormous need for materials for coronavirus testing during the pandemic. Thus, key components for our laboratory processing, including RNA extraction kits and filters, are on extended back order, with expected delivery dates were quite a way out. These shortages delayed this project, as well as our other virus work, and log-jamming lab processing. Ultimately this was successfully completed.
3		
4		

LESSONS LEARNED

Provide recommendations or advice that others may use to improve their performance in implementing similar projects.

During year one of the field trial, we found that Pelletized Poultry Manure was a potentially viable option for control of root lesion nematodes, that corresponded with higher yields. If this can be repeated, this would be a cost-effective approach for growers with additional soil health benefits. For molecular identification, we found optimal primers and conditions for effective identification. We plan to share these parameters with the MSU Diagnostic Laboratory to eventually create efficient and cost-effective protocols for positive identification for plant-parasitic nematodes.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results.

We received funding from the Michigan Craft Beverage Council to continue this project an additional year. In 2021, a field survey of Michigan hopyards for plant-parasitic nematodes was conducted, alongside a repeated year of the nematode and viral efficacy trial on compost management. We are still processing year two data. Once completed, together our team will publish these findings in a relevant peer-reviewed journal, followed by publishing several extension articles for Michigan hop growers.

Dissemination of results:
Darling, E., Nakasato, K., Malmstrom, C., Chung, H., and Quintanilla-Tornel, M. Michigan's Great Beer State Conference. Acme, MI. 12-14 Jan 2022. Oral presentation.

Darling, E. and Quintanilla, M. Great Lakes Hop Working Group. 29 March 2021. Oral presentation.

Darling, E; Chung, H.; Quintanilla-Tornel, M. Impact of manure-based soil amendments on plant-parasitic nematode taxa associated with hop (cv. Centennial). Society of Nematologists Virtual Conference 2020. 15-16 December 2020. Poster.

Darling, E; Cole, E; Quintanilla-Tornel, M. "Evaluation of the Hop Cyst Nematode, *Heterodera humuli*, on Hop (cv. Centennial)". Plant Health 2020: American Phytopathological Society annual meeting. Poster. 3-14 August 2020.

Darling, E. and Quintanilla-Tornel, M. "Plant-Parasitic Nematodes in Michigan Hops". MSU Extension Hops Bine and Dine. Virtual Talk. 3 August 2020. https://mediaspace.msu.edu/media/t/1_b9taevwz

BENEFICIARIES

Number of project beneficiaries: Enter Number of Project Beneficiaries

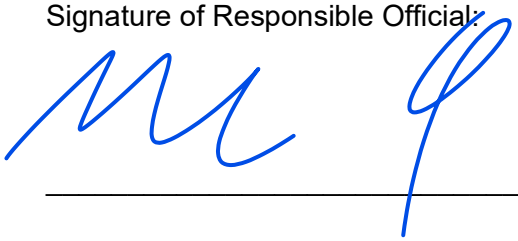
ADDITIONAL INFORMATION

Provide additional information available (i.e., publications, websites, photographs) that is not applicable to any of the prior sections.

The Authorized Individual must sign this statement after the applicable report form is completed.

I certify that the statements and information contained in these documents are true, accurate, and complete.

Signature of Responsible Official: Date:



1/14/2022