Final Technical Report

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Grant 21*1195 *Finding Solutions to Manage Plant-Parasitic Pests of Michigan Hopyards*

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Goals and Objectives

Plant-parasitic nematodes and viral pathogens are both considered to be understudied in comparison to prominent fungal pathogens impacting hops, however they pose a significant risk to perennial crop production. The plant-parasitic nematodes that elicit damage to hop plants and their distribution statewide is unclear in Michigan production. Our project goals were created to understand the plant-parasitic nematode genera prevalent in Michigan hopyards, and to determine if manure-based composted soil amendments would impact nematode populations, viral load, and cone yield. This multi-disciplinary project was achieved through the following specific objectives:

1) To establish a Michigan hop survey to determine distribution and incidence of plant-parasitic nematode pests. This objective aimed to conduct a soil survey of Michigan hopyards, in order to document and determine the plant-parasitic nematodes that may pose a risk to hop growth and development.

2) To establish a multi-year field trial to determine the best potential methods of control of plant-parasitic nematodes impacting Michigan hopyards. Two field trials were established in concurrent seasons, to determine how one nematicide and three composted manure blends would impact plant-parasitic nematode populations and viral load.

3) To report findings to Michigan hop growers, MSU Extension agents, and hop hobbyists. A key pillar of applied research should always involve collaborating with growers and extending research updates to them as the experiments progresses. We aimed to accomplish this goal by establishing good communication with stakeholders in a variety of mixed-media presentations throughout our project timeline.

Results, Conclusions and Outcomes

To address the primary objective, we conducted the first statewide soil survey for plant-parasitic nematodes of hopyards. In 2021, five unique soil samples were each collected from 28 hopyards of eight Michigan counties (Kent Co., Ottawa Co., Berrien Co., Van Buren Co., Grand Traverse Co., Leelanau Co., Presque Isle Co., Monroe Co.). Soil samples were then processed using the sugar centrifugal method to separate soil from nematodes, and nematodes were identified to genus using an inverted light microscope. We identified ten genera in Michigan hopyards: *Pratylenchus, Xiphinema, Heterodera, Helicotylenchus, Tylenchorhynchus, Paratylenchus, Meloidogyne, Criconema, Hoplolaimus,* and *Paratrichodorus* (Table 1). Root lesion nematodes, *Pratylenchus* spp., were the most prevalent plant-parasitic nematode genus, occurring in 96.4% of sampled hopyards. The hop cyst nematode, *Heterodera humuli,* was identified in 50% of Michigan hopyards. The average number of detected cysts per field sample was 21/100cm^3 of soil, with the highest recorded sample possessing 190 cysts. Five nematodes from each field was collected and cataloged with a unique identifier and placed under freezer conditions for long term storage.

Single nematode samples were identified via conventional PCR using primers D2A/D3B from the 28S region and sent to the Genomics Depot at MSU for sequencing. After matching successful sequences to NCBI-BLAST: Dagger nematodes were identified to the *Xiphinema americium* group, root lesion nematodes were identified to *Pratylenchus crenatus* and *Pratylenchus penetrans*, and cyst nematodes were identified to *Heterodera humuli*. Conducting this survey permitted us to identify plant-parasitic, bacterial, fungal, omnivorous, and predatory nematode communities commonly recovered from soil within Michigan hopyards. Most prominently, we recovered >1 full HCN cyst from 50% of sampled Michigan hopyards, which provides strong evidence that HCN could be posing a threat to hop yields of infested Michigan yards.

Our secondary objective was to conduct a multi-year field experiment to investigate how manure-based composted soil amendments and a nematicide, Velum® Prime, would influence plant-parasitic nematode communities, viral load, and hop cone yield. To achieve this, complete randomized block trials were established in the growing season of 2020 and 2021 on Centennial hopyards in Greenville, Michigan. Three plants within each plot were randomly selected and sampled throughout the season (N=75). Soil cores from each of the selected plants were sampled three times thorough out the season: Pre-treatment, 30-days post treatment, and harvest. For both years, soil treatments were applied in early June, and harvest occurred in mid-August. This allowed us to monitor how soil amendments impacted nematode ecology and viral load. Treatments tested were three blends of compost (one dairy manure based, two poultry manure based) provided by Morgan Composting, Herbruck's Poultry Farms, as well as one nematicide applied as a soil drench, Velum® Prime (active: fluopyram). In addition, one plot in each block was left as an untreated control for normal nematode population trends and comparison. To determine what viral pathogens are present in the field and treatment influence, leaf tissue and petiole samples from each of the selected plants were submitted to Dr. Carolyn Malmstrom's laboratory for molecular testing for evidence of hop-related viruses at harvest. This was done to ensure that each plant was monitored for nematode increase/decline throughout the season or unrelated to disease pressure. It also allowed for documentation of viral load in the field.

Nematode communities were influenced by treatments in comparison to untreated control plots. Most notably, the pelletized poultry manure soil amendment significantly reduced root-lesion and dagger nematodes, in comparison to control plots. Additionally, these plots had higher cone yield than non-applied control plots in both years, 2020 and 2021 ($P>0.05$). Deep-sequencing of hop tissue collected in 2020 and 2021 revealed no significant presence of nematode-transmitted viruses, a positive outcome. In both years, the three common hop-infecting carlaviruses (hop latent virus, American hop latent virus, and hop mosaic virus) were represented in all treatments, with lesser detection of apple mosaic virus. Most notably, in both years the proportion of viral reads detected was greatest in hop treated with layer ash blend. This finding suggests the layer ash blend treatment may not be optimal for hopyards with viral exposure or established viral infections. More investigation of the mechanism driving this response is needed.

Time Span

Grant period, 4/1/2021-8/1/2022; project activities carried out 6/1/21-8/1/22.

Work Accomplished/ Methods

We used the following protocols within this research:

- **Sugar-centrifugal soil processing** (Jenkins, 1964). To elucidate nematodes from soil samples to allow identification to genus-level under an inverted microscope.
- **Baermann pan technique**. To elucidate non-sedentary nematodes from root samples via soaking for 48 hours in distilled water and collecting solution post-soaking.
- **Conventional PCR**. To identify several economically relevant plant-parasitic nematodes to species via 28S region primers, D2A/D3B.
- **Deep-sequencing**. To identify virus and viroid presence in hop tissue samples, and to determine viral load of presence viral pathogens.

Communication Activities, Accomplishments and Impacts

Takeaways from this field trial, paired with our findings from the Michigan soil survey, leave our next clear pathway to focus on finding a management solution for the hop cyst nematode, *Heterodera humuli.* For hopyards currently battling high densities of root lesion and dagger nematodes, applying pelletized poultry manure may be a possible way to lower plant-parasitic nematode densities and see a small yield bump. As an additional benefit, viral analysis did not detect increased viral load with this treatment, in contrast to treatment with layer ash blend, in which viral reads were the greatest.

Findings from our experiments were presented orally at the Michigan Great Lake Beer State Conference, as well as the MSU Extension hop field day in 2022. Additionally, we have shared findings with Extension faculty and Great Lake State/Michigan hop growers, and plan to produce an extension article after publishing this data in a peer-reviewed journal. In 2021, our results from the first year of the field trial on plant-parasitic nematode data of hops were presented virtually at both the American Phytopathological Society's annual meeting and the Society of Nematologists' annual meeting, to receive feedback from other plant pathologists and nematologists.

Publications and Outreach:

Darling, E., Nakasato, K., Palmisano, A., Chung, H., Malmstrom, C. and Quintanilla-Tornel, M. 2023. Animal manure-based soil amendments impact yield, nematode communities, and viral load within Michigan hopyards. APS Phytobiomes. *In preparation.*

Lizzote, E., Sirrine, R., Miles, T., Chaudhari S., Quintanilla, M, Issacs, R. 2022. Michigan Hop Management Guide. Michigan State University Extension. https://www.canr.msu.edu/hops/uploads/files/Michigan+Hop+Management+Guide+2022.pdf

News and Media – Public Outreach:

1. Elisabeth Darling and Dr. Marisol Quintanilla interviewed on Michigan hop cyst infestations. https://greatlakesecho.org/2022/03/14/researchers-battle-tiny-menace-to-craft-beers/
2. Elisabeth Darling and Dr. Marisol Quintanilla interviewed on Michigan hop cyst infestations. http://www.keweenawreport.com/featured/michigans-hops-farms-could-be-in-trouble

Budget Narrative

This project was conducted consistent with the budget proposed by the principle investigator and approved by the State of Michigan. Project data and relevant findings were used to leverage further funding to investigate hop cyst nematode distribution and management in United States hop production, a partnership and collaboration with the Pacific Northwest.

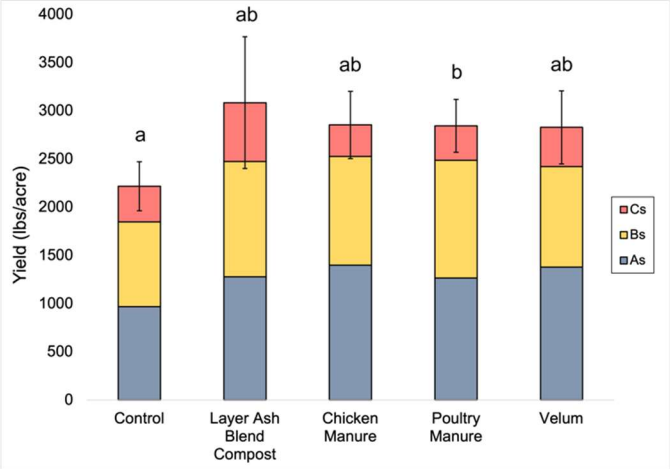

Figure 1. Hop cone yield results from untreated control plots, three soil amendments, and Velum Prime-applied plots (nematicide). Hop cones were graded at harvest due to clearly blighted bracts: A, indicating no disease pressure, B marginal damage, or C, unusable. After submission to MSU Plant Diagnostics Laboratory, cones were infested with *Diaporthe* sp. (Halo Blight) and *Pseudoperonospora humuli* (Hop Downy Mildew).

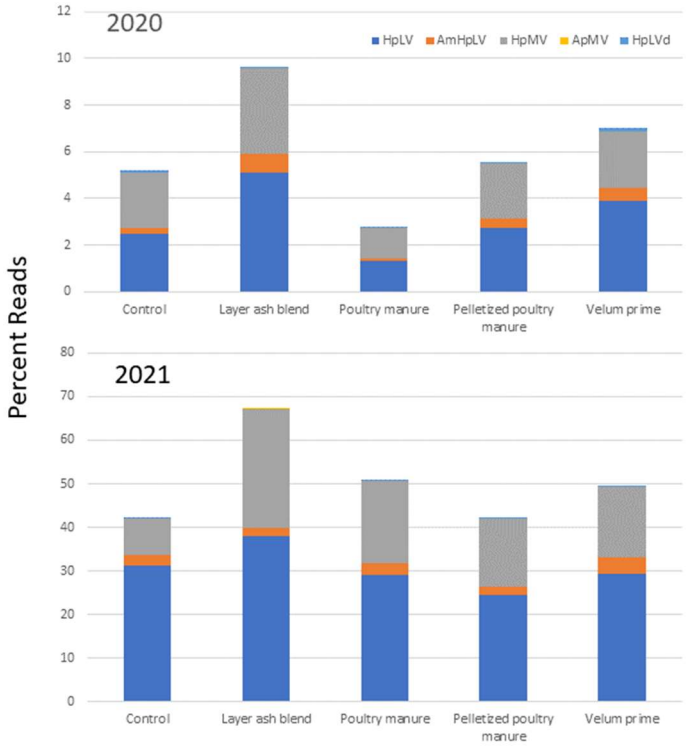

Figure 2. Relative virus detection among treatments sampled in 2020 and 2021, measured as percent reads per library or multiplexed sub-library. 2020 sample processing included a ribosomal RNA removal step that was not highly effective so read percentages overall are smaller, although total reads produced were greater.

Table 1. Plant-parasitic nematode genera identity and prevalence identified within Michigan hopyards (N=28).

Genus	Common Name	Average ± SEM per 100cm3 soil sample*	Percent of fields (%)
Pratylenchus	Root Lesion	5.1 ± 0.6	96.4
Tylenchorhynchus	Stunt	32.1 ± 5.3	89.3
Helicotylenchus	Spiral	59.7 ± 13.7	89.3
Xiphinema	Dagger	16.9 ± 2.8	85.7
Heterodera	Cyst	20.9 ± 4.0	50.0
Paratrichodorus	Stubby Root	2.1 ± 0.5	50.0
Paratylenchus	Pin	15.9 ± 5.5	32.1
Criconema	Ring	8.2 ± 3.4	32.1
Hoplolaimus	Lance	34.8 ± 8.2	14.3
Meloidogyne	Root Knot	16.9 ± 12.6	10.7

***=only fields that contained the corresponding genera were included in the calculated averages.**