Michigan Horticulture Fund FY2017 Final Report Aug. 14, 2018

Title: Root Knot Nematode Management in Ornamental *Hemerocallis* spp. (Daylilies) Bare Root Production.

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Team Members: Kristin Poley, Heidi Lindberg
Collaborator: Walter's Gardens

Objectives:

1. Evaluate the efficacy of new nematicide products and sustainable management practices, including the use of cover crops, manures, and soil solarization on root knot nematodes in daylilies.
    a. Hypothesis: A combination of sustainable management practices with application of new nematicides will provide acceptable nematode control, especially during the second and third year of daylily production without causing phytotoxicity. The number of galls on the bare-roots will be reduced, therefore increasing profit
2. Investigate modifications or alternate strategies to the hot water dip treatment for eliminating root knot nematodes on daylily plugs and adapt methods to different varieties.
    a. Hypothesis: Alternative techniques or modifications to the current hot water dip temperature and duration of dip will reduce plug mortality while still eliminating root knot nematodes from the roots.

Report for each Objective

Objective 1.

Methods:

A replicated field trial was established in second-year daylily plants, variety "Going Bananas". This field was treated with fumigation before daylily planting in year one as is standard in the industry. We treated plants within plots with four nematicides and a control. The products tested were Vydate, Velum Prime, Movento, and Nimitz. Nematicide applications were replicated five times.

Results:

Replicated initial (June) and mid-season (August) soil samples were taken within each treatment at our field plot. A final soil sample was taken at time of daylily harvest, which occurred in October. Nematodes were extracted and identified from each sample. Very few root knot nematodes were counted across all treatments during each sampling point, making it extremely difficult for us to detect any differences that may exist among treatments. To correct this for ongoing and future work in ornamentals and elsewhere, we have modified our methods to include root samples for staining at each sampling date. Root knot nematodes are sedentary endoparasites, which means that upon entering the root for feeding, females remain in place as they molt and produce eggs. Staining roots should give us a better indication of nematode

populations directly affecting roots in each treatment because it will allow us to count the number of females feeding on roots.

In addition to soil samples, plant growth data was collected during the mid-season soil sampling date (Figure 1). There were no differences in plant height among treatments. Plant height data was collected again at harvest and the data follows the same trend as the mid-season data with no differences in the treatments based on 95% confidence intervals (not pictured). It is standard in the bare-root ornamental industry to use root weight as a metric for plant growth and success of treatments. Therefore, at harvest, root weights were taken from one plant in each plot and compared (Figure 2).

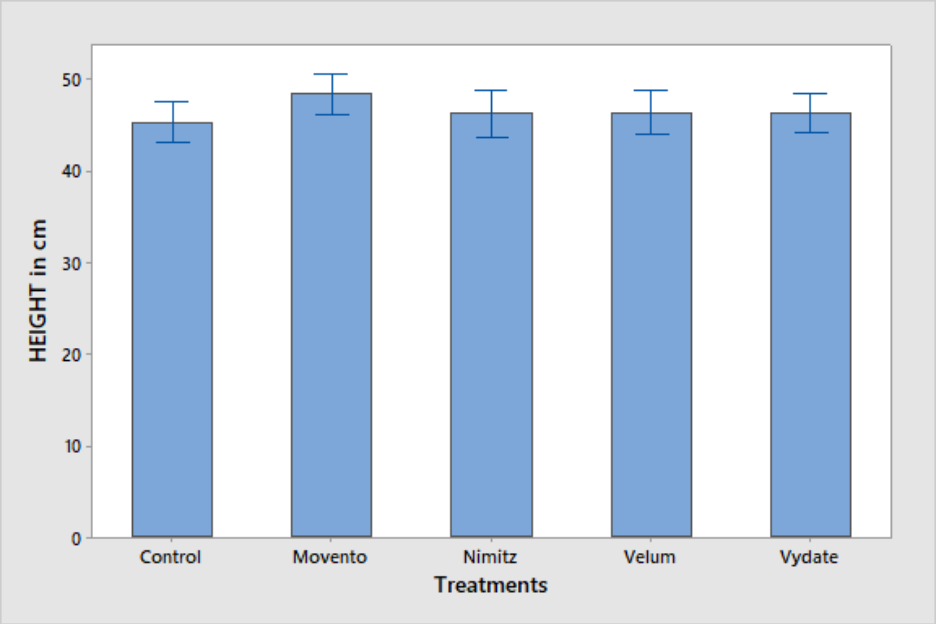

Figure 1. Comparison of mid-season daylily height after soil treatment with four different nematicides (Movento, Nimitz, Velum, and Vydate) and an untreated control. The error bars represent 95% confidence intervals of the mean. There was no significant difference between the treatments.

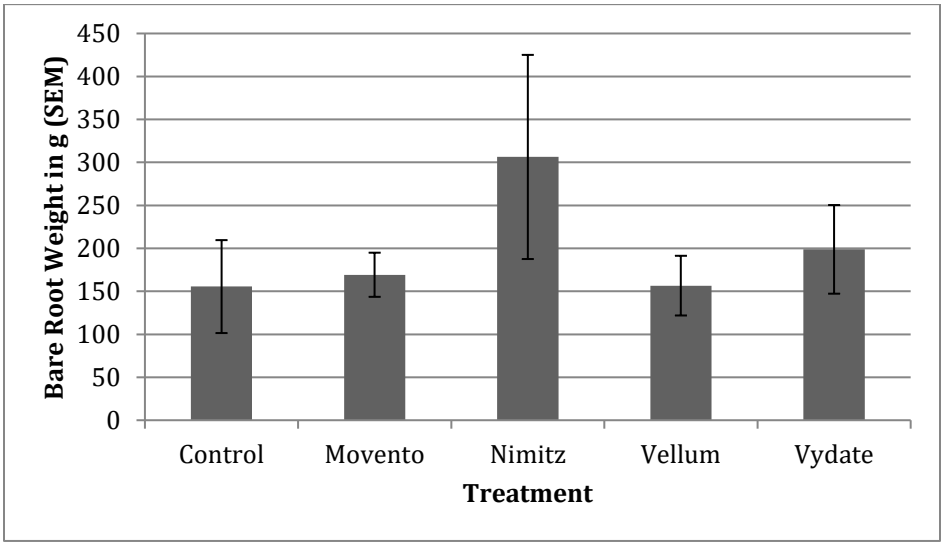

Figure 2. Comparison of bare-root weights at harvest for each treatment. Error bars represent standard error of the mean. Nimitz had a higher average root weight but a high variability in the data. All root weight averages are higher than the control treatment (the average weight of velum is larger than the control by 1g).

Conclusion:

This objective is complete. We were unable to accept our hypothesis due to the low number of nematodes detected per plot, but are continuing this work in an effort to establish best management practices in daylilies. Managers report that root knot nematode damage in daylilies is highest in the second and third years of production, but we were unable to detect enough root knot nematodes during this trial in second-year daylilies to make a confident nematicide management recommendation. This experiment is ongoing and is being repeated at a larger scale using modified methods. In 2018, a second-year daylily plot that was previously fumigated was treated with four nematicides and a control, including two of the same products tested in 2017 – Nimitz and Velum (labeled for use as indemnify). Soil and root samples, as well as plant data, are being taken as part of this effort.

Objective 2.

Methods:

Hemerocallis bare-roots were obtained from Walter's Gardens in June of 2017. The varieties tested were "Rose Katherine" and "Stolen Treasure". Roots were stored in a cooler at 35C for up to two weeks until use in hot water dip alternative treatments. Prior to testing, a small selection of roots were sampled and stained to determine initial root knot nematode infestation. Roots were then subjected to one of 12 treatments (Table 1) and a control.

After treatment, roots were planted in the MSU greenhouse using potting soil provided by the greenhouse. Potting soil did not contain plant-parasitic nematodes. Potted roots were randomly arranged within the greenhouse. Measurements were recorded from each plant weekly for eight weeks. Measurements included plant height, the number of leaves, the number of buds, and the number of flowers.

At the end of eight weeks, plants were removed from the pots, bare roots were weighed, and up to 1 gram of the smallest roots (roots where nematodes are capable of entering) were clipped and stored in petri dishes for root staining.

Table 1. The list of treatments for hot water dipping alternatives and the date the treatment was applied. Hot water dips #1-4 were variations on the standard hot water treatment (Standard HWD) typically employed by Walter's Gardens and were determined based on preliminary results and current literature. All other treatments were chosen based on a review of current literature.

	Treatment	**Date Applied**
1	Preliminary	6/20/2017
2	Control	6/26/2017
3	Standard HWD	6/23/2017
4	Hot Water Dip #1	6/26/2017
5	Hot Water Dip #2	6/26/2017
6	Hot Water Dip #3	6/27/2017
7	Hot Water Dip #4	6/27/2017
8	Bleach Dip	6/23/2017
9	Hot Water and Bleach Dip	6/28/2017
10	UltraViolet Light #1	6/28/2017
11	UltraViolet Light #2	6/28/2017
12	Vellum Prime	6/23/2017
13	Vydate	6/23/2017

Results:

The first treatment tested was a "preliminary" water dip to answer the question: At what time does the inside of the root heat to the media temperature? We found that at 30 minutes of submersion, roots were the same temperature as the water (Figure 3). This influenced the alternatives to the hot water dip we tested.

Treatments were compared only among plant varieties (Figures 4 and 5; Tables 2 and 3). Root staining revealed levels of nematode infestation inside the roots (Figure 6). Bare-root weight is the standard metric for evaluating daylilies. Each root was weighed before clipping for staining (Figure 7).

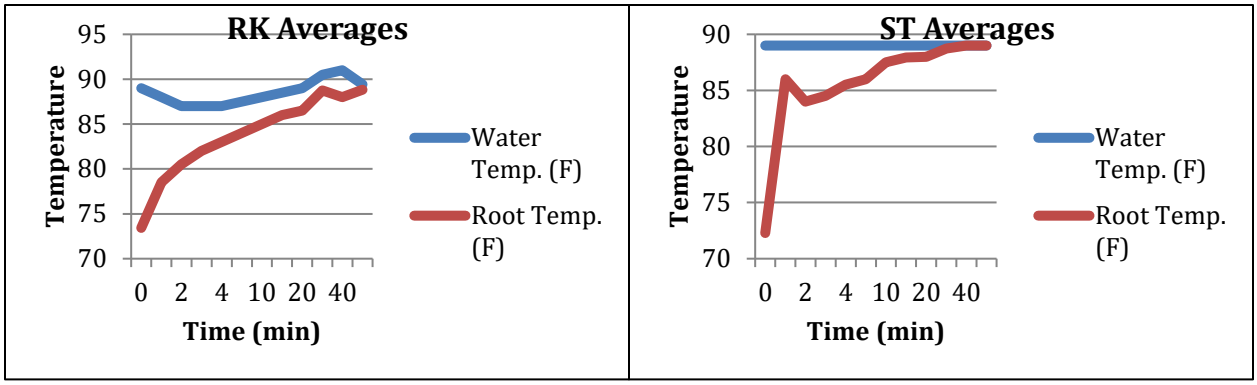

Figure 3. Preliminary hot water dipping results per variety (RK for Rose Katherine and ST for Stolen Treasure.) Preliminary results suggest that daylily roots will reach the same temperature as the water they are immersed in after 50 minutes. These results influenced our variations on the standard hot water dipping method treatments (treatments 4-7 listed in Table 1 above).

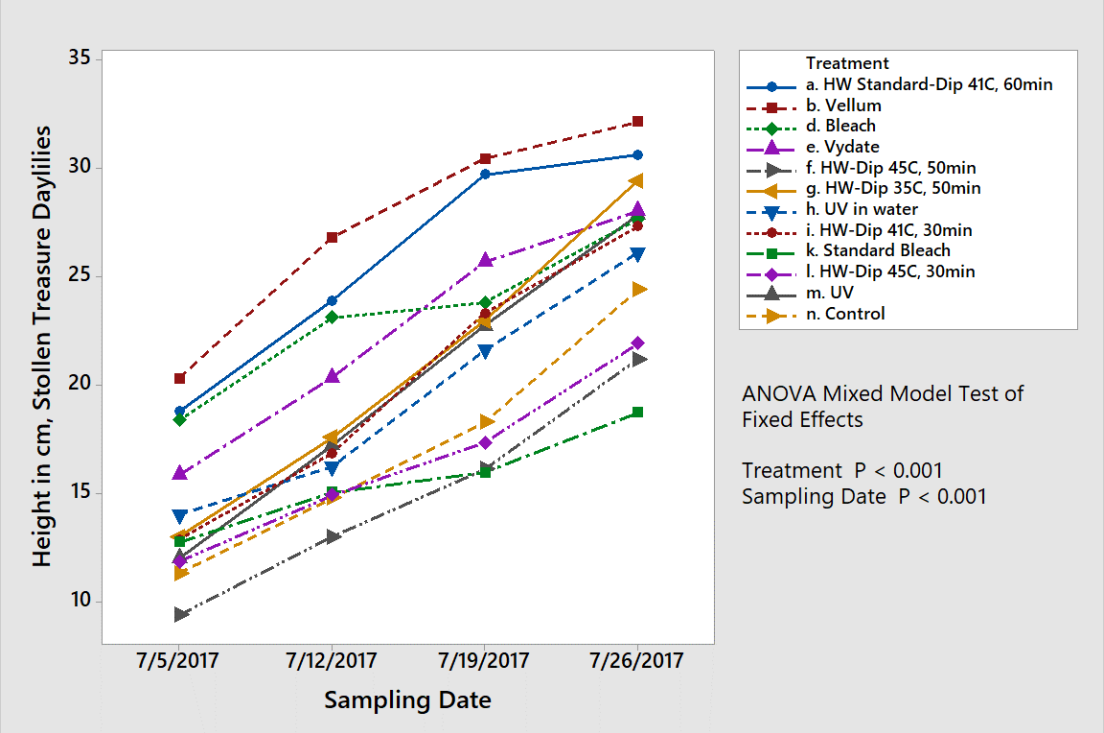

Figure 4a. A comparison of "Stolen Treasure" daylily plant heights across the first four sampling dates. See Figure 4b for graph of complete experimental period. Prior to planting, root-knot nematode infested bare-root daylilies were treated (dipped) with different nematode control strategies. Plants treated with Velum were significantly taller than all other treatments. Standard hot water (HW) dip, Vydate, and Bleach Dip, significantly increased plant growth compared to other treatments. The hot water dips of 45 C, effectively reduced nematodes, but the high temperatures also reduced plant growth. Three treatments (hot water dip at 45C for 30 minutes and 50 minutes and the standard hot water treatment plus bleach) exhibited less plant growth than untreated controls.

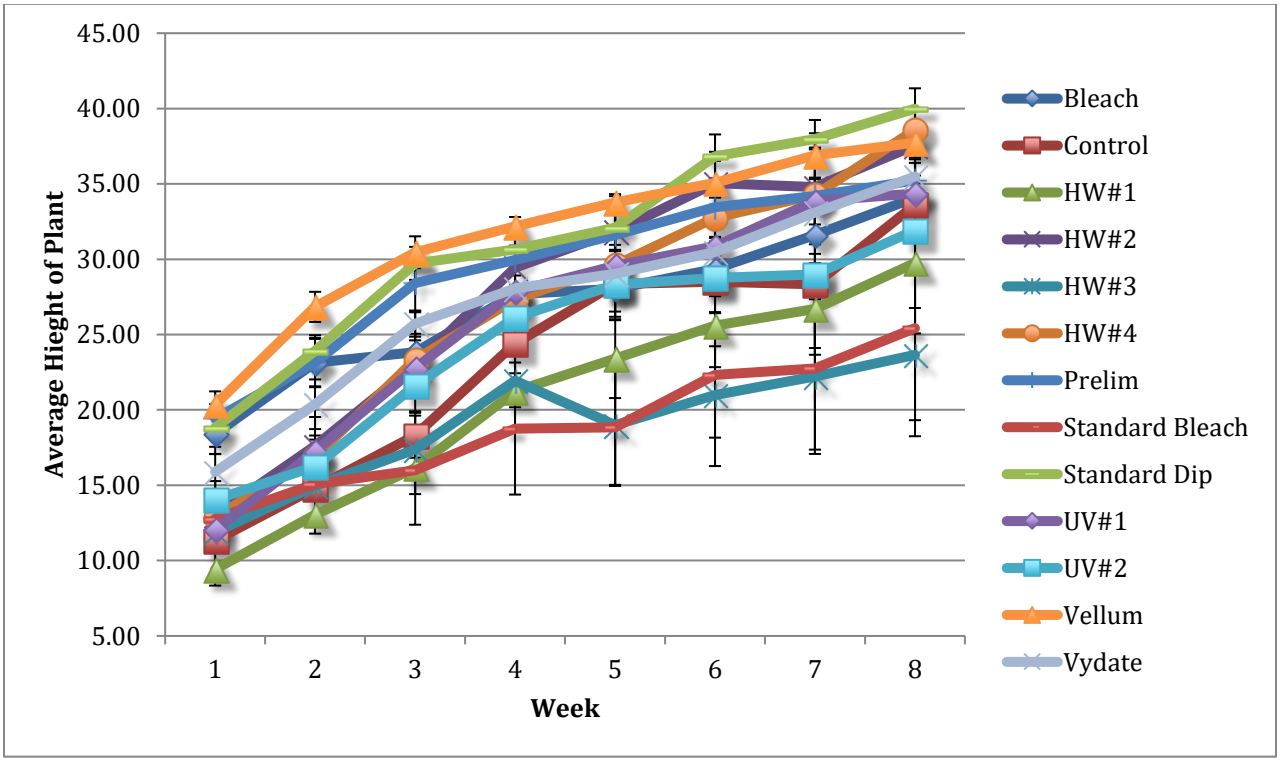

Figure 4b. A comparison of "Stolen Treasure" daylily plant heights across the entire experimental period. The standard dip employed by the industry surpassed velum as the treatment with the highest plants by the end of the experimental period. Analysis is still pending on this data, however these results were used to determine which treatments to drop from the list of possibilities during the second run of the experiment which is ongoing.

Table 2. Stolen Treasure daylily height after treatment with different nematode control strategies applied to bare root plant material after the first four weeks of the experiment. Analysis after complete eight weeks of the experiment is pending.

Treatment	N	Mean	Grouping Tukey 95% Confidence					
b. Vellum	32	27.2035	A					
a. HW Standard-Dip 41C, 60min	32	25.7190	A	B				
e. Vydate	32	23.1124		B	C			
d. Bleach	32	22.6997		B	C			
g. HW-Dip 35C, 50min	32	20.8966			C	D		
i. HW-Dip 41C, 30min	32	20.8164			C	D		
h. UV in water	32	19.7360			C	D	E	
m. UV	32	19.5215			C	D	E	
n. Control	28	16.9344				D	E	F
l. HW-Dip 45C, 30min	32	16.6105					E	F
k. Standard Bleach	32	15.8805					E	F
f. HW-Dip 45C, 50min	32	14.1443						F

Means that do not share a letter are significantly different (P < 0.05).

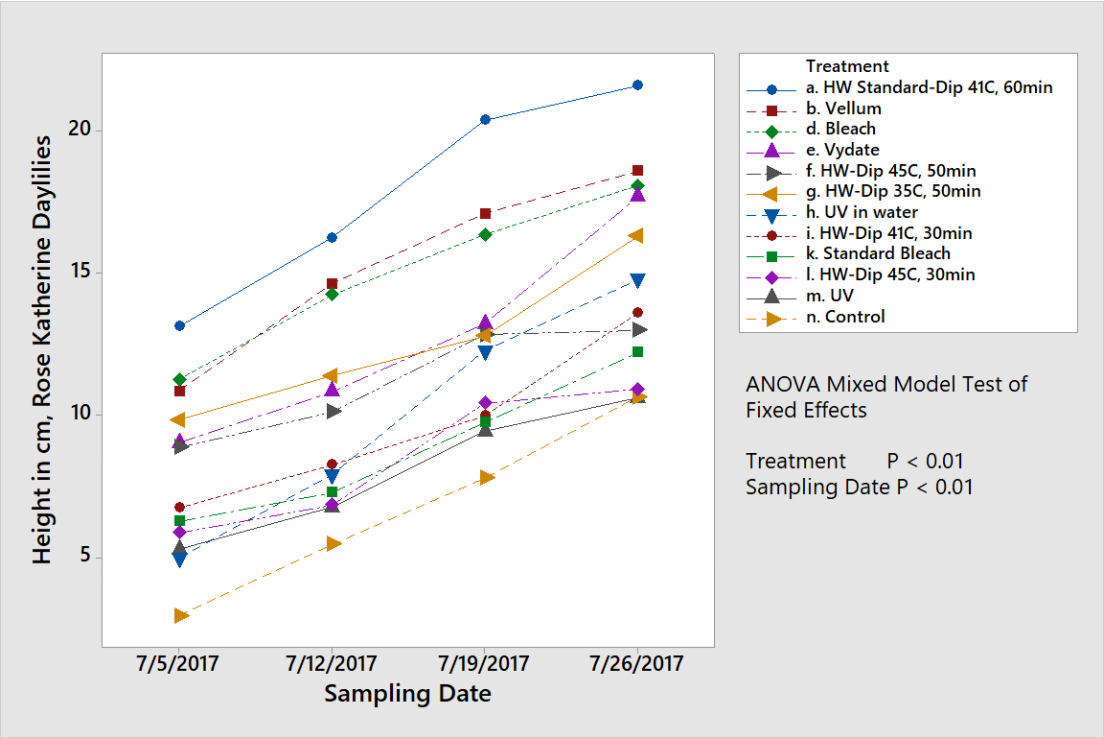

Figure 5a. A Comparison of "Rose Katherine" daylily plant heights across the first four sampling dates. See Figure 5b for graph of complete experimental period. Prior to planting, root-knot nematode infested bare-root daylilies were treated (dipped) with different nematode control strategies. Plants treated with standard hot water (HW) dip, Velum, and Bleach had the greatest heights. Plants left untreated (control) exhibited the least growth.

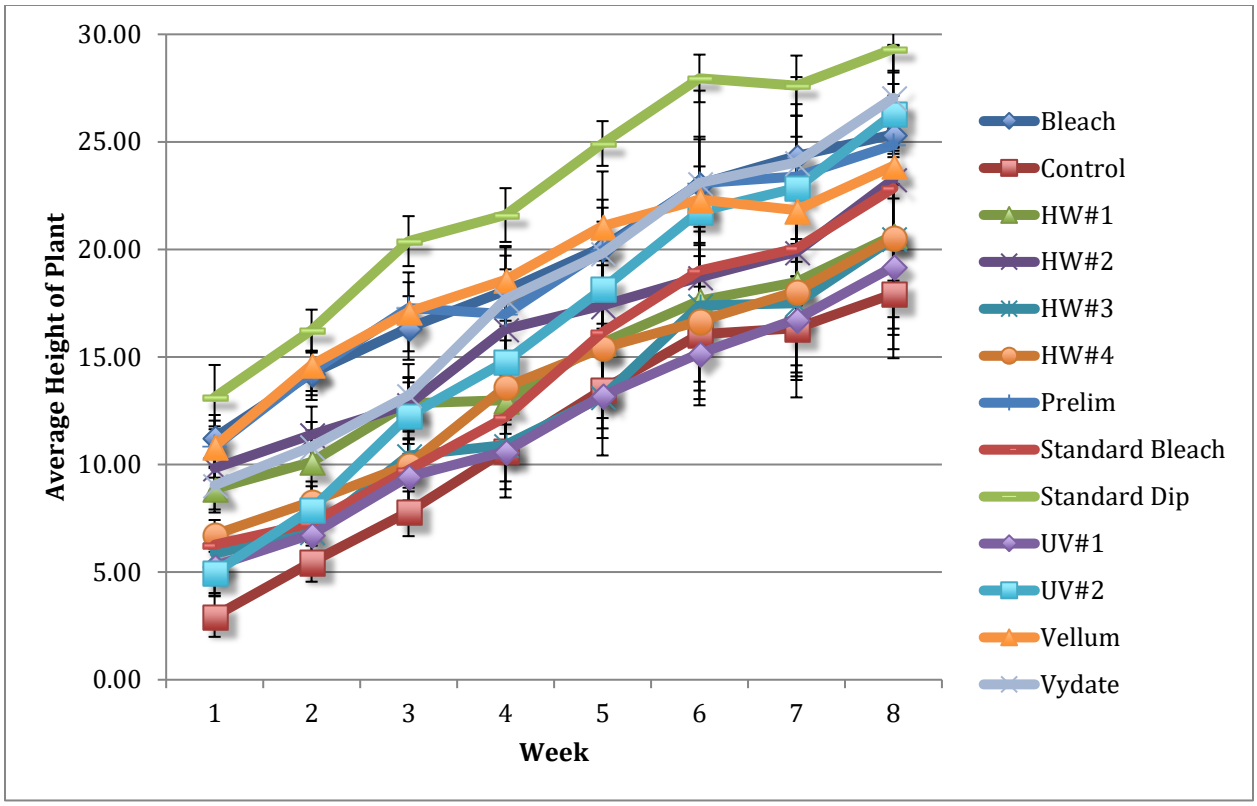

Figure 5b. A comparison of "Rose Katherine" daylily plant heights across the entire experimental period. The standard dip employed by the industry remained the treatment with the highest plants at the end of the experimental period. The nematicide Vydate surpassed Velum in plant height. Analysis is still pending on this data, however these results were used to determine which treatments to drop from the list of possibilities during the second run of the experiment.

Table 3. Rose Katherine daylilies height after treatment with different nematode control strategies applied to bare root plant material after the first four weeks of the experiment. Analysis after complete eight weeks of the experiment is pending.

Treatment	N	Mean	Grouping Tukey 95% Confidence				
a. HW Standard-Dip 41C, 60min	32	17.6387	A				
b. Vellum	32	14.7126	A	B			
d. Bleach	32	14.4287	A	B			
e. Vydate	32	12.8311		B	C		
g. HW-Dip 35C, 50min	32	12.0793		B	C	D	
f. HW-Dip 45C, 50min	32	11.4821		B	C	D	E
i. HW-Dip 41C, 30min	32	9.6650			C	D	E
h. UV in water	32	9.5499				D	E
k. Standard Bleach	32	8.9448					E F
m. UV	32	8.6320					E F
l. HW-Dip 45C, 30min	32	8.5735					E F
n. Control	44	6.1072					F

Means that do not share a letter are significantly different.

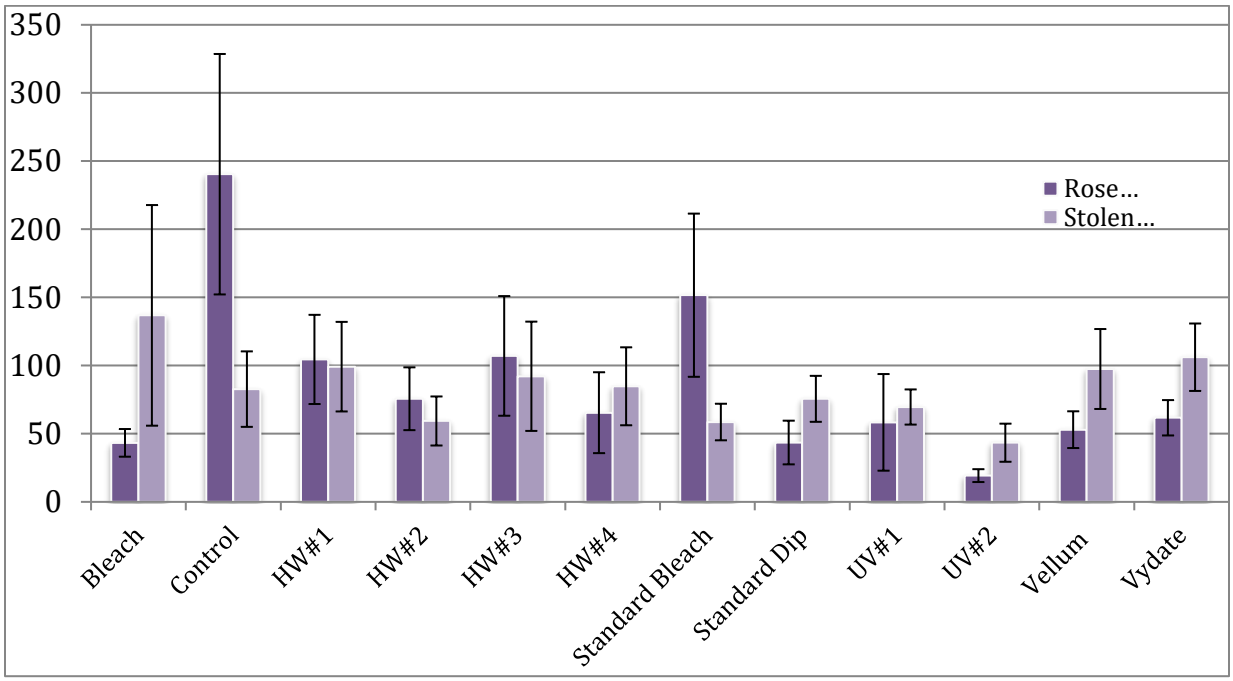

Figure 6. Root-knot nematode counts in stained roots. The darker colored bars represent nematode counts per treatment for the "Rose Katherine" daylily variety and the lighter colored bars represent nematode counts in the "Stolen Treasure" variety. More nematodes were counted in the untreated control treatment than in any other for "Rose Katherine", but this is not the same for "Stolen Treasure" in which the bleach treatment contained the highest number of nematodes. The lowest nematode count across all treatments was the second UV treatment for both varieties.

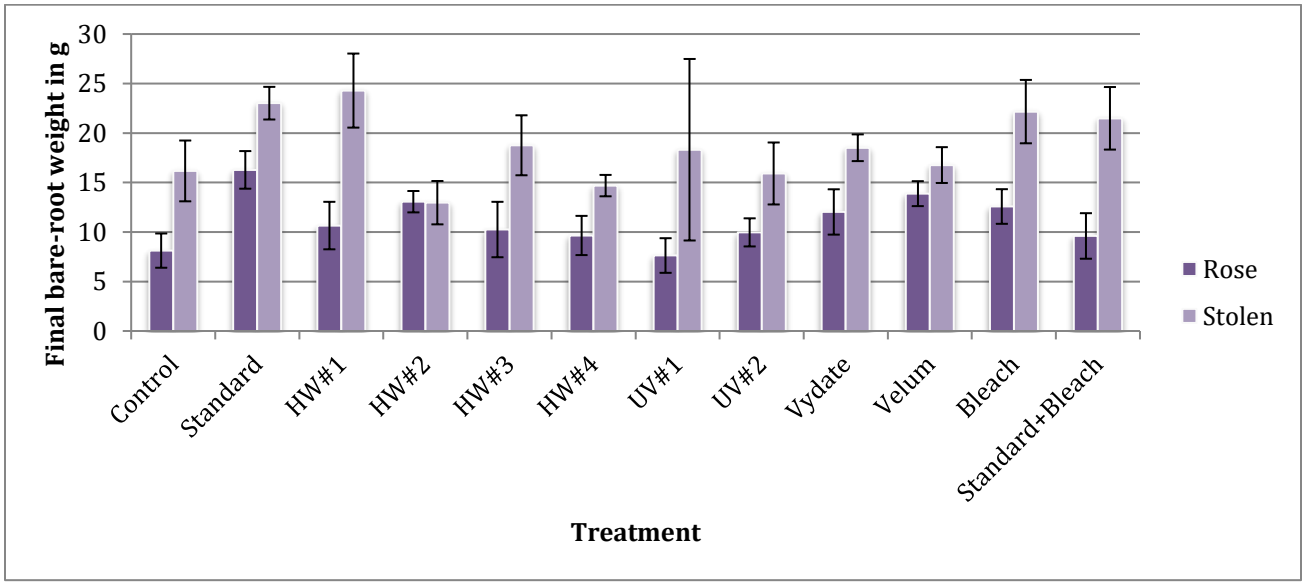

Figure 7. Bare-root weights for each treatment and variety after the experimental period. Root weights for "Rose Katherine" seem to generally follow the same trend by treatment as the plant height data (Figure 5b) with standard hot water treatment the heaviest and control among the lowest. "Stolen Treasure" however is contradictory to the plant height data (Figure 4b), with

HW#1 and Standard Bleach the heaviest average root size. Both of these treatments were among the lowest for plant height.

Conclusion:

This objective is complete. Our hypothesis was accepted. After testing twelve possible hot water dipping alternatives and modifications, we are now able to narrow down our focus to treatments that appear most promising for future experiments. For the most part, nematode counts within plant roots corroborate plant growth data. Although the UV treatment did not produce the tallest plants or the heaviest roots, we found the fewest nematodes in the roots and will continue to look at this strategy as a possibility. Velum and Vydate are both common nematicides used in many cropping systems. Both products produced promising results in this experiment, but we will continue testing with just Velum, as it is a safer product to apply.

Impact of Results:

Perhaps the most interesting result to come from this set of experiments is the disparity among daylily varieties. Treatments that produced excellent plant data or nematode control for one variety performed more poorly for the other plant variety. The bleach dip treatment is a good example of this: bare roots were among the heaviest after this treatment for "Stolen Treasure", but among the lightest for "Rose Katherine". This could mean that experiments of this nature need to be conducted on many of the top-selling varieties in the ornamental industry to really maximize the best management practice. Furthermore, older varieties are being retired while new varieties are continuously being introduced. "Rose Katherine" is currently unavailable through Walter's Gardens.

Thus far, we have been able to validate the hot water dipping procedure that Walter's Gardens typically employs as a top management practice for eliminating root knot nematodes prior to field planting. We have also narrowed down alternatives and modifications that are in the process of being further evaluated in current experiments. In ongoing research trials, we have combined the most effective hot water treatment (the standard) with nematicide dips (velum) in order to eliminate nematodes in the planting material and protect plants from reinfection in the soil.

Michigan is home to one of the largest ornamental bare root producers nationwide. Root knot nematodes are one of the major concerns for bare root daylily production. Nematode free, clean, planting material is required for commercialization, and the low tolerance for plants with nematode-caused galls increases economic losses because of rejected plant material. Additionally, the hot water dip can negatively affect the production of heat sensitive varieties, so finding effective nematode management solutions that do not cause yield loss, would further reduce losses to nematodes and nematode management practices. Fumigation is a standard nematode control practice that has high economic, environmental, and health risk/costs. Finding effective alternative solutions would decrease economic, health, and environmental costs. Using this dataset as a baseline, we have been awarded a continuation grant from the Horticulture fund, and we have leveraged funding from the USDA-APHIS for $91,373 and an IR4 funds to include products in our field trials that are not currently registered in ornamentals.

Financial Summary:

As of August 2018, the total amount of funds awarded, $10,886, has been utilized.

Expenses:

Salaries: $ 10129.52

Supplies (soil sampler's miscellaneous supplies): $ 752.88

Comparison to Grant:

Our work has closely followed the proposal, although this set of experiments did not we include some of the sustainable management practices such as biofumigants and manures that were discussed. Some of these strategies are currently being evaluated in our ongoing field trials at Walter's Gardens. We will continue to work towards finding more sustainable management solutions for daylily production in Michigan through leveraged funds and partnerships with MSU Extension and Walter's Garden.