Washington State Grape and Wine Research Program

FINAL REPORT
Funding July 2020-June 2024 (3 years + 1 NCE)
30 May 2024

Project Title: Alternative Preplant Strategies for Nematode Management in Washington State Wine Grape Vineyards

Principle Investigator: Dr. Michelle M. Moyer, Washington State University; **Co-Investigator:** Dr. Inga Zasada, USDA-ARS

Summary: In this project, we evaluated several alternative approaches for nematode management in Washington State Wine Grape Vineyards. This included continued monitoring of a long-term rootstock block at a commercial vineyard, as well as short-term monitoring of a smaller rootstock trial at another vineyard. We also completed both field and greenhouse evaluations of different cover crops that could serve in a pre-plant management approach, as well as post-planting. Finally, we conducted a grower survey of fallow ground practices to determine their potential impact on the survival of the northern root-knot nematode (*Meloidogyne hapla*). Our decade-long research effort has shown that rootstocks continue to out-perform own-rooted vines in terms of sustained vine productivity when grown under the presence of relatively high nematode pressure, but they are hosts for nematodes. This indicates that the effects of high nematode pressure near the end of a vineyard lifespan, even if the vineyard is on rootstock, might need to be mitigated with alternative nematode management options, or through reduced vineyard stress (e.g., greater monitoring of drought stress, nutrient deficiencies). In our cover crop research, we found that litchi tomato effectively reduced northern root-knot nematode during the season in which it was planted; however, litchi tomato can only be used as a preplant cover crop. Other cover crops, such as specific cultivars of oilseed (daikon) radish worked well at suppressing nematode development and can be used in established vineyards. Finally, we say that keeping a vineyard in a fallow state for at least 1 year assisted in reducing northern root-knot nematode populations in the soil.

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PRINCIPAL INVESTIGATOR / COOPERATORS:

PI Name:	Michelle M. Moyer
Organization	Washington State University
Address	24106 North Bunn Rd., Prosser, WA 99350
Telephone	509-786-9234
Email	michelle.moyer@wsu.edu

CO-PI Name:	Inga Zasada	**CO-PI Name:**	R. Paul Schreiner
Organization	USDA-ARS	**Organization**	USDA-ARS
Address	3420 NW Orchard Ave Corvallis, OR 97330	**Address**	3420 NW Orchard Ave Corvallis, OR 97330
Telephone	541-738-4051	**Telephone**	541-738-4084
Email	inga.zasada@usda.gov	**Email**	paul.schreiner@usda.gov
Cooperator Name:	Julie Tarara	**Cooperator Name:**	Michael Cerio
Organization	Ste. Michelle Wine Estates	**Organization**	Terlato Wine Group
Description of participation:	Vineyard manager of Canoe Ridge Vineyard. Long-term management of rootstock trial. Coordinates additional labor for large project workdays.	**Description of participation:**	VP of operations for managing company of Klipsun Vineyards. They have allowed us to continue to access the property for data collection.

OBJECTIVES AND EXPERIMENTS CONDUCTED TO MEET STATED OBJECTIVES:

The long-term goal of this collaborative nematology research effort is to provide Washington grape growers with more information about the damage potential and management of plant-parasitic nematodes (northern root knot nematode - RKN, *Meloidogyne hapla;* dagger nematode - *Xiphinema* spp.). The specific objectives of this project were:

Objective 1 – Evaluate rootstock selection and preplant fumigation for managing nematode decline in vineyard replant scenarios. (Years 1-3). In this objective, we monitored the long-term rootstock trial established in the Horse Heaven Hills AVA in 2014. This trial allowed us to gather information on the effectiveness of preplant fumigation for nematode management in our perennial cropping system, while simultaneously providing a rootstock evaluation trial. We also expanded our evaluation of rootstocks and pre-plant management practices, by partnering with a vineyard in the Red Mountain AVA. A list of rootstocks evaluated in this objective is available in Table 1.

Table 1 – Summary of rootstocks evaluated, including information from previous Washington-based trials, and general viticulture evaluations of performance in other regions.

Rootstock	Parentage	Sites	*Phylloxera Resistance*	Northern RKN Resistance	Dagger (*X. index*) Resistance*
Own-rooted	*Vitis vinifera*	1,2	*Very low*	Low	Low
101-14 MGT	*V. riparia* x *V. rupestris*	1	*Moderate*	Moderate	Moderate
1103 P	*V. berlandieri* x *V. rupestris*	1,2	*High*	Moderate	Low
Harmony	"1613" (*V. solonis* x "Othello") x "Dog Ridge" (*V. champinii)*	1	*Low*	Moderate	High
Teleki 5C	*V. berlandieri* x *V. riparia*	1	*High*	Moderate	Moderate
3309 C	*V. riparia* x *V. rupestris*	2	*High*	Moderate	Low
1616 C	*V. solonis* x *V. riparia*	2	*High*	N/A	Moderate
GRN-4	(*V. rufotomentosa* x ("Dog Ridge"[V. champinii] x Riparia Gloire (*V. riparia)) x* Riparia Gloire	2	*High*	N/A	Very high
RS-3	"Ramsey" (*V. champinii)* x "Schwarzman" (*V. riparia* x *V. rupestris*)	2	*Low*	N/A	High

* *X. index* is not reported in WA state. WA currently has *X. americanum* group species of dagger nematode.
Rootstock References:
http://iv.ucdavis.edu/files/24347.pdf; https://www.inlanddesert.com/category/rootstock/, http://www.novavine.com/media/11790/Rootstock-Chart-.pdf, https://www.sunridgenurseries.com/index.php/clonal-selections/rootstock-chart

Site 1 – Horse Heaven Hills. This vineyard was planted in 2015 into fumigated (Vapam at maximum rate through the existing drip in 2014), non-fumigated, and non-fumigated with supplemental RKN plots. Vines consisted of Chardonnay as a scion, grafted to four different rootstocks, and two own-rooted controls (self-grafted and non-grafted). There were 10 data vines per treatment replicate (total 720 data vines). At this site, we collected yield, pruning weights, and nematode population information. We also collected vine nutrient status via bloom and véraison tissue tests (these results will not be presented in this report; they are included in the 2023-2026 project cycle). Additional details on this trial are described in East et al. (2021).

Site 2 – Red Mountain. We established a smaller-scale nematode and fumigation trial in the late summer of 2019. Here, Telone II was applied in swath treatments after the existing vineyard infrastructure was removed. Additional experimental details are provided in Moyer et. al (2023). Rootstocks evaluated are listed in Table 1. We also had a smaller-scale Vapam trial in another block (same scion on 1616C). This vineyard was planted in a randomized block design, with four vines per soil treatment (fumigated, non-fumigated), replicated three total times within that soil treatment. In summer / fall 2020, we sampled for nematodes via standard coring techniques, and with deep coring. We had also intended on collecting vine pruning weight information, but the poor establishment status of the vineyard has prevented us from doing so. We continued traditional nematode sampling in the Fall of 2021, but due to additional vine losses at the site in the spring through fall of 2021, we chose to limit any additional effort at this site, and re-direct our focus on expanding other grower-focused rootstock trials (where phylloxera resistance is the primary selection driver). This site was useful, however, as it allowed us to track duration of efficacy of an additional fumigant to complement the work originally done at site 1.

Objective 2 – Evaluate preplant and replant nematode management strategies. (Years 1-3). Under this objective, we evaluated the efficacy of three nematode-management strategies. In the first strategy, we used the non-host litchi tomato as a preplant cover crop to trap RKN and reduce their reproduction. In the second strategy, we evaluated the efficacy of a fallow period, and fallow period management strategies, on reducing nematode populations in the soil. Finally, we evaluated the host status of several post-plant vineyard cover crops against RKN. Specific experiments by subobjective are described below:

Objective 2.1 – Litchi tomato as a preplant cover crop (**Fig. 1**). While the use of litchi tomato *(Solanum sisymbriifolium*) as a trap crop has been well-established in potato, its adoption to a perennial cropping system such as grape needs to be refined. With this objective, we established six treatments at a site of a recently removed vineyard (Mattawa, WA) in 2020, and continued those treatments through 2021. Treatments included: Fallow (mowed), Fallow (unmowed), litchi tomato (year 1, mowed), litchi tomato (year 1, unmowed), litchi tomato (year 1, 2; mowed), and litchi tomato (year 1,2; unmowed). The trial was a randomized block design with four replicates of each treatment. The site was soil-sampled prior to the trial in the spring 2020, including deep-coring and nematode enumeration. The site was routinely managed in both summers for weed control (with a single hand-weeding effort mid-season each year, and perimeter management using herbicide and mowing). The plots were sampled for nematodes again in Fall 2020, Spring 2021, Fall 2021, and the last sampling occurred in Spring 2022.

Fig. 1 - *Litchi tomato plots were prepped and seeded on either May 27 2020, or May 11 2021. Plots were established over previous vineyard rows to maximize potential nematode populations. Plots were also pre-sampled for nematodes, to ensure an even distribution across all proposed treatment*

In 2022-2023, we added an additional objective here, where we conducted a greenhouse experiment to better understand *why* and *how* litchi tomato behaves as a biofumigant against RKN. In other words, is the preplant use of litchi tomato optimized by only allowing the litchi tomato to grow for a short period of time (and then potentially, removal and immediate vineyard replanting, or the option of a second in-season growth cycle of litchi), or does litchi tomato have to develop for a longer period of time before it has activity against RKN?

Greenhouse Experiment: Understanding how long litchi tomato must be grown to achieve maximum RKN suppression. In this experiment, 6-week old 'Roma' tomato (control) and litchi tomato seedlings were planted (separately) in pots, and then inoculated with either *M. hapla* eggs or second-stage juveniles (J2; the free-living soil stage). For the litchi tomato, after 2, 4 or 6 wks of exposure to nematodes, the established plants were removed, and a susceptible Roma tomato was planted in its place. That tomato plant grew for 8 wks, after which it was harvested, and nematode eggs were counted. The initial control tomatoes (Roma, described above) were harvested after 8 wks of exposure to nematodes and eggs counted (this will serve as a control that nematode inoculum was viable). Five plants per inoculum type and exposure duration were used, and the experiment was repeated three times.

Objective 2.2 – Monitoring nematode decline in fallow vineyards. We began soil sampling in 2021 from commercial vineyards that were either fallow or recently undergoing removal with the intention of a fallow period prior to replanting. Five soil samples were collected from each fallow vineyard location A sample consisted of a composite of two cores collected using a manual soil auger (10 cm diameter by 40 cm deep) and three soil probe cores (2.5 cm diameter by 45 cm deep). Within a vineyard site, soil samples were collected using a "W" pattern, and when possible, soil was collected where former vineyard rows were apparent. Soil was collected between June and August in both 2021 and 2022, corresponding to a time of season when the density of RKN eggs are highest. RKN J2 were extracted and counted from soil, but given the potential low RKN J2 density, a tomato bioassay (as described above) was also used for nematode enumeration. Soil subsamples were sent to a commercial lab for basic soil chemistry and nutrient analysis. We collected additional information for modeling: duration of previous planting, soil type, and site fallow management.

Objective 2.3 – Evaluation of the host status of native (and weedy) vineyards ground cover species for RKN. This objective was designed to develop a better understanding of the host status of different native and weedy plant species found in Washington fallow vineyards for RKN and whether the presence of these weedy species is detrimental to the efficacy of that fallow practice. Knowing which plants are alternative hosts to RKN could assist in devising fallow-ground management options (for example herbicide selection) to optimize this control approach. Over the spring to fall in 2020, we collected various native and weedy species for greenhouse assays for RKN host status. Multiple individuals of each species (5 to 10) were collected from a site (dug up, roots intact), and transported to a greenhouse for transplanting. After a few days in the greenhouse to get over transplant shock, the weedy species were inoculated with RKN, and allowed to continue to grow. After approximately 8 weeks, the weedy species were destructively harvested, and the RKN eggs were extracted from roots with bleach and then enumerated. In this process, we noted that most weedy species did not transplant well, and we adjusted efforts in 2021 to focus on in-field inoculations to reduce root disturbance.

In 2021 a site was selected to isolate native weedy ground cover species for RKN. In spring 2021, native and weedy species were isolated in the field using a six-inch diameter, four-inch in height, collar of PVC. This collar was placed around multiples of individual species (5 each). The intent was to isolate five species in spring, summer, and fall, to better capture native species that occur throughout a growing season. Isolated species were to be inoculated with RKN and roots/surrounding soil collected and evaluated for RKN survival at the end of the growing season. After the spring isolation of native species, the area was irrigated once weekly to encourage growth. Without irrigation native species would not have survived the growing season due to high temperatures. This led to all ground cover species germinating and growing at accelerated rates. The isolated plants were quickly overrun with newly germinating plants. Extensive weed management was necessary to control weeds not isolated in PVC isolation collars. Rather than collect data from what became an artificial fallow situation (due to the irrigation necessary to keep vegetation alive during the 2021 heat dome and lack of in-season precipitation), we opted to not continue this objective. We focused our efforts on fallow vineyard surveys (*Objective 2.2).* These surveys, indirectly, provide a similar set of data, and is more representative of the combination of environmental and ground cover effects on nematode survival at a site.

Objective 2.4 – (New to final year of project) *Post-plant cover crop options for RKN management.* Key to IPM for nematode management, will be the addition of post-plant management options. We evaluated post-plant cover crop options for RKN suppression. This trial included an oilseed radish (*Raphanus sativus* 'Dracula'), a mustard (*Brassica juncea* 'Pacific Gold'), and a clover (*Trifolium repens* "Dutch White Clover"). With positive results observed (nematode suppression) in these trials, we conducted a greenhouse trial where a local source of oilseed radish (*R. sativus* 'Image Nematode Control' from Great Basin Seed, Mesa, WA) was evaluated. This oilseed radish cultivar is marketed to reduce populations of sugar beet cyst nematode (*Heterodera schachtii*). The mechanism of plant-parasitic nematode suppression in 'Image Nematode Control' radish is largely unknown. It may be a trap crop, due to ancillary roots that are attractive to plant-parasitic nematodes. For the greenhouse experiment described here, we evaluated seven different cover crops: (i) *R. sativus* 'Dracula' (oilseed radish); (ii) locally sourced *R. sativus* 'Image Nematode Control' (oilseed radish). (iii) *Vicia villosa* (hairy vetch), (iv) x*Tritcosecale* 'Trical 141' (triticale); (v) *T. repens* 'Pipolina' (white clover); (vi) *B. juncea* 'Pacific Gold' (mustard); and (vii) *Eruca sativa* 'Nemat' (arugula). We mimicked greenhouse trials described above in *Objective 2.1 – Greenhouse Experiments*. We seeded different cover crops into five-pot replicates and allowed them to develop for 6 weeks. After this time, they were inoculated with 500 RKN J2 and allowed to grow for another 8 weeks. After that period, plants were removed, roots weighed, and RKN eggs were extracted (from the roots) and counted.

SUMMARY OF MAJOR RESEARCH ACCOMPLISHMENTS / RESULTS BY OBJECTIVE:

Objective 1 – Evaluation of rootstock selection and preplant fumigation on managing nematode decline in vineyard replant scenarios.

This research incorporates the field evaluation of preplant fumigation and rootstock selection on nematode population development and scion productivity. The work in this objective addressed three main questions: (1) What are the **true long-term effects of plant-parasitic nematodes** on wine grape vineyard productivity?; (2) Does preplant fumigation of nematode-infested soils provide an adequate **solution for nematode management** in own-rooted vineyards?; and 3) How do **non-*vinifera* rootstocks** perform under Washington's climate? We already know that the use of rootstocks, in a situation where vines are not under nematode pest pressure, has little negative impact on fruit and wine quality in Washington (Harbertson and Keller 2012, Keller et al. 2012), thus dispelling the common reason (own-rooted is better) that is often used when selecting planting stock. However, trials on rootstocks for nematode management are often only for short periods of time (less than 2 years) (McKenry et al. 2001, Ferris et al. 2012, Zasada et al. 2019), or the evaluation is against nematode species not found in Washington (Sauer 1967, McKenry et al. 2001, McKenry and Anwar 2006, Ferris et al. 2012). Our own work also shows that fumigation may not be a long-term solution for nematode management (East et al. 2021).

Site 1 – Horse Heaven Hills.

NORTHERN ROOT-KNOT NEMATODE (RKN). After 10 years post fumigation, and 9 years of planting, we no longer see a consistent impact on pre-planting fumigation on the total nematode populations. In fact, the lack of response began in Fall 2019. In 2022 there was an odd deviation where there was a fumigation effect, but this was only due to a difference between our non-fumigated (NF) and inoculated (NF+) plots; the NF+ plots had more RKN. However, there was no statistical difference between NF and fumigated (F) plots. By Fall 2023, we also no longer see a major rootstock impact on the number of nematodes the vines host (p = 0.30) (**Fig. 2**). It is interesting to note that since Fall of 2018, we have seen a "leveling off" of the nematode population density in our NF plots, which indicates we have likely reached the natural carry capacity of the soil (**Fig. 2**). However, in our F and NF+ plots, we see cycling "boom and busts" of the nematode population density, particularly in own-rooted vines, potentially indicating a delay in population stabilization. This could be due to either disruption of the predators that would naturally suppress RKN (fumigated), or an overwhelmingly large population of RKN (non-fumigated, inoculated).

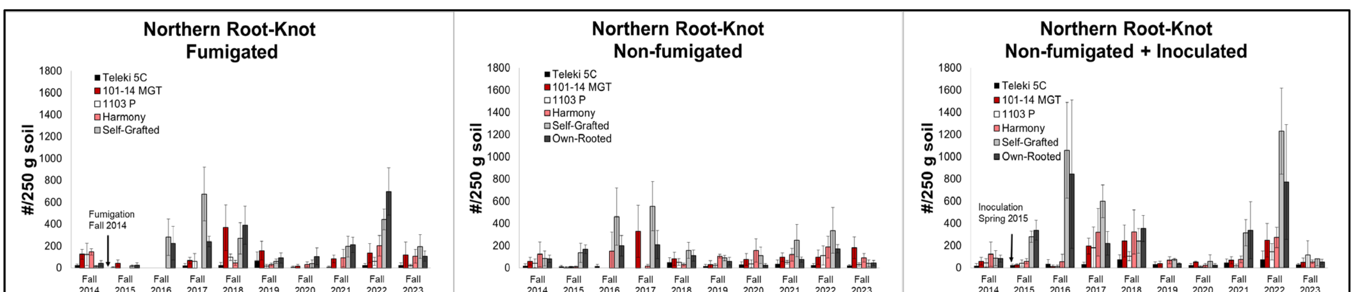

Fig. 2 - *Rootstock performance across different soil treatments. There is no longer a rootstock or soil treatment effect on northern RKN populations, when looking at raw nematode counts from soil samples.*

As we have previously reported, fumigation delays the re-establishment of RKN, but when they do re-establish, they do so at population densities that are much greater than what is seen in NF plots (**Fig. 2**). This is an interesting effect and indicates that rather than initially focusing on what fumigation can do to nematodes in the short-term, perhaps we should focus on how the plant responds to nematodes over the long-term. We should select rootstocks that can tolerate some levels of nematode feeding (without a reduction in vigor), particularly once nematode populations reach the soil's natural carrying capacity.

Using total nematode counts at a site, particularly over the long-term, may not be the best indicator of potential for nematode-induced root damage. Nematode feeding is a chronic stress and must be viewed over time. To do this, we look at "nematode dosage" which is a measurement of total populations over time and considers early exposure (non-fumigated sites), or rapid population increases (fumigated sites). We clearly see a nematode dosage difference between rootstocks (**Fig. 3 – left**), which tells us that even though population levels in Fall 2023 were similar across rootstock, the total pressure has been much lower on the non-*vinifera* rootstocks. Fumigation effects (**Fig. 3 – right**) are also very telling, as at this point the total nematode dosage experienced across fumigated and non-fumigated plots was equivalent; it's as if the fumigated plots were never actually fumigated.

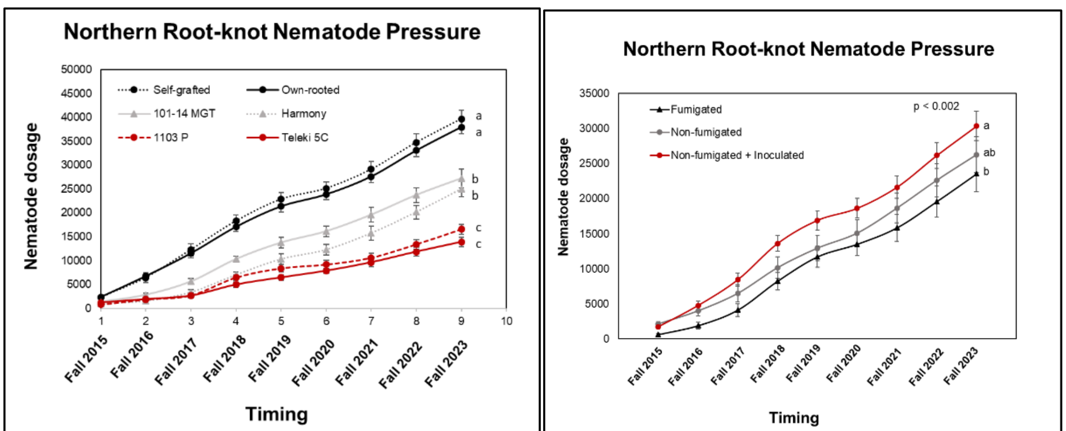

Fig. 3 - *Nematode dosage is a way to look at nematode pressure over time. Populations can spike and crash, but their feeding damage lingers. Here, we can see how* Vitis vinifera *rootstocks have higher nematode pressure over time (left). When it comes to the effects of pre-planting fumigation (right), we see that after several growing seasons, the nematode pressure in the fumigated sites, and the none-fumigated*

Vine response to RKN. We are still seeing positive trends in vine dormant pruning weights, where rootstocks are influencing vine vigor ($p = 0.00001$; **Fig. 4**). Interestingly, those vines that are the most vigorous aren't always the same vines that have the lowest nematode dosage (**Fig. 3**). This reinforces the idea that nematode host status is not the only indicator of potential viability as an acceptable rootstock in Washington.

Capturing own-rooted *vinifera* vine decline in the form of yield is still challenging at this site. Practices like shoot thinning (which is done at this location) can directly influence yields, and vines have a tendency to compensate lower cluster numbers with larger berry size. From a vine health perspective, given these potential manipulations, pruning weights are likely a better indicator of vine health, and yield differences might take longer to visualize.

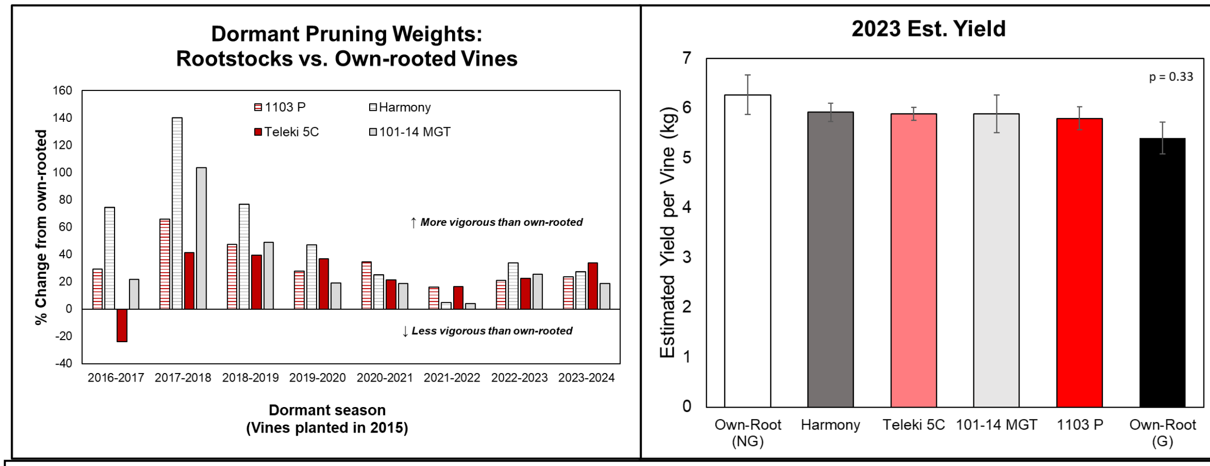

Fig. 4 - *Dormant pruning weights, an indicator for vine vigor, is influenced by rootstock. While there is some rootstock effect on yield, this is still difficult to capture due to several vineyard cultural approaches (i.e., bloom shoot thinning), and weather patterns that confound these early results. But the visible trends are clear – own rooted vines are showing some form of decline, and rootstock vines are supporting healthy canopies.*

Additional information, such as the influence of rootstock on scion tissue nutrient status, is also available from this project, but is included in the 2023-2026 funding cycle reports.

DAGGER NEMATODE. By Fall 2021, there was no fumigation effect (p = 0.09) or rootstock effect (p = 0.29) on dagger nematode populations **(Fig. 5)**. Note, though, that the efficacy

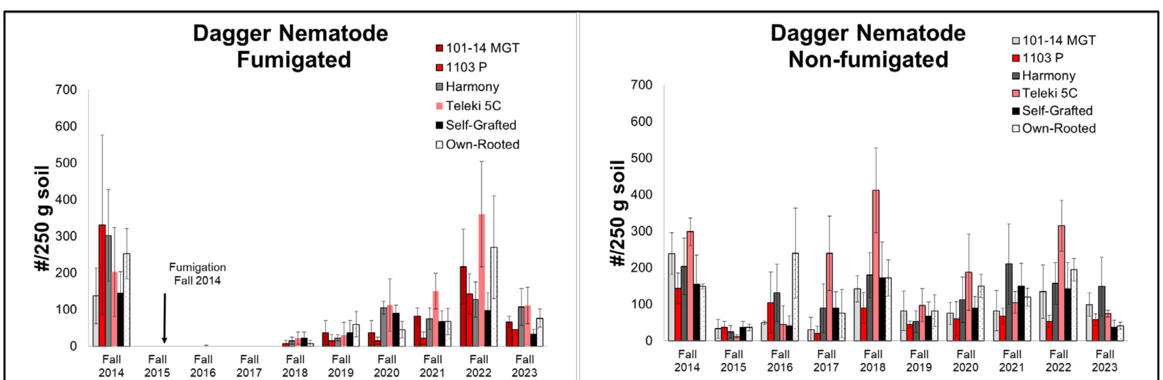

Fig. 5 - *The rootstocks selected for this trial do not have known dagger nematode resistanc. Fumigation has a longer impact on dagger compared to RKN. Disturbance as a part of the replant process also had an effect, as seen in the drop in dagger populations in the non-fumigated plots between vine removal (2014) and establishment (2015).*

of fumigation against dagger nematodes lasted longer than that in RKN – fumigation was a significant factor through Spring 2020.

Site 2 – Red Mountain.

We were initially disappointed in the quality of the plant material delivered for the planting of this site. Arriving in August 2019, much of the material was of poor graft quality. This could be attributed to a short turnaround from grafting to delivery to the site, where insufficient time was allowed for graft union healing at the CA-based nursery. By spring 2020, we had only 68% vine survival for the whole plot (144 vines). The survival rate of each of the rootstocks were as follows: 1103 P (38%), 1616 C (88%), 3309 C (50%), GRN 4 (88%), own rooted (80%), and RS3 (67%). Luckily, the vineyard had overwintered several surplus vines of the same rootstock+scion combination, and we were able to interplant. Unfortunately, this poor development continued through the 2020 growing season, as noted by very few vines achieving enough growth to be trained onto the wire. While vine water status was monitored by the participating grower and used to determine irrigation scheduling, it appears as though the strategy resulted in a strong water deficit. This also resulted in a formation of a caliche layer, which made late October 2020 soil sampling exceedingly difficult (too dry – fall watering had not been applied by late October). Challenges continued through the 2021 growing season, with additional vine losses over the 2020-2021 dormant period, and in-season vine collapse. After the additional vine loss in 2021, and the limited supply of appropriate rootstock+scion combinations for consistent plot replanting, we suspended additional nematode and rootstock monitoring at the site, save for the occasional check-in, particularly if a winter cold event will occur (in the future).

The site did, however, provide decent nematode response-to-fumigation data, as well as initial rootstock performance data. Similar to site 1, we no longer saw a fumigation effect 2.5 years post fumigation (Spring 2019-Fall 2021) ($p = 0.69$; **Fig. 6**). While our Horse

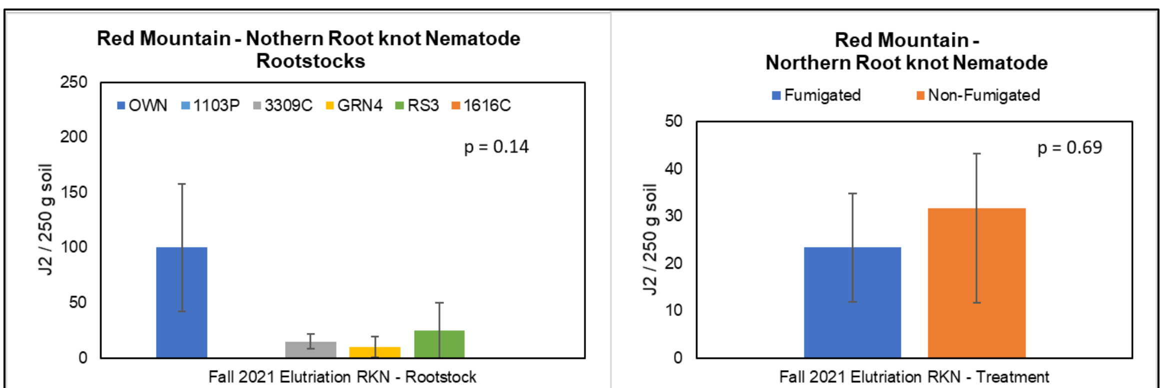

Fig. 6 - *While vine establishment has been challenging at Red Mountain, we still see similar trends to our other trials – preplant fumigation is not a long-term solution for nematode control. We see that these rootstocks support small populations of RKN, but not to the same degree as own-rooted vines. Both 1103P and 1616C had no detectable RKN J2s around their roots in Fall 2021.*

Heaven Hills site was fumigated with Vapam through the drip, and this site was fumigated with Telone II applied via large-swath injection, the results were the same: RKN populations rebound quickly after fumigation.

Objective 2 – Evaluation of preplant and replant nematode management strategies.

In this objective, we evaluated **different preplant and replant strategies to reduce population density of plant-parasitic nematodes in wine grape vineyards.** In the first project of this objective, we used the litchi tomato (*Solanum sisymbriifolium)* as a trap-crop for RKN (Scholte and Vos 2005). This crop is not a host for many nematodes (Zasada *unpublished data*; Hajihassani et al. 2020), but it triggers eggs to hatch and nematodes to invade where they are unable to reproduce. This has been used with success in commercial potato production, as a part of the eradication efforts to clean sites of the quarantine pest Potato Cyst Nematode (*Globodera pallida)* (Dandurand et al. 2019). In vineyard replant scenarios, this might be a good between-planting crop to help reduce total number RKN in the soil prior to replanting.

In the second project of this second objective, we looked at the efficacy of using fallow periods between vineyard replanting for plant-parasitic nematode management. The biggest unanswered question in the use of fallow periods is what duration of fallow is needed, and how "fallow" a fallow practice needs to be to reduce RKN population densities. This has not been evaluated in vineyards, however there are studies from other production systems, such as carrot, that demonstrate the advantage of fallow and cover crops to reduce densities of RKN (Belair and Parent 1996). One of the challenges in fallow period management is maintenance of the site – both from a weed management perspective, but also a soil erosion management perspective. Ideally, the site would should remain weed-free, to not accidentally allow for nematode development on an alternative host. However, in eastern Washington, a lack of vegetation on the site can make it prone to soil erosion from our high winds. If we were able to identify which native and weedy species are non-hosts for the plant-parasitic nematodes of interest, these species might become a tool for soil erosion control during fallow periods, without reducing the efficacy of the fallow practice on nematode reduction.

The third project tried to dissect the weedy-host question, but as described below, we struggled with perfecting the model system that both allowed for adequate nematode enumeration while not creating too-artificial of a growing environment.

In the fourth project (new to 2022-2023) we looked at the impact of oilseed daikon radish (*Raphanus sativus*) as a post-plant nematode management alternative. This new objective complemented recent cover crop trials done by the team, which has shown promising impacts on RKN suppression in vineyards. In other *R. sativus* varieties, the ancillary roots of the main tap root appear as ideal host sites for plant-parasitic nematodes, but once the nematodes begin feeding and establishing a reproduction site, the surrounding plant cells die, trapping and killing the nematode. Cultivars of *R. sativus*, contain isothiocyanates, a compound that degrades to glucosinolates which are toxic to plant-parasitic nematodes (Aydinli and Mennan 2018).

*Objective 2.1 – Litchi tomato as a preplant cover crop***.** Pre-establishment (27 May 2020) of our litchi tomato trial, all treatment plots had equal levels of RKN and *Xiphinema* sp. ($p = 0.43$ and 0.24, respectively). By fall (29 Oct 2020), the litchi tomato cover crop significantly reduced RKN densities ($p < 0.0001$), but not *Xiphinema* sp. densities ($p = 0.183$). However, once plots containing litchi tomato returned to fallow, RKN began to rebuild at the site (**Fig. 7**). Fallow treatments received regular irrigation and had active weed development. This further implicates weedy species as potential refugia for RKN. We did not find a significant effect of mowing on the performance of litchi tomato.

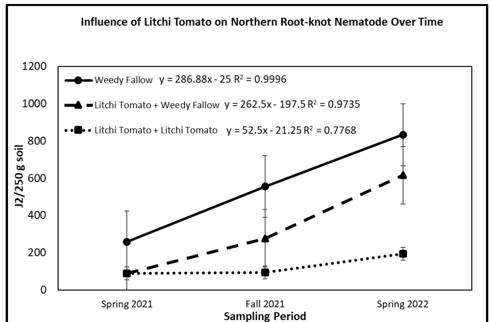

Fig. 7 - *Litchi tomato acts as a good pre-plant cover crop for reducing RKN at sites, but it is only effective for 1 growing season. When litchi tomato plots were allowed to return to fallow (long dashed line), the sites saw a slow return of RKN.*

For deep coring, we looked at sites associated with fallow mowed plots, fallow no-mow plots, and plots that will be under litchi tomato for 2 years, not mowed. Deep cores looked at populations of RKN, *Xiphinema* sp., and root lesion nematode, at 1 ft., 2 ft., and 3 ft. depths. In both pre-establishments, and Fall 2020, there were no differences in population density for RKN or *Xiphinema* sp. as influenced by soil depth or plot treatment. This was also true of root lesion nematode at pre-establishment, but by fall 2020, there were significantly more root lesion nematodes found in the top 1 foot of the fallow-mowed plots than in any other treatment or depth combination ($p < 0.0001$). We did not continue deep coring in Fall 2021.

We also evaluated the efficacy of duration of litchi tomato exposure against both RKN J2 and eggs. In these greenhouse experiences, litchi tomato significantly reduced the ability of RKN to complete a lifecycle, whether RKN was present as a J2 or as eggs. Regardless of duration of exposure to litchi tomato (2,4, or 6 weeks), there were between 93 and 100% fewer nematodes compared to those exposed to susceptible Roma tomato. When RKN J2 were exposed to litchi tomato for various durations of time, the differences between Roma tomato and litchi were stark ($p < 0.0001$). This was also seen in experiments using eggs as inoculum, but the general survivability of eggs as a primary inoculum sources was not always consistent between the three experimental replicates.

Objective 2.2 – Monitoring nematode decline in fallow vineyards. The 38 different sites / soil samples were divided into three different quantities of soil. Samples were sent to Northwest Agricultural Consultants, Kennewick, WA for nutrient testing; USDA ARS in Corvallis, OR for the tomato bioassay and RKN enumeration. We collected information on site management such as irrigation, soil preparation, cover crops, and total years fallow. We also had basic soil chemistry data, including percent organic matter. After categorizing some data due to low sample size within a given metric (i.e., categories for years fallow, categorization of different cover crops such as nematicidal or not), we ran a stepwise regression to determine significant effects on the presence of RKN from our tomato bioassays. We did not use elutriated RKN J2 data as that was very inconsistent site-to-site, and bioassay approaches are more appropriate for low-nematode density soils. Of these comparisons, only one real factor stood out as significantly influencing nematode density: duration of the fallow period ($p = 0.0097$; **Fig. 8**). We categorized vineyard's fallow duration as: >1 year, 1-5 years, and 6+ years fallow. The longer a vineyard is fallow, the fewer RKN we could find, but there was a minimum of one year fallow to achieve this effect.

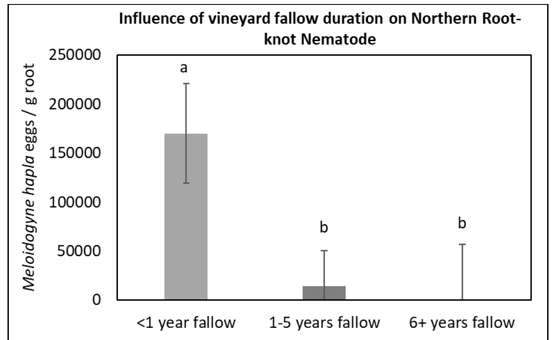

Fig. 8 - *Essentially, allowing a vineyard to be fallow for 1 full year significantly reduces the number of* M. hapla *that survives.*

Objective 2.3 – Evaluating the host status of native (and weedy) vineyard ground cover species for RKN. In 2020, we had three separate dates for collecting vineyard weedy species. In Evaluation #1, we collected cheatgrass (*Bromus tectorum)*, Fiddleneck (*Amsinckia sp.)* and a non-identified Brassica. A highly susceptible tomato host was used as a general comparison. In the Evaluation #2, Groundsel (*Senecio sp.),* Lambsquarters (*Chenopodium sp.),* and Russian Thistle (*Salsola tragus*) were collected. In Evaluation #3 we evaluated Foxtail (*Setaria sp.)*, Marestail (*Erigeron canadensis*), London Rocket (*Sisymbrium sp)*, Redstem Filaree (*Erodium cicutarium)*, and Bigbract Verbena (*Verbena vracteata*). Reproduction factor (RF), which is a calculation that divides the number of RKN eggs at the end of the experiment by the number of RKN eggs used for inoculation, is a common means for ranking plant host status (**Fig. 9)**. A RF of greater than one (>1) indicates a host -- and the larger the number, the more suitable the host. Groundsel appears to be a good RKN host. Many of the other evaluated weedy species were hosts but did not have large RFs. In some cases, the associated RF value was less than one. However, given the small surviving sample size of some of these weedy species, we are not sure if that low RF is due to lack of poor host status due to the inherent plant species, or lack of poor host status due to extreme host stress. In 2021, all attempts to establish a trial to evaluate weedy hosts in-field did not work. The complications of the 2021 heat dome, lack of natural precipitation, and the need for

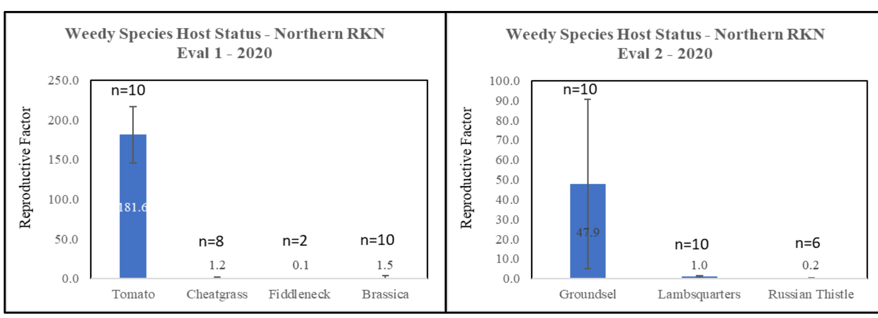

Fig. 9 - *Weedy plant species are known for their aggressive development but they do not like being transplanted. While our preliminary screen for RKN host status has provided some interesting results, we did not continue this evaluation due to the artificial conditions needed to support weedy growth.*

supplemental irrigation created too-artificial of a system to evaluate the survival and host status of weedy species for RNK. We did not pursue further inoculation and evaluation of our selected weedy hosts and opted to continue to focus on fallow vineyard sampling, along with additional cover crop evaluations.

Objective 2.4 – Post-plant cover crop options for nematode management.

Over the course of this project, we routinely struggled with access to adequate greenhouse facilities to conduct our greenhouse screens. This Objective was not spared, and we had to repeat the trial several times to compensate for conditions that resulted in freezing, or over-heating, of plants.

We found that almost all the cover crops evaluated, except for hairy vetch and perhaps Pipolina white clover, supported less RKN development than the susceptible tomato (**Fig. 10**). We continued to be impressed with the performance of both Image and Dracula daikon radishes (earlier field trials had equally impressive results) especially given the other horticulturally-relevant properties they can impart as a cover crop (nitrogen scavenging, soil conditioning).

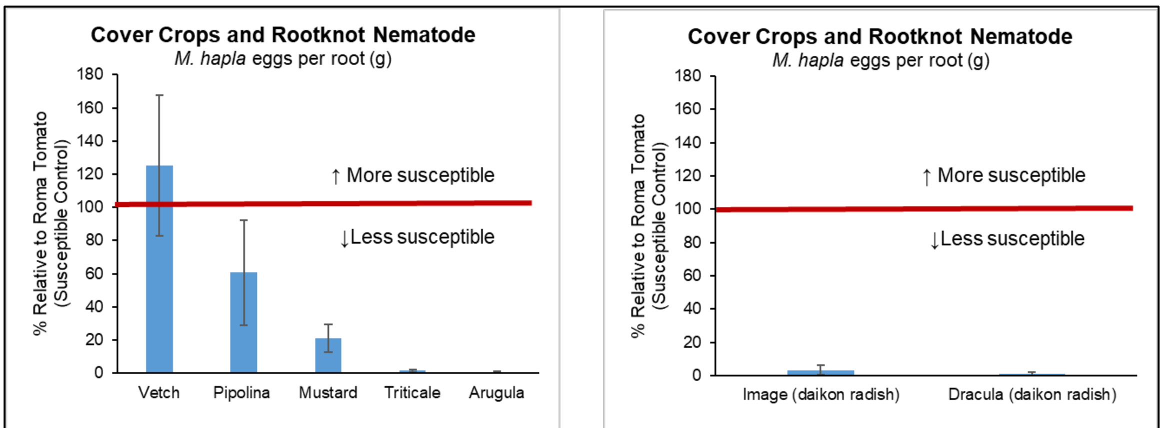

Fig. 10 – *While still preliminary, several of the cover crops evaluated, including the daikon radishes, mustard, triticale and arugula, supported less RKN development than the susceptible Roma tomato control plants, indicating their potential value as a post-plant, undervine cover crop in established or establishing vineyards.*

OUTREACH AND EDUCATION EFFORTS - PRESENTATIONS OF RESEARCH

Information dissemination needs to be a multi-pronged approach, where repetition and familiarity are key. As such, our team focuses on presenting trial results (once they are ready to present, of course!) in multiple formats, from posters to presentations, from trade articles to podcasts. Below is a listing of a few key Extension efforts the team has completed over the course of this project.

Posters:

- "Annual Update: Effect of Rootstock on Scion Nutrient Status". 2023 Washington State Grape Society Annual Meeting, Grandview, WA, USA.
- "Fumigation and Rootstocks: Managing Plant-Parasitic Nematodes in Vineyard Replant Scenarios." 2023 Washington State Grape Society Annual Meeting, Grandview, WA, USA.
- "Effect of Rootstock on Scion Nutrient Status." Poster. 2023 Washington WineVit Conference, Kennewick, WA, USA. **(*Award: 1st Professional*).**
- "Cover Crop Alternatives for Nematode Management in Washington Vineyards." Poster. 2023 Washington WineVit Conference, Kennewick, WA, USA. **(*Award: 1st Graduate Student*).**
- "Annual Update – Rootstocks for Vineyard Nematode Management." Poster. 2022 Washington WineVit Conference, Kennewick, WA, USA. **(*Award: 1st Professional*).**
- "Plants Helping Plants: Cover Crop Alternatives for Nematode Management in Washington Vineyards." Poster. 2022 Washington WineVit Conference, Kennewick, WA, USA. **(*Award: 1st Graduate Student*).**
- "Nematode Management in Vineyards Using Cover Crops." Nov 2021. Washington State Grape Society, Grandview, WA, USA.
- "Annual Update – Rootstocks for Vineyard Nematode Management." March 2021. Poster. WineVit2021 - Virtually presented. **(*Award: 1st Professional*).**
- "Alternative Strategies for Nematode Management." Poster. March 2021. WineVit2021 - Virtually presented. **(*Award: 1st Graduate Student – B. Gagnier*).**

Presentations:

- "What to Integrate with Nematode IPM." 11 Apr 2024. WAVE Connect. Richland, WA, USA. (Moyer)
- "What Looms Below – Pest in Vineyard Soils." 25 Jan 2024. Wilbur Ellis Grower's Webinar.
- "Litchi Tomato as a Fumigation Alternative in Washington State Wine Grape Vineyards." 22nd International Meeting of Viticulture G*i*ESCO. 17-21 July 2023. Ithaca, NY, USA (Gagnier)
- "Preplant Fumigation Only Temporarily Reduces Northern Root-knot Nematode." 22nd International Meeting of Viticulture G*i*ESCO. 17-21 July 2023. Ithaca, NY, USA (Moyer)
- "Designing (Rootstock) Field Trials." 13 Jul 2023. Washington Rootstock Field Day. Benton City, WA. (Moyer)
- "Screening Rootstocks Against the Northern Root-knot Nematode (*Meloidogyne hapla*)." 74th American Society for Enology and Viticulture National Conference. 27-29 June 2023. Napa, CA, USA. (Gagnier)
- "Cover Crops for the Management of the Northern Root-Knot Nematode." Walla Walla Vit Tech 2023. Walla Walla, WA, USA (Gagnier)

- "Plant-parasitic nematode impacts in wine grape production." Sustainable Agriculture Expo 2023, San Luis Obisbo, CA. Online seminar (Zasada)
- "Nematode Management: To Fumigate or Not?" 20 Apr 2023. WAVEx. Virtual. (Moyer)
- "Cover Crops for the Management of the Northern Root-Knot Nematode." G.S. Long Annual Grower's Meeting 2023. Yakima, WA, USA (Gagnier)
- "Cover Crops for the Management of the Northern Root-Knot Nematode." G.S. Long Fertility Meeting 2023. Yakima, WA, USA (Gagnier)
- "Cover Crop Alternatives for Nematode Management in Washington Vineyards." Washington State Grape Society Annual Meeting 2022. Grandview, WA, USA (Gagnier)
- "Cover Crop Alternatives for Nematode Management in Washington Vineyards." Society of Nematology Annual Meeting 2022. Anchorage, AK, USA (Gagnier)
- "Playing the Long Game for Nematode Management in Wine Grapes." 4 Oct 2021. Department of Plant Pathology. Washington State University, Pullman, WA, USA. (Moyer)
- "Integration of rootstocks into PNW semi-arid grape production system for the management of soilborne insects and nematodes." 4 Aug 2021. Walla Walla Viticulture working group. ZOOM webinar. (Zasada)
- "Water Movement through Soil – Implications for Nematicides." 29 July 2021. WSU – WSGS Viticulture Field Day. Prosser, WA, USA. (Gagnier)
- "Are nematodes taking a sip out of your glass of wine?" 6 April 2021. Cornell AgriTech, Geneva, NY. Zoom seminar. (Zasada)
- "Own Rooted Grapevines – An Expert Panel Discussion." 15 Mar 2021. SommCon 2021. Zoom webinar. (Moyer)
- "Washington and Rootstocks: The Vineyard Conversation of the 2020s." 16 Mar 2021. 2021 WineVit. ZOOM Delivered. (Moyer)

Web and Other Media:

- "(S2:E31) Pathogens sneaking around your vines!? Act now and protect the wine! (Part 2)". 4 Oct 2023. I See Dead Plants Podcast: The National Crop Protection Network. https://sites.libsyn.com/416264 (Moyer)
- "(S2:E30) Pathogens sneaking around your vines!? Act now and protect the wine! (Part 1)". 27 Sept 2023. I See Dead Plants Podcast: The National Crop Protection Network. https://sites.libsyn.com/416264 (Moyer)
- "Soil Pests in Vineyards." Washington Ag Network Radio Show (radio broadcast only). 2 October 2023. (Moyer)
- Honcoop, D. (Host) (2019 – present). Real Food Real People. Bernadette Gagnier #079 [Audio Podcast]. 12 Oct 2021. https://content.blubrry.com/rfrp/Bernadette_Gagnier_Full_Episode.mp3

Publications:

- Gagnier, B., I. Zasada, and M. Moyer. Impact of Vineyard Fallow Practices on Reducing *Meloidogyne hapla* Population Densities. Plant Health Progress. (*Submitted for review, March 2024*).
- Gagnier, B.K. , M.S. Mireles, A.B. Peetz, I.A. Zasada and M.M. Moyer. 2024. Greenhouse Evaluation of Rootstocks Against the Northern Root-Knot Nematode (*Meloidogyne hapla*). Am. J. Enol. Vitic. 75:0750006. DOI: 10.5344/ajev.2024.23061
- Gagnier, B., I. Zasada, M. Mireles, and **M.M. Moyer**. 2023. Litchi Tomato as a Fumigation Alternative in Washington State Wine Grape Vineyards. (talk). 22nd International Meeting of Viticulture G*i*ESCO. 17-21 July, 2023. Ithaca, NY, USA. https://ives-openscience.eu/34235/
- Moyer, M.M., M. Mireles, B. Gagnier, K. East, and I. Zasada. 2023. Preplant Fumigation Only Temporarily Reduces Northern Root-knot Nematode. (talk). 22nd International Meeting of Viticulture G*i*ESCO. 17-21 July, 2023. Ithaca, NY, USA. https://ives-openscience.eu/34221/
- Schreiner, P., M.M. Moyer, K.A. East, and I.A. Zasada. 2023. Managing Arbuscular Mycorrhizal Fungi in Arid Columbia Basin Vineyards of the Pacific Northwest United States. Am. J. Enol. Vitic. 74:0740020. DOI: 10.5344/ajev.2023.23005
- East, K.E., I.A. Zasada, R.P. Schreiner, and M.M. Moyer. 2022. Irrigation and Rootstocks to Manage Northern Root-Knot Nematode in Wine Grape Vineyard Establishment. Plant Health Progress 23:49-56. DOI:10.1094/PHP-06-21-0097-RS
- East, K.E., I.A. Zasada, J. Tarara, and M.M. Moyer. 2021. Field Performance of Wine Grape Rootstocks and Fumigation During Establishment Phase in Washington Vineyards. Am. J. Enol. Vitic. 72: 113-125. DOI:10.5344/ajev.2020.20023

Trade Publication Interviews / Mentions:

- Good Fruit Grower - *Hansen: The Fumigation Fade*. 15 April 2023.
- Good Fruit Grower – *Hansen: Long-term Investment in Nematode Research Pays Off*. 1 Feb 2022.
- Good Fruit Grower – *Changes taking root in Washington vineyards.* 10 Nov 2021.
- Good Fruit Grower – *WineVit session focuses on rootstock solutions for Washington.* 16 Mar 2021.

RESEARCH SUCCESS STATEMENT:

The results from these trials are foundational as the industry begins transitioning to rootstocks in future plantings. We are building the necessary background to truly understand the efficacy of different preplant (and replant) management tools that have been industry staples in the past (fumigation), and that are of interest for those looking for reduced inputs (trap crops, cover crops). Combined, the impacts of using rootstocks and practicing alternative site remediation approaches (cover crops, fallow periods) may prove to provide enough reduction in nematode pressure to eliminate the perceived need of chemical inputs in vineyard renovation / replanting.

FUNDS STATUS:

We had requested a 1 year no-cost extension in spring of 2023. Those funds have been expensed.

LITERATURE CITED

Aydinl G., and Mennan S. 2018. Biofumigation Studies by Using *Raphanus sativus* and *Eruca sativa* as a Winter Cycle Crops to Control Root-knot Nematodes. Brazilian Archives of Biology and Technology, 61.

Belair G, and Parent LE. 1996. Using crop rotation to control *Meloidogyne hapla* Chitwood and improve marketable carrot yield. HortScience 31: 106-108.

Dandurand LM, Zasada IA, LaMondia JA. 2019. Effect of trap crop, *Solanum sisymbriifolium*, on *Globodera pallida, Globodera tabacum* and *Globodera ellingtonae*. J. Nematol. 51: 1-11.

East KE, Zasada IA, Schreiner P, and Moyer MM. 2019. Developmental Dynamics of *Meloidogyne hapla* in Washington Wine Grapes. Plant Dis. 105: 966-971.

East KE, Zasada IA, and Moyer MM. 2021. Field Performance of Wine Grape Rootstocks and Fumigation During Establishment Phase in Washington Vineyards. Am. J. Enol. Vitic. 72:113-125.

Ferris H, Zheng L and Walker MA. 2012. Resistance of grape rootstocks to plant-parasitic nematodes. J Nematol 44: 377-386.

Hajihassani A, Rutter WB, Schwarz T, Woldemeskel M, Ali ME and Hamidi N. 2020. Characterization of Resistance to Major Tropical Root-Knot Nematodes (*Meloidogyne* spp.) in *Solanum sisymbriifolium*. Phytopathology. (Online only)

Harbertson JF, and Keller M. 2012.Rootstock effects on deficit-irrigated winegrapes in a dry climate: Grape and wine composition. Am. J. Enol. Vitic. 63: 40-48.

Keller M, Mills LJ, and Harbertson JF. 2012.Rootstock effects on deficit-irrigated winegrapes in a dry climate: Vigor, yield formation, and fruit ripening. Am. J. Enol. Vitic. 63: 29-39.

McKenry MV and Anwar SA. 2006. Nematode and grape rootstock interactions including an improved understanding of tolerance. J. Nematol. 38:312-318.

McKenry MV, Kretsch JO and Anwar SA. 2001. Interactions of selected rootstocks with endoparasitic nematodes. Am. J. Enol. Vitic. 52:310–316.

Sauer MR. 1967. Root knot tolerance in some grape vine rootstocks. Aust. J. Exp. Agric. 7:580-583.

Scholte K, Vos J. 2005. Effects of potential trap crops and planting date on soil infestion with potato cyst nematodes and root-knot nematodes. Ann. Appl. Biol. 137:153-164.

Zasada IA, Howland AD, Peetz AB, East K and Moyer M. 2019. *Vitis* spp. rootstocks are poor hosts for *Meloidogyne hapla*, a nematode commonly found in Washington winegrape vineyards. Am. J. Enol. Vitic. 70:1-8.