Litchi Tomato as a Fumigation Alternative in Washington State Wine Grape Vineyards

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Abstract:

Context and purpose of the study – The northern root-knot nematode (*Meloidogyne hapla*) is one of the most prevalent plant-parasitic nematodes affecting Washington State *Vitis vinifera* vineyards. This nematode induces small galls on roots, restricting water and nutrient uptake. In new vineyards this can impede establishment. In existing vineyards, it can exacerbate decline in chronically stressed vines. While preplant fumigation is a common strategy for *M. hapla* management, its efficacy is temporary and relies on broad-spectrum chemicals that undergo frequent regulatory scrutiny. The trap crop litchi tomato (*Solanum sisymbriifolium*) showed promise in reducing plant-parasitic nematode densities in potato. This prompted field greenhouse experiments to evaluate its potential to reduce *M. hapla* in *V. vinifera*.

Material and methods – A field experiment was conducted in a commercial vineyard block with vines recently removed in Mattawa, WA, USA. Ground cover treatments consisted of plots: 1) seeded to litchi tomato for one year; 2) seeded to litchi tomato for two consecutive years; or 3) a weedy fallow control, all replicated 4 times in a randomized complete block design. Plots were watered with overhead irrigation and soil samples were collected in spring and fall. In a separate greenhouse experiment, 6-week-old litchi tomato and a 'Roma' tomato control were transplanted into 4-inch pots, inoculated with 500 *M. hapla* eggs per pot, and allowed to grow to specific development stages before destructively harvesting at two-week intervals to evaluate the duration of root development on *M. hapla densities* in the soil.

Results – In the field experiment, after one season of growth, litchi tomato reduced *M. hapla* densities by 74.5% relative to the weedy fallow. This significant effect was also seen the following spring with a 65.3% reduction. By the end of the second season of growth, plots that were planted to litchi tomato for two years had an 83.1% reduction in *M. hapla* compared to weedy fallow plots. Soil samples collected in the spring after the second season has reduced levels (76.6%) of *M. hapla* in plots that were seeded for two years relative to control plots. This field experiment demonstrates that litchi tomato reduced *M. hapla* densities relative to allowing a site stay as a weedy fallow. Greenhouse trials are currently underway investigating how long litchi tomato needs to grow to be the most effective. Used in conjunction with *M. hapla* resistant or tolerant rootstocks and other post-plant nematicidal or nematistatic cover crops, our results demonstrate that litchi tomato has potential as part of an integrated pest management approach in the management of *M. hapla* in Washington State wine grape vineyards.

Keywords: wine grapes, Cover Crops, Sustainability, Northern Root-knot nematode, integrated pest management

1. Introduction

The northern root-knot nematode, *Meloidogyne hapla,* is the primary plant parasitic nematode in Washington state vineyards (Zasada et al. 2012). *Meloidogyne hapla* attacks and penetrates grape roots as second-stage juveniles (J2). Once in the root, feeding induces the formation of galls which reduces the vine's ability to take up water and nutrients (Esmenjaud 2003). While pre-plant fumigation is a common strategy for managing this nematode, its efficacy is temporary (East et al. 2021). In addition to temporary success, fumigation options for plant parasitic nematode management are progressively limited. Post-plant nematicides are another option, but their application must align with specific nematode biological development windows (East et al. 2019), which can prove challenging in commercial vineyard settings.

An alternative approach for nematode management in vineyard systems is the use of cover crops. Litchi tomato (*Solanum sisymbrifolium*) is one example. Litchi tomato has been demonstrated to reduce densities of potato cyst nematode (*Globodera* spp.) in the soil, functioning as a as a trap crop (Dandurand and Knudsen 2019). Litchi tomato functions as a trap crop, as its root exudates trigger potato cyst nematode egg hatching, but it is a non-host for the nematode. Unable to establish a feeding site, the newly hatched potato cyst nematodes starve, halting the reproduction cycle (Dandurand and Knudsen 2019). There are many biological similarities between potato cyst nematodes and *M. hapla*; both are sedentary endoparasites with a soil mobile juvenile second stage; both utilize effectors to manipulate root cells for feeding and reproduction sites; and, both hatch from eggs as J2 when signaled by exudates from plants that can serve as a host. Therefore, we decided to explore litchi tomato as a management strategy for *M. hapla*.

2. Material and methods

Field evaluations: The trial was established in fallow ground that was previously planted to *Vitis vinifera* 'Cabernet Sauvignon' that were removed after 15 years of production. Treatments consisted of fallow ground, litchi tomato (planted for 1 year or 2 years consecutively), for a total of three cover crop treatments grown over two growing seasons. In the case of the 1-year litchi tomato, in the second growing season, those plots were allowed to return to fallow. Each cover crop treatment was duplicated so that cover crops were either mowed or unmowed each season. All six treatments were arranged in a randomized complete block design with treatments replicated four times. Litchi tomato seeds were broadcast seeded in spring 2020 and 2021 at a rate of 100 seeds/m^2. Irrigation was necessary for seed germination and was applied via overhead sprinklers in 8 hr sets that were adjusted as necessary throughout the growing season; fallow plots also received irrigation. Mowing, when applicable, occurred at flowering; this treatment was selected to reduce potential reseeding of the litchi tomato.

Soil samples (2.5 cm diam. by 25 cm deep) were collected in spring 2020 prior to litchi tomato seeding. This established initial population densities of *M. hapla* in the soil. Soil samples were again collected in fall of 2020 after one growing season. Sampling was repeated in spring 2021, prior to the second season of litchi tomato seeding and samples were collected again in fall 2021. Final soil samples were collected in spring 2022. Soil samples were processed, and nematodes elutriated via the use of a semi-automated elutriator (Seinhorst, 1962). Nematodes were extracted by elutriation and sugar centrifugation (Zasada et al. 2012) before enumeration under an inverted light microscope. Data will be evaluated using a modified ANOVA to analyze interactive effects. When appropriate, Tukey's HSD was used as a post-hoc means separation.

Greenhouse evaluations. Six-week-old litchi tomato and a 'Roma' tomato (*Solanum lycopersicum*) control were transplanted into 15 cm pots containing a loam-sand soil mix. Tomato is a favored host for *M. hapla* and was used as a positive control. Ten of each plant were inoculated with *M. hapla* eggs, while the other half were inoculated with *M. hapla* J2. Plants were allowed to grow for two, four or six weeks before being removed and replaced with a tomato plant to complete a tomato bioassay for an additional xx weeks (Singh and Gaur 1994). Tomato roots were then destructively harvested and evaluated for *M. hapla* eggs.

Eggs were extracted from roots and dyed with Acid fuchsin before enumeration under an inverted light microscope. Reproduction factor (final population density/initial population density) was used as a response variable for statistical evaluation. Reproduction factor values were evaluated using one-way ANOVA. To further differentiate means Tukey's HSD used as a post-hoc measure.

3. Results and discussion

3.1 - *Litchi tomato ability to reduce* M. hapla *population densities in vineyard soil.*

After one growing season, litchi tomato reduced *M. hapla* populations in plots where it was grown, relative to plots that remained fallow ($p<0.0001$; Fig.1A). Mowing of the plots did impact cover crop efficacy against *M. hapla* (*data not shown).* In the second year of the study, where there were three different cover crop treatments (2 years of fallow, 1 year of litchi tomato + 1 year of fallow, and 2 years of litchi tomato), plots that were consecutively seeded to litchi tomato for two years reduced *M. hapla* densities in soil ($p=0.0035$; Fig.1B). Plots that were seeded to litchi tomato in year 1, but were fallow in that second growing season, had an intermediary effect on *M. hapla* densities, transitioning from reducing *M. hapla* densities as a result of growing litchi tomato in year 1, to a rebuilding of a densities as the plots returned to fallow in year 2. These results suggest that like fumigation, the effects of litchi tomato are transient, and that vineyard replanting would likely need to occur by the season immediately following the use of litchi tomato in order to capitalize on its suppressive effect. Litchi tomato might be a viable preplant option in vineyards selecting multiple integrated pest management approaches. It is important to remember that litchi tomato is not appropriate for use as a post-plant vineyard cover crop, as once *M. hapla* eggs hatch, litchi tomato will not be the only plant attracting the nematodes – grapevine roots will also be present, and *M. hapla* will be able to continue its life cycle.

3.2 -*Greenhouse studies give insight into effective growing periods of Litchi tomato against* M. hapla *populations.*

Greenhouse studies are currently underway, with the target of better understanding the litchi tomato – *M. hapla* interaction. While we assume, based on the literature, that litchi tomato is effective by inducing egg hatch and disrupting subsequent J2 invasion, this is not well established for *M. hapla* (Dandurand and Knudsen 2019). Using both *M. hapla* egg and J2 inoculum, we hope to uncover how litchi tomato acts against *M. hapla*. We also do not know how long a litchi tomato plant needs to grow and produce root exudates to be effective. By evaluating different time periods of litchi tomato growth, we can better prescribe the use of litchi tomato as a fumigation alternative, such as recommending multiple plantings within a season to ensure soil depletion of *M. hapla* inoculum (Perpétuo et al. 2021). This fundamental information may lead to quicker turnover of fallow periods, and planting or replanting vineyards when *M. hapla* populations are most effectively reduced after the use of litchi tomato.

4. Conclusions

These results suggest that litchi tomato is a potential candidate as a pre-plant management option for the suppression of *M. hapla* in *V. vinifera* vineyards relative to using a pre-plant fallow period. When timing and mode-of-action against *M. hapla* become clearer, litchi tomato may become a valuable tool for vineyard growers as part of an integrated management approach of plant parasitic nematodes.

5. Acknowledgments

Funding for this project was provided through the Washington State Grape and Wine Research Program, including Washington State Wine Commission, Auction of Washington Wines, State Liter tax, and/or WSU Agriculture Research Center and partially funded through USDA National Institute of Food and Agriculture Hatch project 1016563. Collaboration with personnel from USDA-HCRU Corvallis OR has made these experiments possible.

6. Literature Cited

Dandurand L.-M., Knudsen G.R. 2016. Effect of the trap crop *Solanum sisymbriifolium* and two biocontrol fungi on reproduction of the potato cyst nematode, *Globodera pallida*. Ann.Applied Bio., 169(2), 180-189.

East K. E., Zasada I.A., Moyer M.M. 2021. Field performance of wine grape rootstocks and fumigation during establishment phase in Washington vineyards. Am. J. Enol. Vitic. 72, 113-125.

East K.E., Zasada I.A., Schreiner R.P., Moyer M.M. 2019. Developmental dynamics of *Meloidogyne hapla* in Washington wine grapes. Plant Dis. 103(5), 966-971.

Esmenjaud D. 2003. Insects and other animals, nematodes. Encyclopedia of Rose Science. Elsevier 444-448.

Gautier C., Montarry J., Piriou C., Renault L., Porte C., Yvin J-C., Nguema-Ona E., Fournet S. 2021. Impact of the genetic diversity of three cyst nematodes on the effectiveness of root exudates to induce hatching. European J. Plant Path. 161(3), 553–563.

Perpétuo L.S., Da Cunha M.J.M., Batista M.T., Conceição I.L._2021. *Solanum linnaeanum* and *Solanum Sisymbriifolium* as a sustainable strategy for the management of *Meloidogyne chitwoodi*. Sci. Rep. 11, 3484–3484.

Seinhorst J.W. 1962. Modifications of the elutriation method for extracting nematodes from soil. Nematologica 8, 117-128.

Singh J., Gaur H.S. 1994. A quick bioassay technique for estimation of root-knot infestation levels in soil. Ann. Plant Protec. Sci. 2, 52–54.

Zasada I.A., Riga E., Pinkerton J.N., Wilson J.H., Schreiner R.P. 2012. Plant-parasitic nematodes associated with grapevines, *Vitis vinifera*, in Washington and Idaho. Am. J. Enol. Vitic. 63, 522-528.

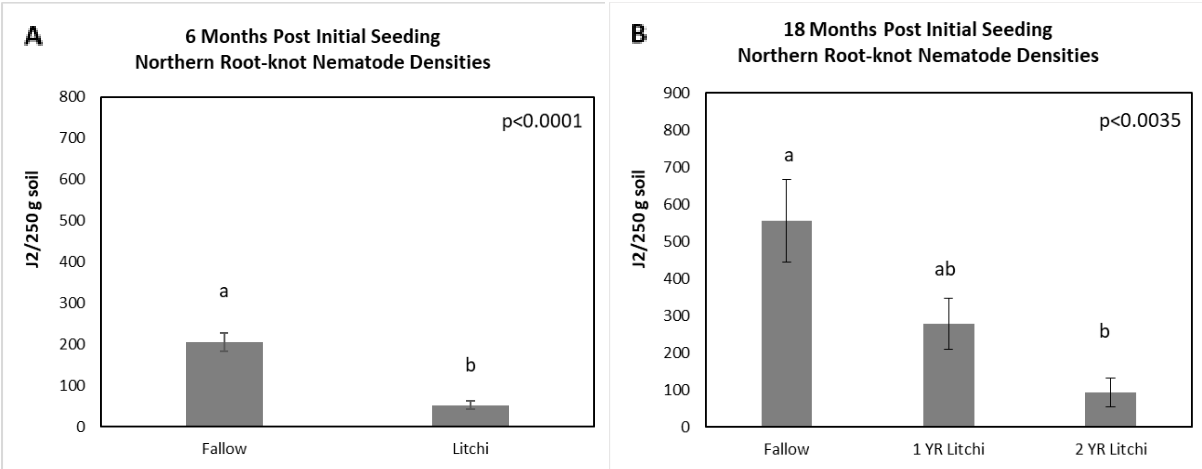

Figure 1: *Meloidogyne hapla* second-stage juveniles (J2) per 250 g of soil throughout the growing seasons in a field experiment in Mattawa, WA. **(A)** *M. hapla* J2 densities in fall 2020, **(B)** *M. hapla* J2 densities in fall 2021.