

Nitrogen inputs and irrigation frequency influence population dynamics of *Mesocriconema xenoplax* under grapevines

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Abstract

Nitrogen (N) fertilization and irrigation are critical for tree fruit and grape production in semi-arid regions of Western North America. Growers are increasingly considering more conservative fertilization and irrigation practices in order to optimize fruit quality while minimizing environmental impacts. The implications for pest populations of such shifts in production practices are not well known and warrant consideration. The objective of this research was to determine the effects of drip irrigation frequency (daily vs approximately every third day) and N fertilizer rate (ranging from 0 to 64 kg N/ha/year) on population densities of the ring nematode, *Mesocriconema xenoplax*, in a vineyard. The experiment was a split-plot randomized complete block design with irrigation frequency applied as whole-plot treatments and N input applied as subplot treatments. Nematode populations in root zone soils were assessed in spring, summer and fall of 2010 and 2011. There was a significant irrigation frequency × N input interaction, with *M. xenoplax* population densities increasing with N input under daily irrigation but not under low frequency irrigation. The data suggest that reductions in fertilizer N input and irrigation frequency, that have minimal impacts on fruit quality and yield, can also minimize *M. xenoplax* population buildup.

Keywords

Grape, Irrigation, Nematode management, Nematode ecology, *Mesocriconema xenoplax*, *Vitis*.

The ring nematode, *Mesocriconema xenoplax*, is an economically important parasite of woody perennial fruit crops including *Vitis* spp. wine-grape (Pinkerton et al., 2004) and *Prunus* spp., such as peach and plum (Ferris et al., 2004; Cao et al., 2006). Most wine-grape and tree-fruit production in western North America occurs in semi-arid regions with nutrient-poor soils and is dependent on irrigation and supplemental nitrogen (N) inputs for optimal productivity. For reasons of optimizing fruit quality and minimizing environmental impacts, new and generally more conservative N fertilization (Hannam et al., 2013) and irrigation practices (Bowen et al., 2012; Hannam et al., 2013) are increasingly being evaluated and adopted by growers. Little is known, however, of the non-target and possibly longer-term influences of

such changes in N inputs and irrigation practices on the development of pest and pathogen populations, particularly for plant-parasitic nematodes such as *M. xenoplax*.

As herbivores, the fecundity of plant-parasitic nematodes is likely to be positively influenced by the N status of their hosts (Mattson, 1980), but relationships between fertilization of woody perennial crops and the population dynamics of *M. xenoplax* have never been studied directly. Previous research suggests that *M. xenoplax* population growth is favored by moist soil conditions and therefore also likely to be influenced by irrigation practices. For example, Howland et al. (2014) reported that *M. xenoplax* population densities were spatially aggregated under drip emitters in a Washington vineyard. In three Spanish vineyards,

population peaks of *M. xenoplax* population densities coincided with periods of high precipitation within a year (Pinochet and Cisneros, 1986). Similarly, changes in *M. xenoplax* population densities in a South Carolina peach orchard were more closely related to soil moisture than to temperature (Nesmith et al., 1981).

Because *M. xenoplax* has the potential to cause significant damage to perennial fruit crops at high population densities (Ferris et al., 2004; Pinkerton et al., 2004; Cao et al., 2006), it is important to determine how variation in N fertilization and irrigation practices affect its population dynamics. A field experiment designed to examine the effects of N fertilizer application rate and irrigation frequency on productivity and fruit quality of wine-grapes was established in the Okanagan Valley of British Columbia in 2006 (Hannam et al., 2013). Subsequently, the site was found to be infested with *M. xenoplax*. The objective of this research was to determine the interactive effects of N fertilizer application rates and drip irrigation frequency on population densities of *M. xenoplax* in the root zone of wine-grape in the semi-arid Okanagan Valley of British Columbia.

Materials and methods

Experimental design

The field experiment was initiated in 2006 in a three year-old experimental vineyard at the Summerland Research and Development Centre (SRDC), located in the Okanagan Valley, near Summerland, British Columbia (49° 34'N, 119° 39'W). The soil was an Osoyoos loamy sand (Wittneben, 1986) with 90% sand, 9% silt and 1% clay. The vines were variety Merlot (*Vitis vinifera* L.) grafted onto SO-4 rootstock (*Vitis berlandieri* × *V. riparia*), and planted at 1.2m inter-vine spacing, and 3m inter-row spacing. A 1 m herbicide strip was maintained on both sides of the vine rows, and disease and insect control measures followed standard industry practices (BC Tree Fruit Production Guide; www.bctfpg.ca; accessed November 9, 2018).

The experimental design was described previously (Hannam et al., 2013). Briefly, the planting was divided into six blocks, each of which was a section of row consisting of two irrigation whole-plots encompassing 20 vines each. The irrigation system was set up as two parallel systems with two separate lines running down each vine row, with each line used to irrigate one of the two irrigation whole-plots via two 4Lh⁻¹ drip emitters per vine, located approximately 0.3m to each side of every vine within the irrigation whole-plot. The two irrigation treatments randomly applied to the two whole-plots in each block were: daily

irrigation, in which 100% of the previous day's losses to evapotranspiration (ET) were replaced each day; and reduced frequency irrigation, in which irrigation was withheld for at least three days and water losses to evapotranspiration were only replaced when a minimum of three hours of irrigation were required. Irrigation treatments were initiated between two and four weeks pre-bloom and were maintained until harvest each year. The intention of the treatments was to alter irrigation frequency while applying the same total amount of water in the two treatments (100% ET). However, the actual quantity of water applied to the reduced irrigation frequency treatment plots was 87 to 93% of that applied to the daily irrigation treatments each year (Hannam et al., 2013). An equipment failure resulted in the low frequency treatment receiving only 61% of the water applied to the daily irrigation treatment plots in July 2010.

Evapotranspiration was measured using an electronic atmometer (ETGage Co, Loveland, CO) linked to irrigation valves via a CR10X datalogger (Campbell Scientific, Logan, UT), and a seasonal crop coefficient curve for grape was used to convert atmometer readings to an estimate of vineyard ET as described in Hannam et al. (2013). Soil moisture was monitored periodically over three weeks in August of each year, 2007 through 2010 (no data were collected in 2011 due to equipment failure). Soil moisture was measured using time domain reflectometry (TDR – Trase TDR, Soilmoisture Corp., Santa Barbara, CA). TDR probes were 20cm in length and were placed within the vine row, approximately 0.15m from a vine, in six daily irrigation plots and six reduced frequency irrigation plots. Summary data for the four years of soil moisture monitoring (2007 to 2010) were used to compare overall differences between the two irrigation frequency treatments (Table 1).

Each of the two irrigation whole-plots in each block was further sub-divided into four 5-vine split-plots to which the four N input treatments were randomly allocated. The N treatments were applied as urea (46-0-0) at four levels: control – 0g urea-N/vine, 5g urea-N/vine, 10g urea-N/vine and 20g urea-N/vine. These rates are equivalent to 0, 16.6, 32.2 and 64.4 kgN/ha, respectively. The urea was applied to the soil surface directly below the two emitters on either side of each vine in June of each year. Leaf petiole N concentrations were determined each year of the study as described in Hannam et al. (2013).

Nematode sampling and data analyses

In May, July and October of 2010 and 2011, five and six years after initiation of the experiment, six

Table 1. Effects of nitrogen (N, as urea) input and irrigation frequency on grapevine petiole N concentrations (% dry weight) determined at full bloom.

Fertilizer N input	Irrigation frequency	
	Daily (% N)	Low frequency (% N)
0 kg N ha ⁻¹ year ⁻¹	0.91 (0.02)	0.89 (0.02)
16.6 kg N ha ⁻¹ year ⁻¹	0.91 (0.02)	0.94 (0.04)
33.2 kg N ha ⁻¹ year ⁻¹	1.01 (0.05)	0.94 (0.04)
66.4 kg N ha ⁻¹ year ⁻¹	1.12 (0.06)	1.11 (0.06)
ANOVA table	Pr>F	
Irrigation frequency	0.63	
N fertilization	<0.0001	
Irrigation × N fert	0.61	

Values are averages from 2007 to 2011 ($n=30$; five years × six blocks), followed by standard error in parentheses.

2-cm diameter × 30-cm deep cores were taken from within the herbicide strip around the base of the three central vines in each plot and composited. The six cores were collected as follows: two cores from along the vine row, two from approximately 45° off the vine row, and two perpendicular to the vine row.

Soil samples were first passed through a 6mm sieve to remove stones and root fragments. Nematodes were extracted from a 100 cm³ subsample using a wet-sieving sucrose centrifugation procedure (Forge and Kimpinski, 2007), and the *M. xenoplax* in each sample extract were counted using a gridded counting dish on an inverted microscope. Root fragments were separated into coarse (>2mm diameter) and fine (<2mm diameter) size fractions, dried, weighed and data were expressed as g roots per kg dry soil.

Mesocriconeema xenoplax population density data, root biomass data and leaf petiole N data were subjected to a mixed-model blocked two-way split-plot repeated measures analysis of variance using Proc Mixed in SAS (Statistical Analysis Systems, Cary, NC). Fixed factors were irrigation frequency and N input treatment. Block was treated as a random factor in the model and sample date was considered in the model as repeated measures. Population

densities of *M. xenoplax* were log-transformed by log(X+100) prior to analysis of variance to minimize heteroscedasticity and improve model fit.

Results and discussion

While sample date had a significant main-factor effect on population densities ($p<0.001$), there were no significant interactions of sample date with N input or irrigation frequency treatments. Representing the main-factor effect of sample date, overall mean *M. xenoplax* population densities (averaged over four N input rates, two irrigation frequencies and six blocks; $n=48$) for the May, July and October sample dates were 1181, 690 and 850 nematodes/100cm³ soil in 2010, and 255, 945 and 1423 nematodes/100cm³ soil in 2011, respectively.

Because there was no interaction of sample date with N input or irrigation frequency, further presentation and discussion of the data is focused on the N input and irrigation frequency treatment combinations, averaged over sample dates and blocks (Fig. 1). There was a significant irrigation frequency × N input interaction effect on *M. xenoplax* population densities ($p=0.01$). Under the high frequency (daily) irrigation treatment, *M. xenoplax*

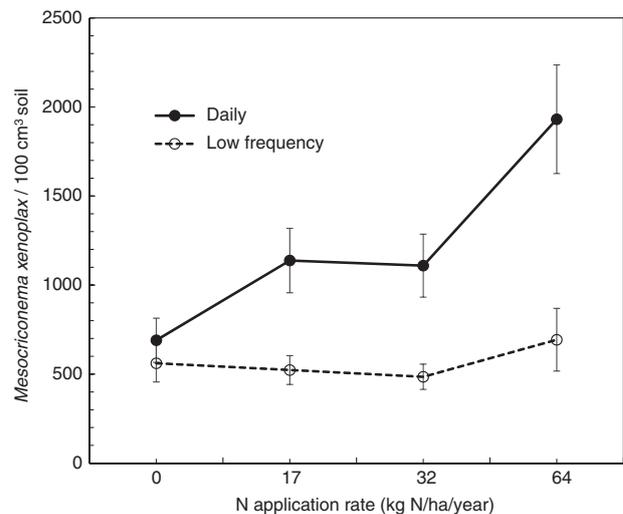


Figure 1: Effects of nitrogen (N, as urea) input and irrigation frequency on population densities of the ring nematode, *Mesocriconeema xenoplax*, in root zone soils of wine-grape. Values are averages, (+/-) standard errors of means, from six blocks and six sample dates during 2010–2011 ($n=36$).

population densities tended to increase with N input rate while they did not increase with N input rate under the low frequency irrigation treatment (Fig. 1). The main-factor effect of N input was significant ($p=0.03$) and the main-factor effect of irrigation frequency was marginally significant ($p=0.06$). There were no significant effects of N input, irrigation frequency or N input \times irrigation frequency interaction on either total root biomass or fine root biomass (data not shown).

Because there were no significant interactions of sample date with N input or irrigation frequency treatments, we conclude that the treatment-induced changes in *M. xenoplax* population densities occurred mostly before 2010, year five of the experiment, when assessment of *M. xenoplax* population densities began. It is not possible to relate changes in *M. xenoplax* population densities to actual soil moisture regimes or plant N status in specific years preceding 2010. Consequently, discussion of *M. xenoplax* population data in relation to soil moisture regimes or plant N status is limited to data summarized over the first five (plant N status, Table 1) or four (soil moisture, Table 2) years of the experiment.

Our data, demonstrating an increase in *M. xenoplax* population densities with N fertilizer application rate up to 64kgN/ha (20g per vine), are consistent with previous studies documenting increased population densities of plant-parasitic nematodes in response to N fertilization of grasslands (Todd, 1996; Sarathchandra

et al., 2001; Forge et al., 2005). It is important to note that this body of research contrasts with numerous previous studies documenting nematicidal effects of ammonium-based fertilizers and organic amendments with high N contents (e.g. Rodriguez-Kabana, 1986; Oka et al., 2006; Su et al., 2015). This latter group of studies generally involved incorporation of organic amendments or fertilizer N into the soil at relatively high rates (>200kgN/ha) prior to planting annual crops, with the intent of generating nematicidal levels of ammonium in soil solution (Rodriguez-Kabana, 1986; Oka et al., 2006; Su et al., 2015). In perennial systems involving surface application of fertilizers, sometimes split over multiple applications per year (Forge et al., 2005), it would likely be difficult to achieve such nematicidal levels of ammonium within the soil profile without exceeding optimal fertilization rates for wine-grape. In fact, this would probably be true for many woody perennial fruit crops, which generally require lower N inputs for optimal production than other crops.

We speculate that the positive influence of increasing N inputs on *M. xenoplax* population growth observed in this system was mediated through improved grapevine N status rather than via direct influences of ammonium or nitrate ions on nematodes in soil. The fecundity of herbivorous invertebrates is generally responsive to the N status of host plants (Mattson, 1980), and we hypothesize that this also applies to plant-parasitic nematodes. While the N input did not have consistent effects on root biomass (this study), canopy growth or yield (Hannam et al., 2013), petiole N concentrations generally increased with N input throughout the experiment (Table 1), and grapevine root N status can be correlated with both N fertilization rates and leaf petiole concentrations (Pino et al., 2012).

Alternatively, N inputs could have altered the composition of the soil food web, by reducing the activity of ring nematode antagonists such as nematophagous fungi or predacious nematodes. Previous research has shown that soil suppressiveness to plant-parasitic nematodes increases with the C:N ratio of organic amendments (Stirling, 2014), suggesting that soil N availability could regulate the activity of such suites of nematode antagonists. Indeed, Tenuta and Ferris (2004) found that omnivorous and predacious nematodes in the family Dorylaimida were more sensitive than other groups of nematodes to dissolved nitrate and ammonium. The direct effects of mineral N inputs on other antagonists such as nematophagous fungi or overall suppressiveness have not been determined.

We speculate that irrigation frequency had a direct effect on *M. xenoplax* population growth. Over the

Table 2. Effects of daily and low frequency irrigation treatments (irrigated to replace evapotranspiration loss) on volumetric soil moisture content of the top 20 cm of soil.

Soil moisture	Irrigation frequency	
	Daily	Low frequency
Mean (cm ³ /cm ³)	14.25 (0.62)	11.39 (0.76)
CV ^a	0.11	0.16
Maximum (cm ³ /cm ³)	17.81	16.30
Minimum (cm ³ /cm ³)	11.37	8.03

Values are averages from six plots over multiple dates in August of each year from 2007–2010, followed by standard error in parentheses. ^aCoefficient of variation (standard deviation/mean).

course of this experiment, soil moisture was more variable and, in general, lower with reduced irrigation frequency than with daily irrigation (Table 2). This is consistent with trends in soil moisture observed over two growing seasons in an adjacent apple orchard with two irrigation frequency treatments: daily irrigation and irrigation every two days (Fentabil et al., 2016). In general, nematode activity is known to be directly influenced by soil moisture (Seshadri, 1964; Simons, 1973). In an analysis of the spatial distribution of *M. xenoplax* in a drip-irrigated vineyard in Washington, Howland et al. (2014) found that greatest population densities were concentrated under drippers and positively correlated with soil water content. Our data extend these observations to show that daily irrigation, which resulted in overall slightly greater and more consistent soil moisture contents than the reduced frequency irrigation, led to greater *M. xenoplax* population densities in the entire plot area. It is not possible to determine from our data if the *M. xenoplax* population responded primarily to the differential average soil moisture, or to the differential magnitude of fluctuation in soil moisture, as these two aspects of irrigation frequency were confounded in this field experiment. Future research to determine which of these two aspects of changing irrigation frequency have the greatest impact on *M. xenoplax* population growth could reveal novel means of using irrigation scheduling to minimize *M. xenoplax* population growth while minimizing any negative impacts on vine water relations.

Population densities of *M. xenoplax* at this site greatly exceeded the damage threshold of 50 nematodes/100cm³ soil proposed by McKenry (1992). There was, however, no obvious relationship between *M. xenoplax* population densities and measurements of vine vigor in this study. While *M. xenoplax* population densities increased with N input and irrigation frequency, root biomass (this study) and other key indices of vine vigor (berry yield, berry weight, canopy density) did not vary significantly across treatments in this study (Hannam et al., 2013). It is of interest to note that overall vine vigor and productivity was expected to increase with N input and irrigation frequency in this field experiment, which was established prior to knowing that the site was infested with *M. xenoplax*. We speculate that, in the context of *M. xenoplax*-infested soil, vine vigor did not increase with N input and irrigation frequency because *M. xenoplax* population densities also increased with N input and irrigation frequency, and effects of the increased *M. xenoplax* population densities may have negated the expected direct benefits of such treatments on vine vigor. Micro-plot experiments directly comparing

responses of grapevines in *M. xenoplax*-infested and non-infested soil, to N input and different irrigation treatments, would prove whether increased population growth of *M. xenoplax* negates the expected benefits of increased N inputs and irrigation frequency on grapevine vigor.

Shifts in fertilization and irrigation practices have environmental and economic benefits (e.g. improved resource-use efficiency, improved fruit quality), and here we demonstrate that they can also affect population densities of *M. xenoplax*. We propose that it may be possible to strategically reduce N inputs or irrigation frequencies in vineyards extensively infested with *M. xenoplax*, in order to suppress population buildup and reduce longer-term impacts of nematode parasitism on grapevines.

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