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Shifts in water use in grapevine due to an invasive sap-feeding planthopper persist following insect removal

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Abstract:	Increasing outbreaks of invasive insect pests pose a substantial threat to the functioning and viability of cultivated and wild woody perennial species worldwide. In the eastern U.S., the spotted lanternfly (Lycorma delicatula White; SLF), an invasive phloem-feeding planthopper, was reported to be able to negatively impact late-season plant carbon dynamics in various woody perennial species following repeated or prolonged infestation and feeding events. However, it remains unclear if SLF infestations also impact plant water relations and if SLF-mediated effects persist when populations are controlled and feeding stops. This study investigated how late-season exposure to SLF impacts whole-plant water relations by assessing diurnal sap flow and trunk radius changes in grapevines subjected to varying infestation densities of adult SLF. In two seasons, vines exposed to high infestation densities (an average of 180 SLFs per vine) for up to 31-32 days of cumulative SLF exposure had significantly lower sap flow rate than those with no SLF, resulting in up to 38% less daily total water use. Trunk diurnal amplitudes increased under SLF infestation, but impacts were less dependent on infestation density, suggesting that grapevines may be utilizing trunk water storage during infestation to meet both grapevine water use and SLF sap ingestion. In both cases, SLF-mediated effects persisted following removal of SLFs, suggesting that exposure to high populations of SLF can alter patterns of late-season grapevine water use, at least when populations are not effectively controlled. These results indicate that SLF can modify both whole-plant water relations and carbon dynamics concurrently, further defining the implications that intensive infestations by high populations of SLF has for woody perennial whole-plant physiology.
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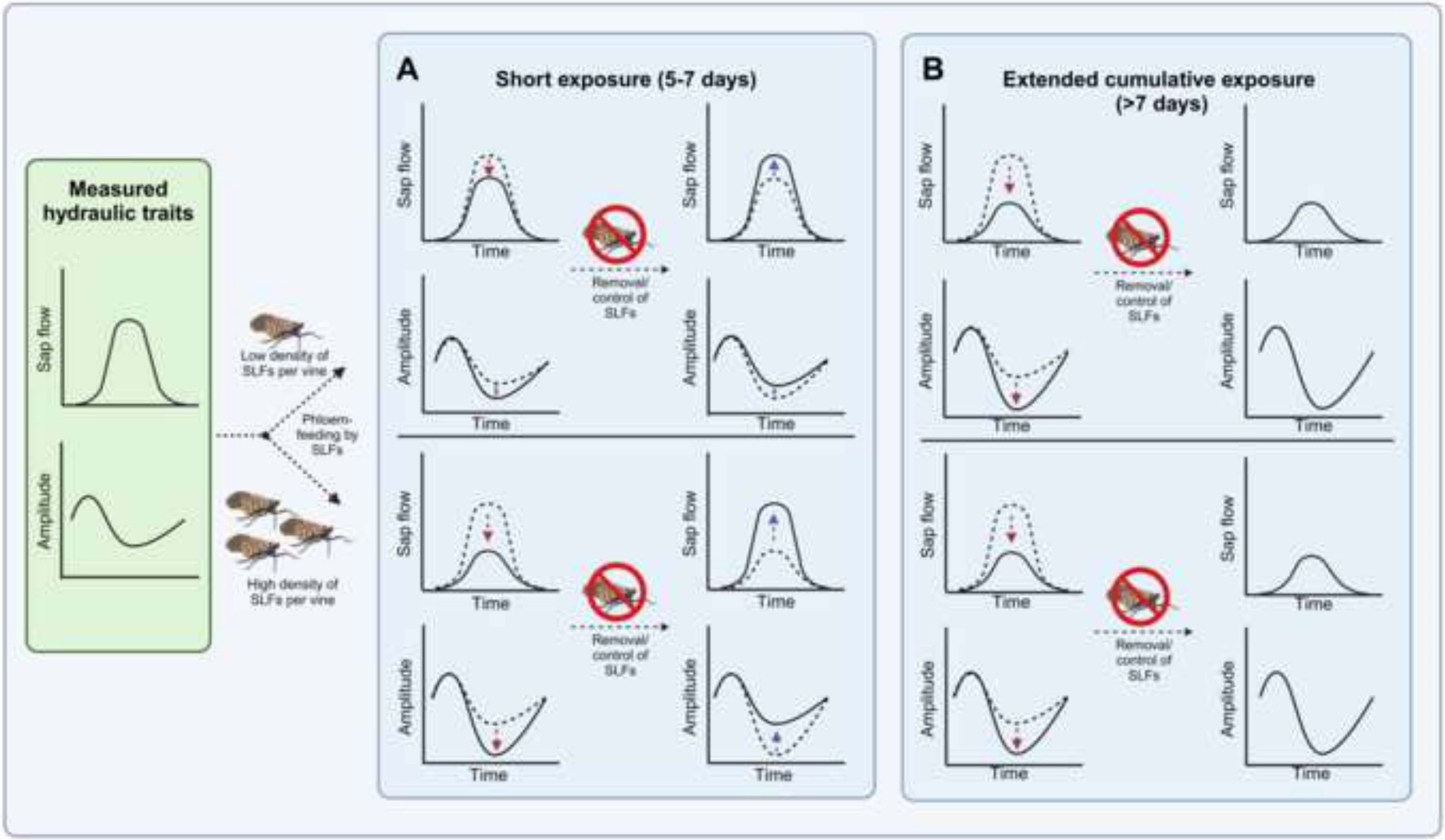
Title: Shifts in water use in grapevine due to an invasive sap-feeding planthopper persist following insect removal

Authors: Andrew D. Harner[1,4*], Heather L. Leach[2,5], Lauren Briggs[1], Donald E. Smith[1], Roman Zweifel[3], and Michela Centinari[1,*]

Highlights:

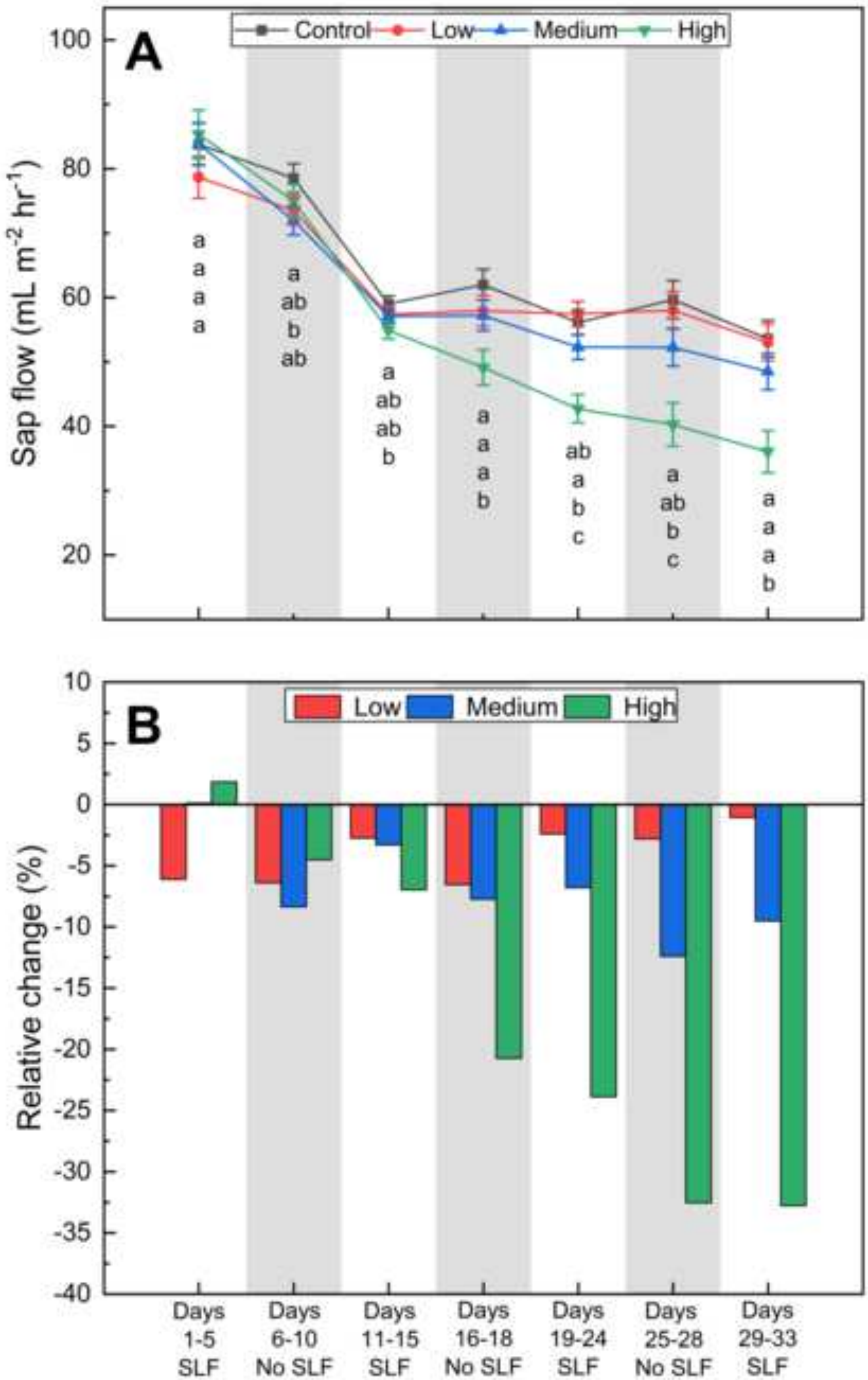
- Prolonged exposure to high populations of spotted lanternfly can lower grapevine water use
- Trends in lower water use persisted following insect removal
- Exposure to spotted lanternfly also increased diurnal trunk amplitudes, regardless of insect density
- Implications of spotted lanternfly on water relations may vary by region and climate

Figure 1



Measured hydraulic traits
Sap flow
Amplitude
Low density of SLFs per vine
Phloem-feeding by SLFs
High density of SLFs per vine
A
Short exposure (5-7 days)
Sap flow
Time
Amplitude
Removal/ control of SLFs
B
Extended cumulative exposure (>7 days)

Figure 2



A
Control
Low
Medium
High
Sap flow (mL m-2 hr-1)
100
80
60
40
20
a a a a
a ab b ab
a ab ab b
a a a b
ab a b c
a ab b c
a a a b
B
Low
Medium
High
Relative change (%)
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
Days 1-5 SLF
Days 6-10 No SLF
Days 11-15 SLF
Days 16-18 No SLF
Days 19-24 SLF
Days 25-28 No SLF
Days 29-33 SLF

Figure 3

Click here to access/download;Figure;New Figure 3.tif

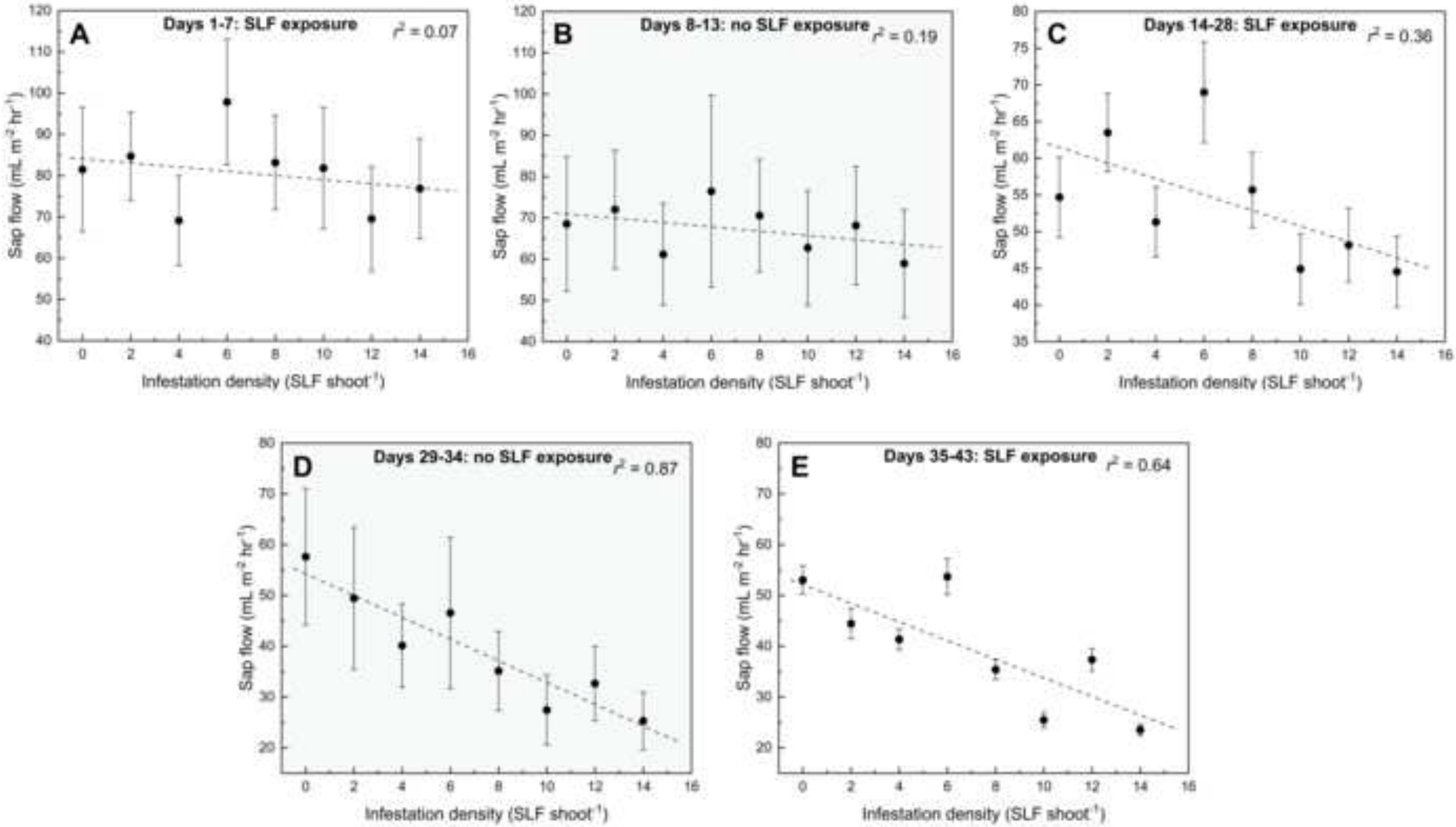


Figure 4

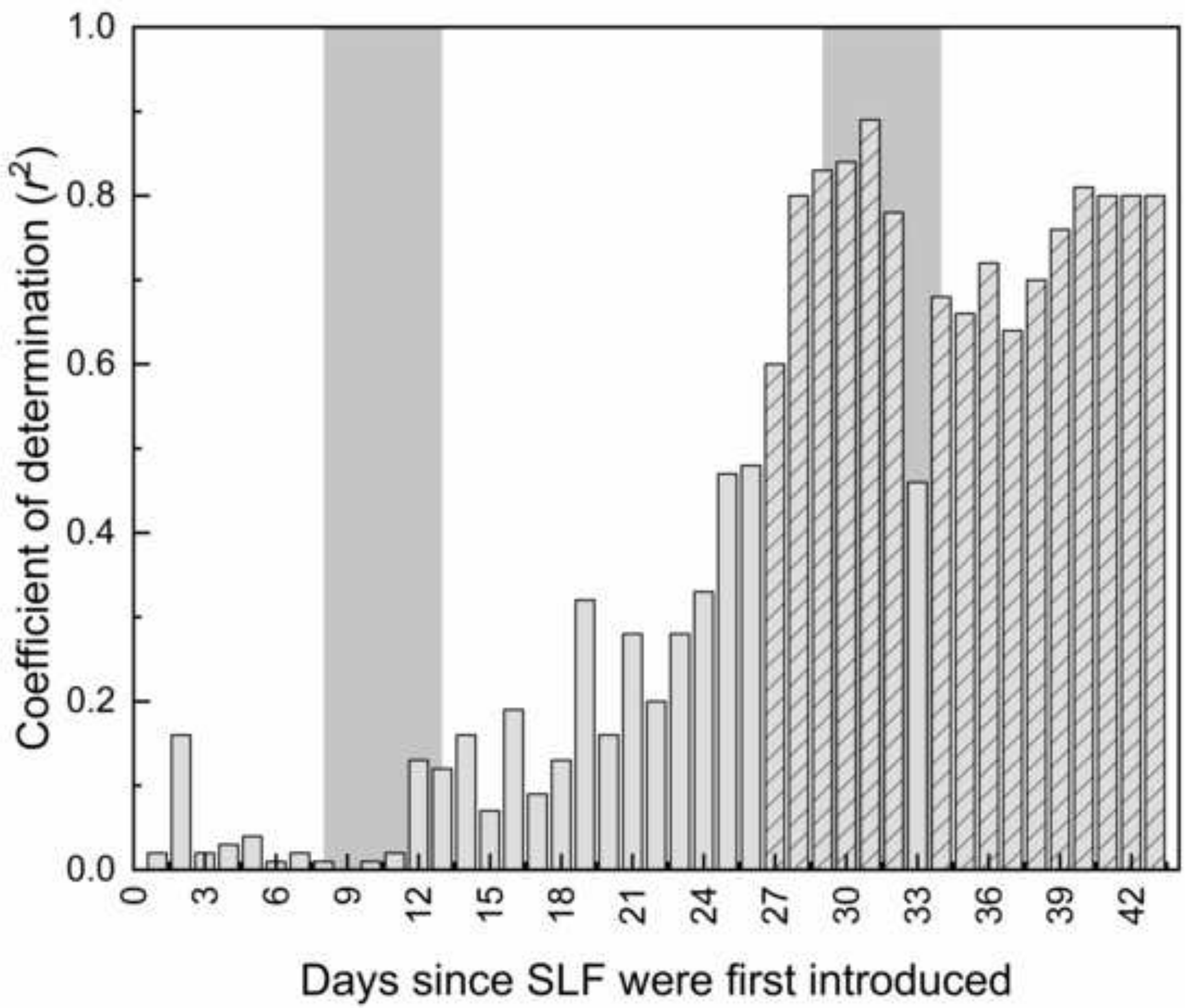
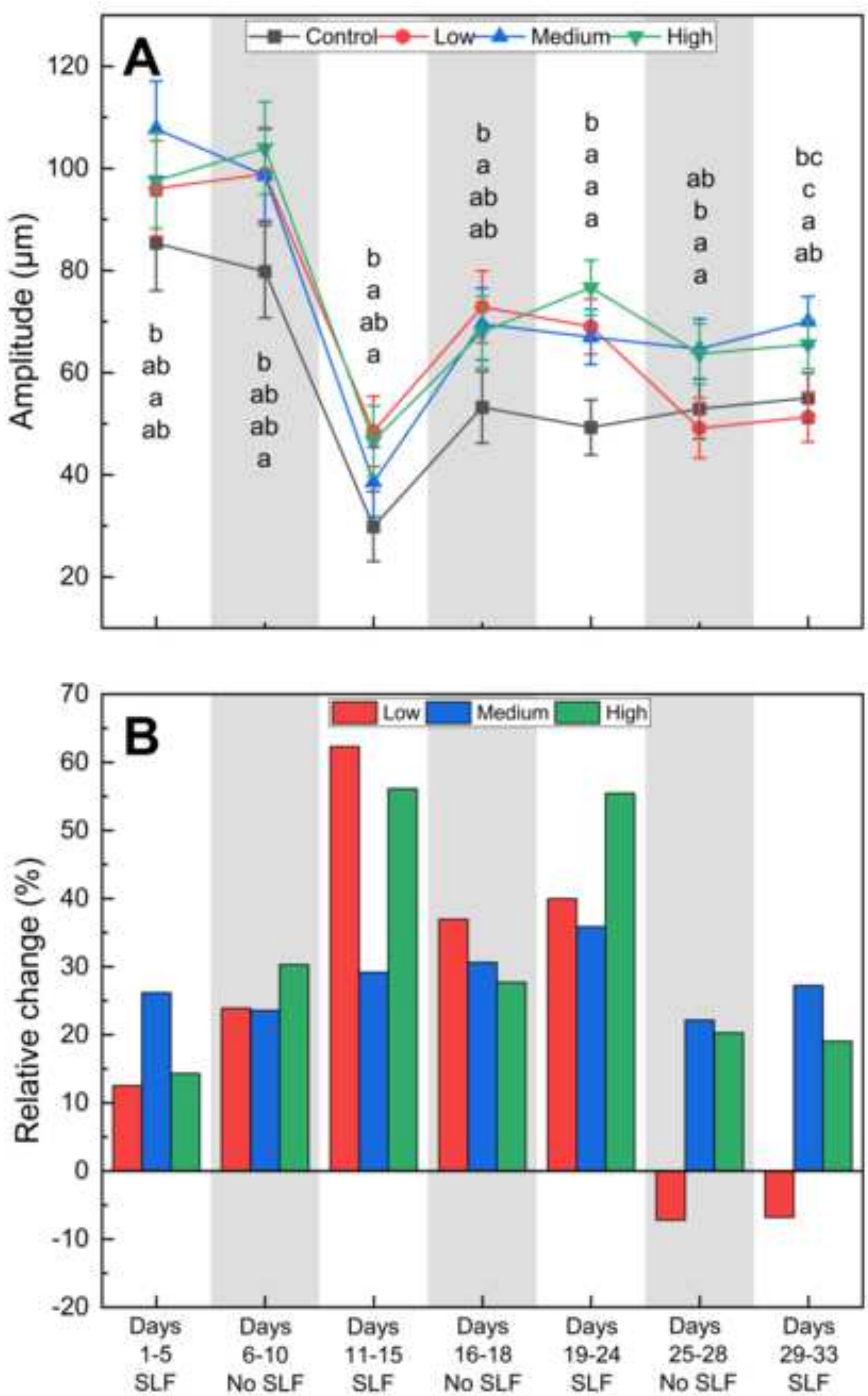
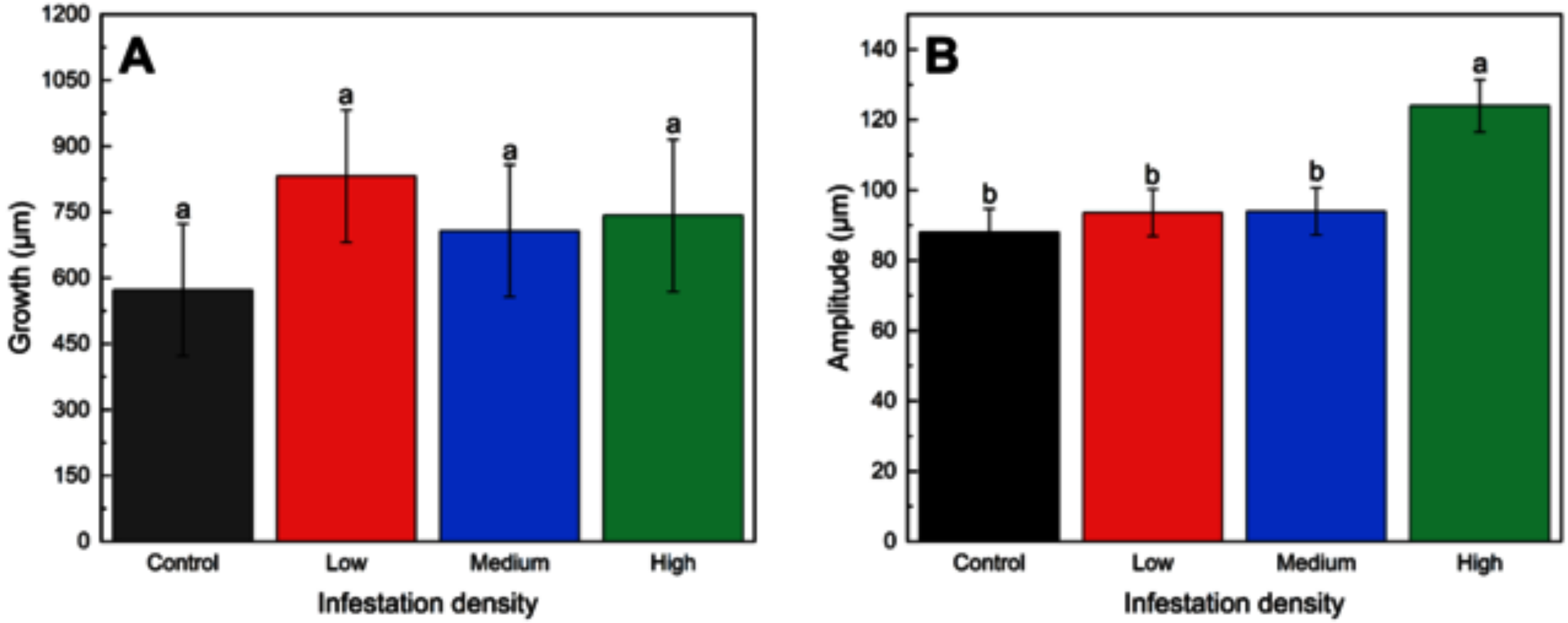

1.0
0.8
0.6
0.4
0.2
0.0
Coefficient of determination (r^2)
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
Days since SLF were first introduced

Figure 5



A
Control
Low
Medium
High
Amplitude (μm)
120
100
80
60
40
20
b
ab
a
ab
b
ab
ab
a
b
a
ab
a
b
a
ab
ab
b
a
a
a
ab
b
a
a
bc
c
a
ab
B
Low
Medium
High
Relative change (%)
70
60
50
40
30
20
10
0
-10
-20
Days 1-5 SLF
Days 6-10 No SLF
Days 11-15 SLF
Days 16-18 No SLF
Days 19-24 SLF
Days 25-28 No SLF
Days 29-33 SLF

Figure 6


A
Growth (µm)
1200
1050
900
750
600
450
300
150
0
a
a
a
a
Control
Low
Medium
High
Infestation density
B
Amplitude (µm)
140
120
100
80
60
40
20
0
b
b
b
a
Control
Low
Medium
High
Infestation density

Supplemental Figures

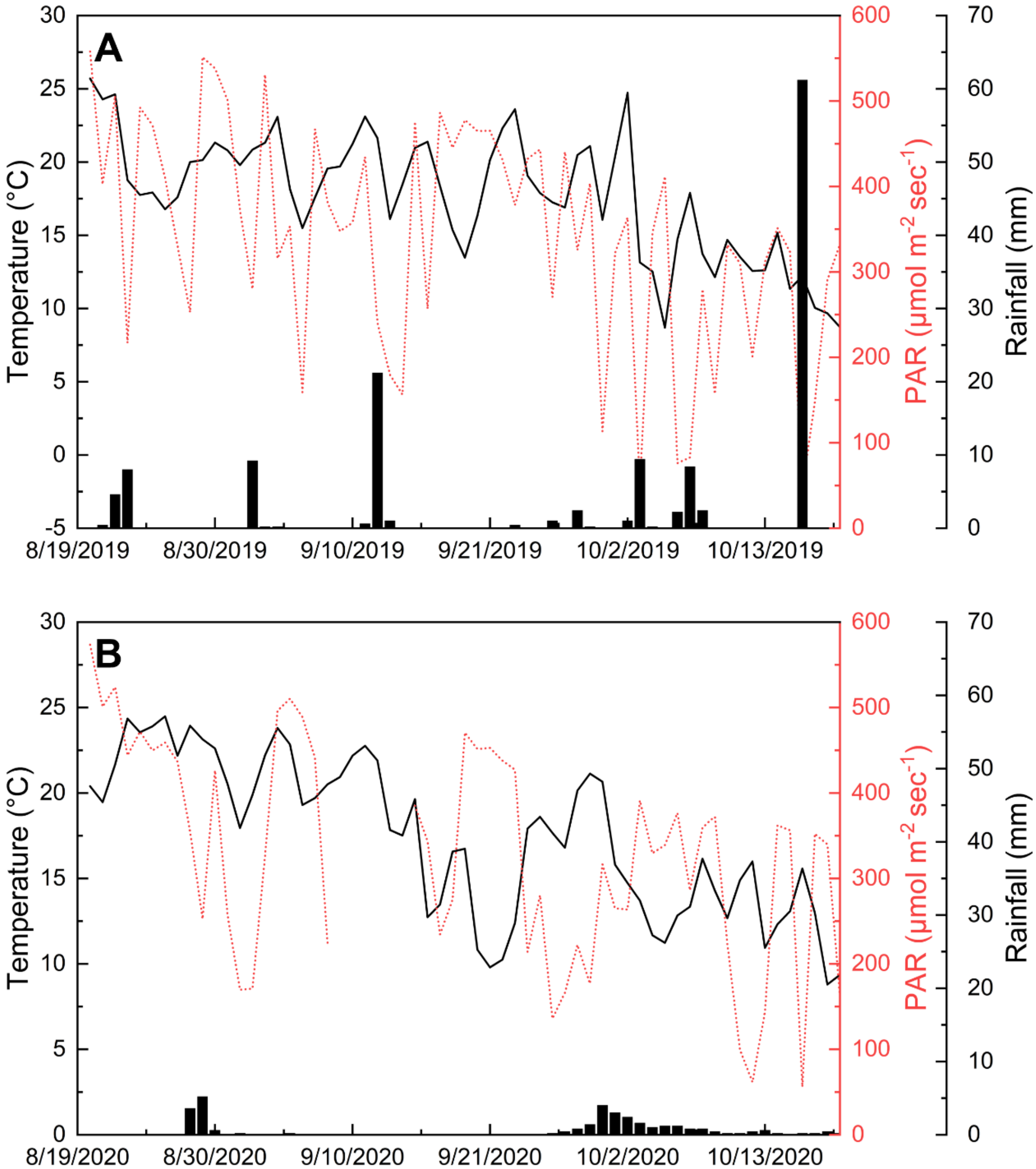


Figure S1. Vineyard daily average temperatures (°C; solid black line), PAR (µmol m^{-2} sec^{-1}; dotted red line), and rainfall (mm; black vertical bars) for the experimental periods in 2019 (A) and 2020 (B).

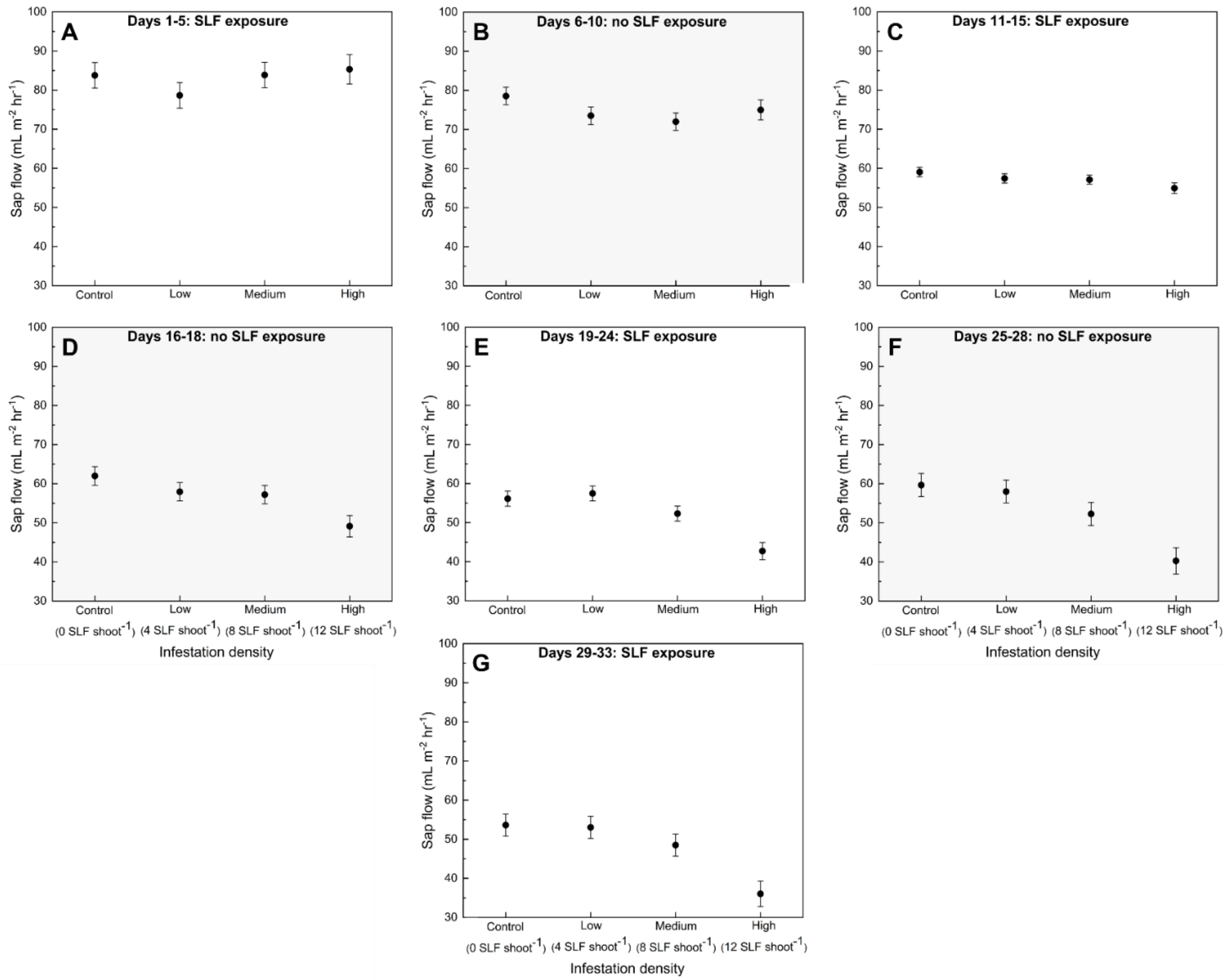


Figure S2. Mean hourly sap flow rate (mL m^{-2} hr^{-1}) averaged across each day for four infestations (i.e., days with SLF; A, C, E, and G) and the time between infestation (i.e., days with no SLF; B, D, F), from August 26 to September 28, 2019 for grapevines exposed to zero (control; 0 SLF $shoot^{-1}$), low (4 SLF $shoot^{-1}$), medium (8 SLF $shoot^{-1}$), and high (12 SLF $shoot^{-1}$) SLF density treatments, respectively. The data from this series of graphs corresponds with Figure 2A.

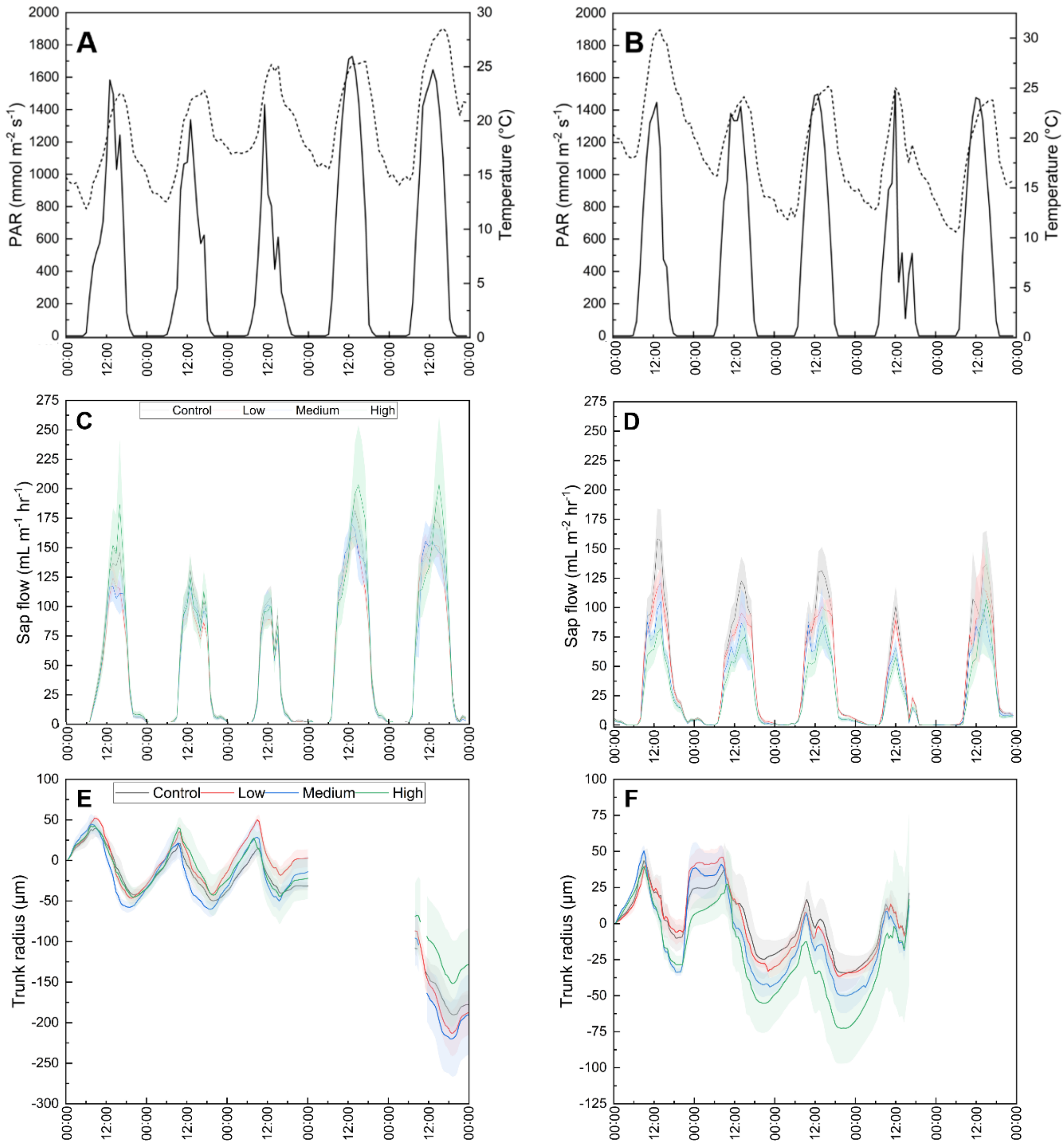


Figure S3. (A, B) Diurnal trends for air temperature (°C) and photosynthetically active radiation (PAR, mmol m^{-2} s^{-1}) measured at the experimental vineyard; (C, D) hourly sap flow rates (mL m^{-2} hr^{-1}) and (E,F) continuous trunk radial changes (µm) measured for Riesling grapevines exposed to zero (control; 0 SLFs $shoot^{-1}$), low (4 SLFs $shoot^{-1}$), medium (8 SLFs $shoot^{-1}$), or high (12 SLFs $shoot^{-1}$) infestation densities of adult SLFs during two infestations in 2019. Panels A, C, and E show trends from August 26 to August 30, when the first infestation occurred (1-5 days of cumulative SLF exposure) and panels B, D, and F show trends from September 23 to September

27, 2019, when the fourth infestation occurred (17-21 days of cumulative SLF exposure). For sap flow and trunk radius data, black, red, blue, and green lines represent the means per control, low, medium, and high SLF infestation density treatments, respectively, (black, control; red, low; blue, medium; and green, high) and shaded areas represent the standard error of the mean for each line. Gaps in data in panels E and F represent brief periods when dendrometer sensors were offline and not transmitting data.

Supplemental Table 1. Mean average sap flow rate (mL m^{-2} $hour^{-1}$) per infestation and the days in between for Riesling vines exposed to SLFs in 2019 from August 26 to September 28. Data was analyzed using a mixed model in PROC GLIMMIX and since there was a significant interaction, differences between treatments were analyzed using simple effects *t*-tests by the SLICEDIFF option. Different letters between treatments indicate significant differences between treatments ($p < 0.05$).

Treatment	Days 1-5 SLF	Days 6-10 No SLF	Days 11-15 SLF	Days 16-18 No SLF	Days 19-24 SLF	Days 25-28 No SLF	Days 29-33 SLF
Control	83.78 a	78.54 a	59.04 a	61.99 a	56.12 ab	59.67 a	53.61 a
Low (4 SLFs $shoot^{-1}$)	78.67 a	73.52 ab	57.42 ab	57.94 a	57.47 a	58.00 ab	53.04 a
Medium (8 SLFs $shoot^{-1}$)	83.88 a	71.98 b	57.10 ab	57.20 a	52.31 b	52.27 b	48.51 a
High (12 SLFs $shoot^{-1}$)	85.34 a	75.00 ab	54.93 b	49.14 b	42.72 c	40.26 c	36.04 b
p-value$_{SLF}$	**< 0.0001**						
p-value$_{CYCLE}$	**< 0.0001**						
p-value$_{SLF*CYCLE}$	**0.0015**						

Supplemental Table 2. Mean trunk amplitude (µm) per infestation and the days in between for Riesling vines exposed to SLFs in 2019 from August 26 to September 28. Data was analyzed using a mixed model in PROC GLIMMIX and since there was a significant interaction, differences between treatments were analyzed using simple effects *t*-tests by the SLICEDIFF option. Different letters between treatments indicate significant differences between treatments ($p < 0.05$).

Treatment	Days 1-5 SLF	Days 6-10 No SLF	Days 11-15 SLF	Days 16-18 No SLF	Days 19-24 SLF	Days 25-28 No SLF	Days 29-33 SLF
Control	85.38 b	79.80 b	29.88 b	53.22 b	49.30 b	52.93 ab	55.06 bc
Low (4 SLFs $shoot^{-1}$)	96.07 ab	98.89 ab	48.50 a	72.89 a	69.02 a	49.12 b	51.32 c
Medium (8 SLFs $shoot^{-1}$)	107.73 a	98.61 ab	38.60 ab	69.52 ab	66.98 a	64.65 a	70.06 a
High (12 SLFs $shoot^{-1}$)	97.62 ab	103.99 a	46.65 a	67.96 ab	76.65 a	63.67 a	65.65 ab
p-value$_{SLF}$	**< 0.0001**						
p-value$_{CYCLE}$	**< 0.0001**						
p-value$_{SLF*CYCLE}$	**0.0905**						

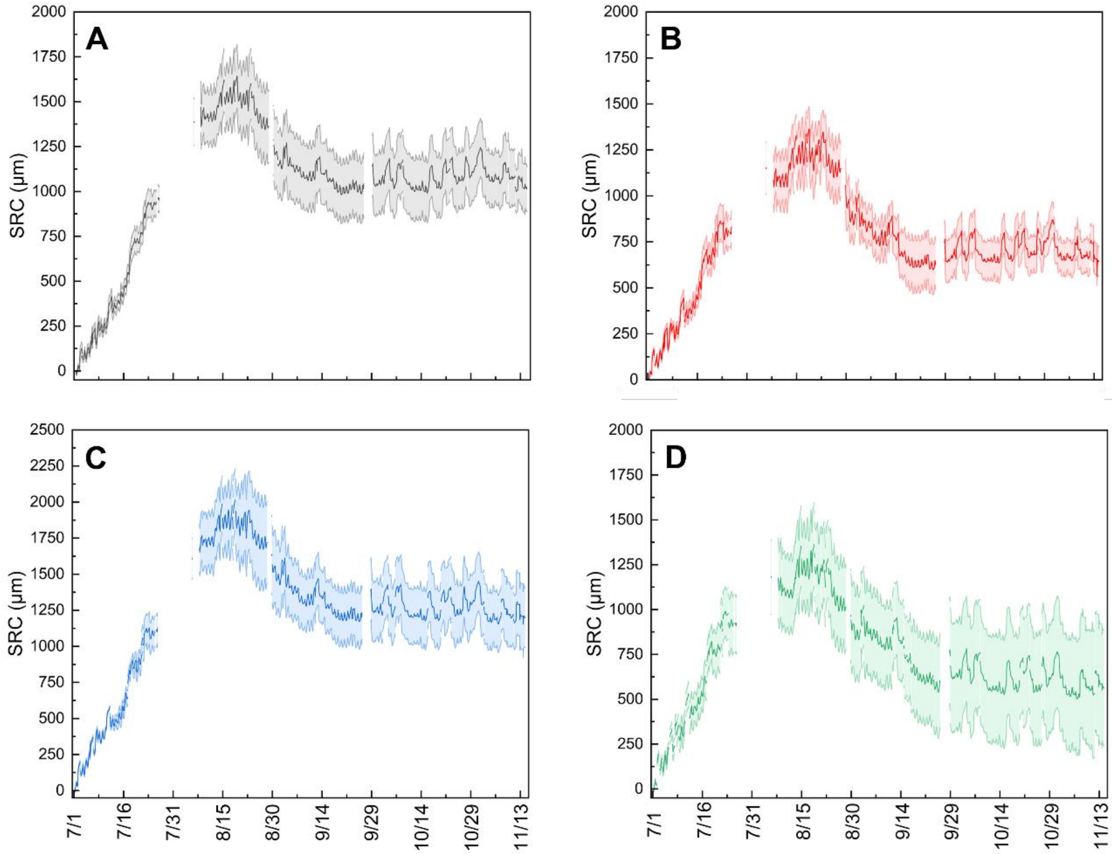


Figure S4. Seasonal stem radius changes (SRC; µm) for non-infested vines (control; 0 SLFs shoot^{-1}; A) and vines exposed to low (B; 4 SLFs shoot^{-1}), medium (C; 8 SLFs shoot^{-1}), and high (D; 12 SLFs shoot^{-1}) adult SLF densities from July 2 – November 15 in 2019. Lines represent mean values while shaded areas indicate the standard error per treatment.

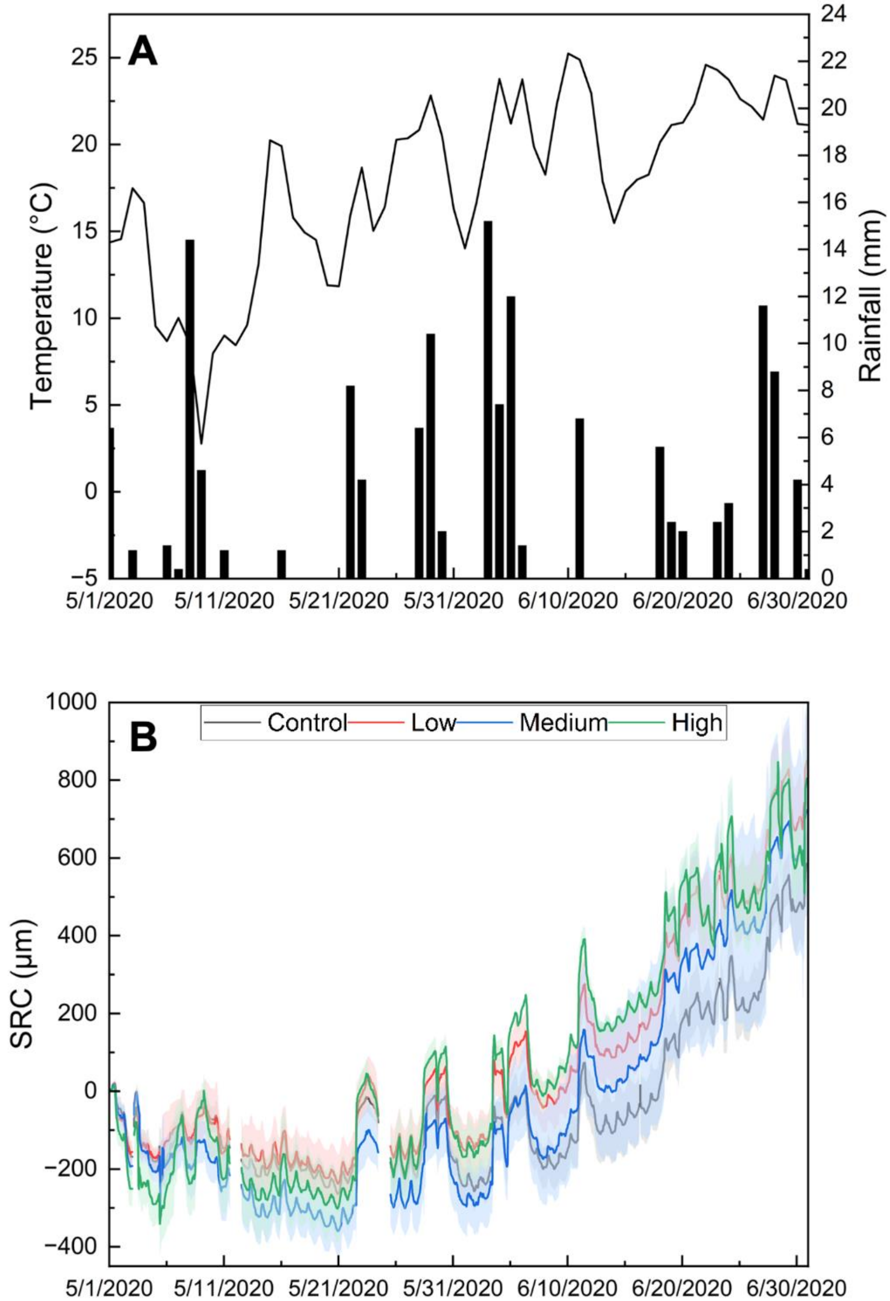


Figure S5. (A) Average daily temperature (°C) and cumulative rainfall (mm) and (B) continuous trunk radius changes (SRC, μm) during the late spring and early summer (May 1 – June 30) of 2020 for non-infested (control; 0 SLFs shoot^{-1}) and vines previously exposed to low (4 SLFs shoot^{-1}), medium (8 SLFs shoot^{-1}), and high (12 SLFs shoot^{-1}) SLF densities in 2019. Lines in B represent mean values per SLF treatment while shaded areas indicate the standard error of the mean per treatment.

Supplemental Table 3. Mean daily total sap flow (mL m^{-2} day^{-1}) per infestation for Riesling vines exposed to SLF infestation in 2019, from August 26 to September 28. Data presented was not statistically analyzed and instead computed only as a numerical reference for total vine water use per day.

Treatment	**Days 1-5 SLF**	**Days 11-15 SLF**	**Days 19-24 SLF**	**Days 29-33 SLF**
Control	991.0	771.0	672.0	985.4
Low (4 SLFs shoot^{-1})	832.3	671.2	640.8	862.0
Medium (8 SLFs shoot^{-1})	887.5	655.7	543.5	672.3
High (12 SLFs shoot^{-1})	983.7	687.2	494.5	611.6

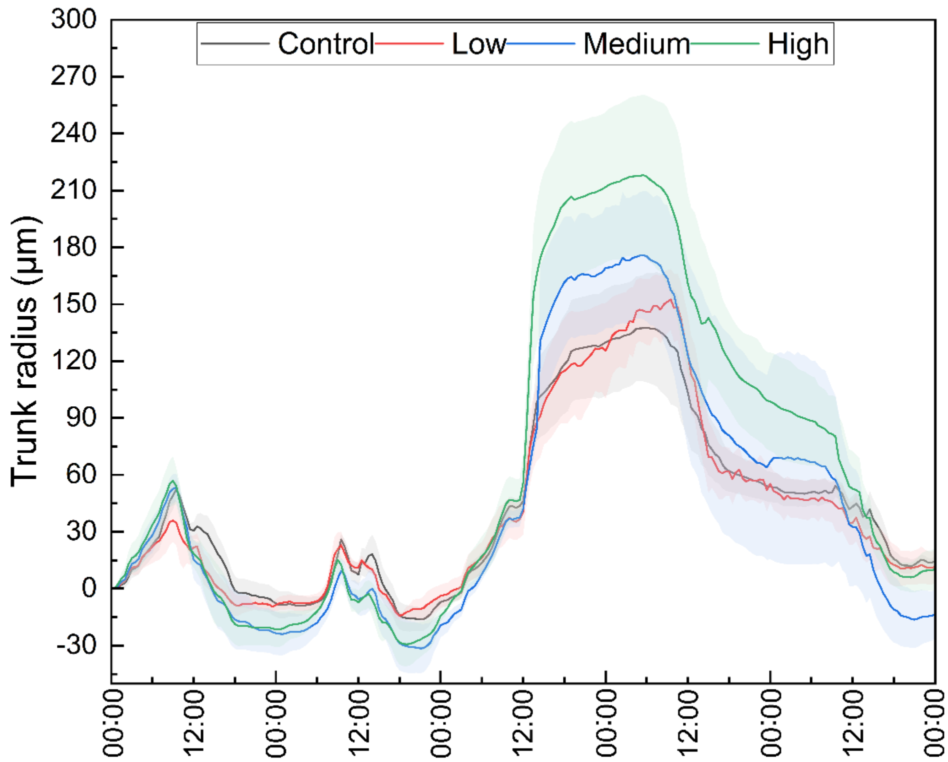


Figure S6. Changes in trunk radius (µm) for non-infested grapevines (control; 0 SLFs shoot^{-1}) and grapevines subjected to low (4 SLFs shoot^{-1}), medium (8 SLFs shoot^{-1}), and high (12 SLFs shoot^{-1}) SLF infestation density from October 14-18, 2019 (28-32 days of SLF exposure), when multiple rain and fog events occurred. Lines represent mean values per treatment while shaded areas represent the standard error of the mean per treatment.

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Michela Centinari reports financial support was provided by National Institute of Food and Agriculture. Michela Centinari reports financial support was provided by Pennsylvania Department of Agriculture. Michela Centinari reports financial support was provided by Pennsylvania Wine Marketing and Research Board. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Title: Shifts in water use in grapevine due to an invasive sap-feeding planthopper persist following insect removal

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Key words: plant-insect interactions**;** point dendrometers; sap flow; source-sink relationships; *Vitis*

Running title: Late season sap-feeding and grapevine water use

Abstract

Increasing outbreaks of invasive insect pests pose a substantial threat to the functioning and viability of cultivated and wild woody perennial species worldwide. In the eastern U.S., the spotted lanternfly (*Lycorma delicatula* White; SLF), an invasive phloem-feeding planthopper, was reported to be able to negatively impact late-season plant carbon dynamics in various woody perennial species following repeated or prolonged infestation and feeding events. However, it remains unclear if SLF infestations also impact plant water relations and if SLF-mediated effects persist when populations are controlled and feeding stops. This study investigated how late-season exposure to SLF impacts whole-plant water relations by assessing diurnal sap flow and trunk radius changes in grapevines subjected to varying infestation densities of adult SLF. In two seasons, vines exposed to high infestation densities (an average of 180 SLFs per vine) for up to -31-32 days of cumulative SLF exposure had significantly lower sap flow rate than those with no SLF, resulting in up to 38% less daily total water use. Trunk diurnal amplitudes increased under SLF infestation, but impacts were less dependent on infestation density, suggesting that grapevines may be utilizing trunk water storage during infestation to meet both grapevine water use and SLF sap ingestion. In both cases, SLF-mediated effects persisted following removal of SLFs, suggesting that exposure to high populations of SLF can alter patterns of late-season grapevine water use, at least when populations are not effectively controlled. These results indicate that SLF can modify both whole-plant water relations and carbon dynamics concurrently, further defining the implications that intensive infestations by high populations of SLF has for woody perennial whole-plant physiology.

1. Introduction

Plant water status and carbon reserves play leading roles in determining the resilience of plants to biotic and abiotic stress. In woody perennial systems, research has focused on how shifts in plant water use and/or patterns of carbon assimilation and storage can influence plant decline under atypical environmental conditions (i.e., prolonged or severe drought) (Adams et al. 2017; Choat et al. 2018; Peltier et al. 2023), pathogen and/or insect pest outbreaks (Deslauriers et al. 2023), and combinations of the two (McDowell et al. 2008; Anderegg et al. 2015; Erbilgin et al. 2021; Hajek et al. 2022). Those that have investigated the role of these processes in insect-facilitated decline of woody perennials have targeted various species of bark beetles and defoliating insects, given their prevalence in forest ecosystems and the considerable damage that they can cause during severe outbreaks (Anderegg et al. 2015). However, the number of invasive species introductions and outbreaks are projected to rapidly increase in temperate regions and will likely include both defoliating and sap-feeding insect species (Bradshaw et al. 2016; Yan et al. 2017), the latter of which have been less studied in temperate woody perennial plant systems, including grapevines. Improved understanding of the combined responses of plant water relations and carbon dynamics in woody perennial species to sap-feeding insects could improve future management of potential outbreaks and how plant species cope with severe uncontrolled infestation.

The spotted lanternfly (*Lycorma delicatula* White; SLF) is a phloem-feeding planthopper that was discovered in North America (Pennsylvania, Eastern U.S.) in 2014. Populations are now present in 17 states across the eastern and midwestern United States (NYS IPM) and with projections of continued spread (Wakie et al. 2019). The spotted lanternfly is a polyphagous insect that produces one generation per year, but preferred hosts in the Eastern U.S. include tree of heaven (*Ailanthus altissima* Mill. Swingle), wild and cultivated grapevine species (*Vitis* spp.), black walnut (*Juglans nigra* L.), and red and silver maples (*Acer rubrum* L. and *Acer saccharinum* L., respectively) among others (Barringer and Ciafré 2020). Following its establishment, commercial wine grape growers in Pennsylvania reported substantial reductions in crop yield and, in some cases, plant decline and mortality after severe, heavy infestations over multiple successive seasons (Urban 2020). Prolonged exposure to adult SLFs can substantially affect woody plant carbon dynamics, namely by reducing foliar carbon assimilation and transpiration (Harner et al. 2022; Lavely et al. 2022), lowering fruit sugar accumulation (Harner et al. 2022), and decreasing belowground end-of-season starch storage in woody roots (Harner et al. 2022; Hoover et al. 2023). However, it is unknown how SLF infestations affect plant water relations and whether the effects of SLF on plant water use can contribute to plant decline following heavy, successive infestation across seasons, as reported by commercial wine grape growers in Pennsylvania.

Similar to plant responses to abiotic stressors like drought, the impacts of tissue- and sap-feeding insect pests on plant water relations can vary with severity and duration, with effects also influenced by the type of organ damaged. For instance, heavy defoliation of plant canopies by chewing insects can substantially reduce whole-plant water use, since the total canopy surface area of transpiring leaves is directly reduced (Cunningham et al. 2009). These effects are not always permanent, however, as plants exposed to periodic defoliation can recover to pre-infestation rates of water use if canopy regrowth occurs immediately following defoliation, as documented in tamarisk trees (*Tamarix* spp.) exposed to defoliation by saltcedar leaf beetles (*Diorhabda carinulata* Desbrochers) (Hultine et al. 2010). Comparatively, the effects of sap-feeding insects on whole-plant water relations are less documented, but they broadly tend to decrease foliar transpiration (Mobley and Marini 1990; Schaffer and Mason 1990), mainly by stimulating stomatal closure (Sun et al. 2015; Lin et al. 2022). In the case of adult SLF, it is unclear if reductions in foliar transpiration reflect broader shifts in plant water use strategy (Harner et al. 2022). Additionally, it is unknown if impacts on transpiration due to adult SLFs persist following control or removal of SLFs.

Sap-feeding insects consume large volumes of sap to meet their own dietary requirements (Douglas 2006). It is likely that large populations of SLFs feeding on a single plant can impact host-plant water relations by directly removing substantial volumes of sap, in addition to the possibility that phloem-feeding may also impact overall vascular tissue integrity and functioning. This is an increasingly important consideration as SLF continues to expand its range throughout the U.S. to drier, more arid regions (Wakie et al. 2019, Jones et al. 2022) where grape and other perennial fruit and nut production utilizes irrigation, and where management efforts for SLF populations may need to consider its effects on both plant carbon and water resources.

In this study, we measured whole-plant water use in field-grown mature grapevines repeatedly exposed to different population sizes of adult SLFs to determine if water use responses and potential recovery to intermittent exposure are influenced by SLF density and exposure time. To assess whole-plant water relations in high resolution we used sap flow sensors and point dendrometers (Steppe et al. 2015a). Sap flow sensors have been widely applied to estimate water use in perennial plants, often for capturing the effects of prolonged or intermittent drought on plant water status (Steppe et al. 2015b). However, their applications to assessment of insect-driven shifts in perennial plant water use have been mostly limited to defoliating insects (Cunningham et al. 2009; Hultine et al., 2010). Dendrometers have been used to measure trunk radius and diameter changes and assess plant water relations and carbon dynamics (De Swaef et al. 2015). When combined with sap flow data, they can provide insights into patterns of whole-plant water use and its relation to diurnal fluctuations in trunk tissue water stores under variable conditions (Steppe et al. 2015a). Dendrometers have been used to assess diurnal and seasonal usage of tissue water storage in response to defoliating insects in tree species (Balducci et al. 2021; Deslauriers et al. 2023), in addition to impacts on growth (Deslauriers et al. 2019). However, to date, these approaches have not been widely adapted to study of sap-feeding insects in woody perennial systems, in addition to scenarios involving repeated infestation and feeding, like in the case of SLF.

Based on reported ecophysiological responses of woody plants to SLF (Harner et al. 2022; Lavely et al. 2022; Hoover et al. 2023), we hypothesized that plant water use would be negatively affected by SLF in a density- and time-dependent manner (Figure 1). First, in response to short-term (5-7 days) exposure, we expected low SLF densities to mildly reduce daily water use (Figure 1A), with greater SLF infestations (i.e., 80-100 SLFs vine^{-1} and above) reducing daily water use to a much greater extent (Figure 1A). However, we also hypothesized that reductions in water use would be temporary following short exposure (i.e., 5-7 days), with use recovering to rates comparable to non-infested vines once SLFs are removed (Figure 1A). Comparatively, we anticipated that an extended cumulative exposure (i.e., repeated short exposures, > 7 days total exposure) will induce a reduction in vine water use that persists post-infestation, when vines are not exposed to SLF. Similarly to the short exposure scenario, greater SLF densities will yield greater reductions, mirroring trends in gas exchange previously reported for grapevines infested with SLFs (Harner et al. 2022) and likely a byproduct of sustained stomatal closure often caused by sap-feeding insects. In addition, we hypothesized that the substantial removal of sap by adult SLFs would result in increased diurnal trunk radial shrinkage (amplitude), as plants rely more on stored tissue reserves to meet daily insect and plant water needs. Like impacts on daily water use, we expected high densities of SLFs to have a more severe impact than low densities, with amplitude and trunk radial shrinkage recovering to a pattern like that of non-infested vines following short-term exposure only (Figure 1A). An improved understanding of the magnitude and duration of the impacts of small to large populations of adult SLFs on late season water relations can help determine if, and when, control strategies should be adopted.

2. Materials and Methods

2.1 Study site and experimental design

The study was conducted in 2019 and 2020 at an unirrigated vineyard in Coopersburg, Pennsylvania, U.S. (40.492644° N, 75.456533° W) on Riesling (*Vitis vinifera* L.) grapevines planted in 2016 and grafted on 101-14 Mgt (*Vitis riparia* x *Vitis rupestris*) rootstock. Standard canopy and disease management practices for the region were implemented (Wolf 2008), but no insecticides were applied to the grapevines throughout the trial. In summer 2019, 16 grapevines were randomly selected from two adjacent vineyard rows and fully enclosed with custom-built insect exclusion cages in August. Details about cage construction and design are available in Harner et al. (2022). Infestation densities were chosen based on reported population levels of adult SLFs in infested vineyards (Leach and Leach, 2020). In 2019, the experimental design was a randomized complete block design (RCBD) with four blocks and four SLF density treatments (i.e., four vines per treatment): control (0 SLF shoot^{-1}; 0 SLF vine^{-1}); low (4 SLFs shoot^{-1}; average of 60 SLFs vine^{-1}); medium (8 SLFs shoot^{-1}; average of 120 SLFs vine^{-1}); and high (12

SLFs shoot^{-1}; average of 180 SLFs vine^{-1}) density. The number of shoots per experimental vine varied from eight to 17, with most vines having 14-15 shoots, as is standard for commercial grapevines in this region. The total number of SLFs per vine ranged from 0-204 insects per vine.

In 2020, a new group of 16 Riesling grapevines from the same vineyard were used. New vines were selected to avoid cumulative effects of SLF exposure from the year prior. In 2020, the experiment layout was modified to a completely randomized design (CRD) with 16 infestation densities ranging from 0-15 SLFs shoot^{-1}, using an increment of 1 SLF shoot^{-1} (i.e., one vine per infestation density). The number of shoots of the experimental vines varied from 11-20, with most vines having between 14-15 shoots, and the number of SLFs per vine was between 0 and 195. The new design was selected to confirm results from 2019 and improve understanding of density-dependent effects (Harner et al. 2022).

No resident populations of SLFs were present in the vineyard in 2019 and 2020. In both years, adult SLFs of mixed sex were collected from nearby untreated woodlots the morning of each introduction. Over the course of the experiment, they were counted and then introduced into the caged vines in repeated infestation events to mimic the observed phenology of adult SLF infestation in vineyards. This was necessary since infestation typically occurs near fruit harvest and resident SLF populations can only be controlled using insecticides with little or no residual activity, resulting in SLF reinfestation shortly after insecticide application (Leach and Leach, 2020). Insects that died following introduction were counted and replaced every other day with insects collected from wooded area on the same day, to maintain assigned infestation densities. Across both years, insect mortality during 2019 ranged between 0.0% to 32.8% and between 0.0% to 27.1% in 2020. At the end of each infestation, all SLFs were manually removed, and the vines were not exposed to any further SLFs until the beginning of the following infestation. In

2019, six infestations lasting 5-6 days each were implemented between 24 August and 18 October, resulting in a cumulative 32 days of exposure to SLF across 54 consecutive days. The number of days between each infestation (i.e., days with no SLF) varied between 3 and 6 days. In 2020, caged vines were infested with adult SLFs between August 25 and October 6, with three infestations lasting between 7 and 15 days each, for 31 total days of cumulative exposure to SLF across 43 consecutive days. The number of days between each infestation was 6 days. Due to travel and labor restrictions in response to the global COVID-19 pandemic in 2020, adjustments to the infestation length were necessary. However, the total number of days that SLF were in the cages was similar between the two years (e.g., 32 days in 2019 and 31 days in 2020).

Weather data were collected for the duration of the experiment using an onsite weather station, outfitted with a remote-accessible HOBO microRX datalogger system (Onset Computer Corp, Bourne, MA, USA). Every 15 minutes, air temperature, precipitation, and photosynthetic active radiation (PAR) were recorded. Data were uploaded to and accessed via the HOBOlink online platform and downloaded in daily and 30-min time intervals.

2.2 Grapevine leaf area

Total canopy leaf area per grapevine was measured to standardize vine sap flow rates to a per m^2 leaf area basis. Grapevine leaf area was estimated using linear regression at the end of the season (Lopes and Pinto, 2005). On September 19, 2019, 17 shoots of a wide range of length were sampled from non-experimental Riesling vines from the same vineyard and transported back to the laboratory. Each shoot was manually defoliated, and the leaf area of each main and lateral shoot was measured separately using a LI-3000c scanning leaf area meter (LICOR

Biosciences, UT, USA). The main and lateral shoot length was measured using a flexible tape measure. The leaf area of each main and lateral shoot was plotted against the corresponding shoot length and linear regression was used to produce equations for estimation of main ($y = 14.856x + 71.588$, where y = main leaf area per shoot and x = shoot length; $r^2 = 0.92$) and lateral ($y = 16.78x + 6.8662$, where y = lateral leaf area per shoot and x = shoot length; $r^2 = 0.99$) shoot leaf area of the experimental vines. Shoot length of all experimental vines was measured following leaf senescence on November 7 in 2019 and on November 10 in 2020 and total leaf area per vine was estimated (data not shown).

2.3 Sap flow measurement

Vine sap flow rates were continuously measured using the heat balance (HB) method (Baker and Nieber 1989). Sap flow sensors (EXO-Skin Sap Flow Sensor, Dynamax, Inc., Houston, Texas, USA) were installed on the vine's trunk following manufacturer instructions. Each vine had two trunks and one was randomly selected for sap flow measurement in 2019 since only 16 sensors were available. Sensors were installed on August 8 and removed on November 21, but only data from August 8 to September 28 (harvest and 21 days of cumulative SLF exposure) were included for analysis since mean sap flow rates were extremely low (i.e., 15-30 mL m^{-2} hr^{-1}) after this date and towards the end of the season and canopy senescence occurred prior to November 21. In 2020, eight of the sixteen vines were selected for sap flow measurements and two sensors were installed per vine (i.e., one on each trunk of the vine) on 24 July and removed on 4 December. Only data from July 24 to October 6 (31 days of cumulative SLF exposure) were included for analysis, since mean sap flow rates after this time were also extremely low, similar to 2019. Vines assigned to SLF infestation densities of 0, 2, 4, 6, 8, 10, 12, and 14 SLF shoot^{-1} were chosen to represent a wide range of SLF infestation density. Sap flow rates (mL hr^{-1}) were measured every 5 minutes and logged via a Dynagage Flow32-1K Sap Flow System (Dynamax, Inc., Houston, TX, USA). Only sap flow data from 7:00 AM through 7:00 PM were used for analysis, to target the daytime hours when SLF are most active.

Average hourly sap flow rates per day were calculated and used to assess how SLF affected sap flow during each infestation and if sap flow trends change between infestation when SLFs were not present. In both years, average hourly sap flow rates of each trunk were standardized per unit of leaf area (mL hr^{-1} m^{-2}) using the respective section of the vine. In 2020, the standardized average hourly sap flow rates of the two trunks per vine were averaged.

2.4 Point dendrometer measurements

In 2019, each vine was equipped with a point dendrometer installed at the base of the vine and below the graft union, about 10 cm above the ground. Each dendrometer consisted of potentiometer mounted on a single thermal-resistant, carbon fiber ring-shaped frame (Model ZN12-O-2WP, Natkon Dendrometers, Oetwil am See, Switzerland) and potentiometers were placed on a sanded section of trunk bark to monitor trunk radius changes (Zweifel et al. 2014). Point dendrometers were installed on June 27, 2019, and removed on June 30, 2020. Data were continuously recorded at 10-minute intervals using wireless transmitters and wirelessly uploaded to a central online server database for data access and storage (Decentlab GmbH, Dübendorf, Switzerland). Raw stem radius changes (SRC, μm) were downloaded in 15-minute intervals and daily amplitudes (μm) were calculated by subtracting the minimum radius value from the maximum radius value recorded on each day throughout the experiment (Epron et al. 2021).

Average daily amplitudes for each infestation and the periods in between were calculated separately to determine if exposure duration and SLF infestation density had an impact on daily amplitudes and whether these effects persisted following the removal of SLFs. In the spring of 2020, daily amplitudes were also calculated to determine if SLF infestation the previous year had carry-over effects on amplitude and trunk growth. Measurement started on May 1, around the time of budbreak (resumption of growth) to June 30 when vegetative growth of the vines typically starts to slow down in our region. Total trunk growth (μm) was also measured during this period.

2.5 Statistical analysis

Sap flow rates and trunk amplitudes were analyzed in SAS v. 9.4 (SAS Institute, Cary, NC, USA). Average hourly sap flow rates (mL hr^{-1} m^{-2}) in 2019 during infestations and the time in between without SLF were analyzed using PROC GLIMMIX and a mixed model to determine the impacts of SLF exposure on sap flow rates and test if impacts varied across time (i.e., following repeated infestations). In the case of a significant treatment by time interaction, the SLICEDIFF option was used to perform post-hoc treatment comparisons of least-square means by a simple effects *t*-test (Marini et al. 2019). In 2019, assessment of pre-infestation baseline (i.e., early-to-mid August) sap flow rates indicated that rates significantly differed ($p < 0.001$) before SLF infestation treatments began. Consequently, the average hourly sap flow rate per vine for the baseline period was calculated from data spanning 15 August through 24 August and used as a covariate in statistical analysis of average hourly sap flow data. Sap flow rates did not significantly differ for vines in 2020 and no baseline covariate was used in statistical analysis. In 2020, sap flow data were analyzed in two ways: first, hourly data were averaged per day, like for data collected in 2019, and PROC REG was used to perform linear and polynomial regression to determine if the daily relationship between SLF density and average hourly sap flow rate changed over time; secondly, average hourly rates per day were averaged for each infestation and the time in between and linear and polynomial regression models were assessed using PROC REG to determine the nature of the relationship between SLF infestation densities and sap flow rates.

Raw dendrometer data were time-aligned, cleaned of outliers and unexpected artificial shifts, and gap-filled using R (R Core Team, 2021) and the treenetproc package, according to Knüsel et al. (2021). Daily trunk amplitudes were calculated by subtracting the minimum radius value from the corresponding daily maximum radius value. Trunk amplitude data collected in 2019 and 2020 was analyzed using PROC GLIMMIX using the method outlined above for sap flow data in 2019. Trunk growth measured in 2020 was analyzed using PROC MIXED. If there was a significant treatment effect, post-hoc multiple comparisons were assessed using Tukey's honest significant difference (HSD). In 2019, relative changes (%) between the non-infested control and SLF-infested vines were determined for average hourly sap flow rate and daily amplitude. Relative change was calculated by normalizing the difference between the non-infested and infested treatment values to the non-infested vine value. Graphs and plots were generated using OriginPro v. 2024b (OriginLab Corporation, Northampton, MA, USA), and conceptual figures constructed using BioRender.com (BioRender, Toronto, ON, CA).

3. Results

3.1 Vineyard weather conditions

In both seasons, average daily temperatures and PAR were highest at the beginning of the experiment, around mid-August, and then trended lower as summer ended, and the beginning of fall approached (Figure S1). In 2019, the mean daily temperature for the infestation period (August 24-October 18) was 17.5° C, with a maximum and minimum temperature of 24.7° C and 8.7° C, respectively (Figure S1A), while mean, maximum, and minimum daily temperatures in the infestation period in 2020 (August 25-October 6) were 18.3° C, 24.5 °C. and 9.8 °C. Total rainfall during the infestation period was greater in 2019 , as 121.2 mm of rainfall occurred during this time in 2019 and only 27.0 mm in 2020.

3.2 Sap flow trends

In general, grapevines exposed to high numbers of SLFs had significant reductions in sap flow rates (mL m^{-2} hr^{-1}) starting approximately one week (i.e., 7-8 days) after SLF introduction, with effects increasing with further exposure to SLFs. Comparatively, effects were less severe with lower densities in both years (Figures 2 and 3). Regardless of treatment, sap flow rates decreased throughout the experiment as the season progressed and autumn approached. In 2019, exposure to low SLF density (4 SLFs $shoot^{-1}$) did not significantly affect sap flow rates throughout the experiment (Figure 2; Supplemental Table 1; Figure S2). Comparatively, exposure to high SLF density (12 SLFs $shoot^{-1}$) had the greatest effect, as average hourly sap flow rates of these vines were significantly lower than control and low SLF density vines by the end of the second infestation (i.e., 10 days of cumulative exposure). These negative effects persisted throughout the experiment, even when vines were not exposed to SLFs, and increased in severity following further exposure; by the end of the fourth infestation (21 days of cumulative exposure), sap flow rates of high SLF density vines were up to 32.8% lower than that of control vines (Figure 2B). Comparatively, medium SLF density (8 SLFs shoot^{-1}) vines did not have lower sap flow rates than control vines during the infestations. However, sap flow rates of medium SLF density vines were significantly lower than the control when vines were not exposed to SLFs, specifically between the first and second infestations (days 6-10) and again between the third and fourth infestations (days 25-28), although differences were never over 15% (Figure 2B). Additionally, sap flow rates of medium SLF density vines were significantly greater than those of vines subjected to high SLF density starting approximately 16 days after the first introduction to the study's end. Contrary to our hypothesis, once vines exposed to SLFs had significantly lower sap flow rates than non-infested control vines, they did not show a clear or consistent subsequent increase in sap flow comparable to that of control vines once the SLFs were removed (i.e., in between infestations), which would otherwise suggest a recovery (Figure 2; Figure S2). We expected vines to be able to recover following short-term exposure to SLFs, but this was not the case.

Reductions in average hourly sap flow rate measured in 2019 appeared to be driven by reductions in sap flow rates between 10 am and 4 pm, when sap flow rates were typically at their highest each day (Figure S3). The data in Figure S3 show the progression of diurnal trends of hourly sap flow rates of vines exposed to different SLF densities for a short (1 to 5 days exposure) and long time (17-21 days of cumulative exposure). As the season progressed, the magnitude of daily sap flow rates decreased for all vines due to late-season weather conditions and vine phenological stage. In addition to seasonal effects, daily peaks in hourly sap flow rate, typically around midday (1:00 pm), were always numerically lower for high SLF vines than the control vines when exposed to SLF for 17-21 days cumulatively (Figure S3D). For example, on the third day (i.e., 19 days of cumulative exposure), high SLF vines had 45.5% lower hourly sap flow rate at 1:00 pm, relative to the control vines (p = 0.080).

In general, sap flow trends in 2020 confirmed the effects of SLFs reported for the previous year: hourly sap flow rate was negatively affected by exposure to SLF, with exposure to increasing SLF densities leading to more severe reductions in sap flow and after a shorter cumulative exposure length (Figure 3). Reductions were neither detected following limited SLF exposure (1-7 days) nor during the following 6 days when vines were not exposed to SLF (Figure 3A, 3B). However, linear reductions were measured by the end of the second infestation (22 days of cumulative exposure), with high SLF densities (10-14 SLFs shoot^{-1}) strongly reducing sap flow and at a similar rate, while there was no clear trend with lower SLF density (0-8 SLFs shoot^{-1}) (Figure 3C). As the exposure to SLF increased (31 days of cumulative exposure) reductions in sap flow rates were more apparent for lower densities as well (Figure 3E). Again, once reductions in sap flow were detected, they persisted for the remainder of the experiment and there was no indication of sap flow recovery, i.e., an increase in sap flow to rates comparable to those of the 0 SLF vine. This is supported by linear regression analysis performed on average hourly flow rates for each day of the experiment (Figure 4); the responses of sap flow to SLF were linear, negative, and increasingly related to SLF density as the days of exposure progressed, evidenced by increasing r^2 values for daily regressions, ranging from 0.00 (6 days of cumulative exposure) to 0.89 (14 days of cumulative exposure).

3.3 Trunk radius changes

In 2019, exposure of vines to SLFs from the beginning of the experiment through fruit harvest (i.e., August 25 through September 28, 2019) had a significant impact on daily trunk radius amplitudes ($p < 0.0001$), but the effects did not appear to be dependent on SLF density or the length of exposure, as we hypothesized (Figure 5A; Supplemental Table 2). Instead, exposure to low and high SLF density had similar effects on amplitudes during infestation. For example, trunk radius amplitudes for low and high SLF treatments were greater than those of control vines only following 10 days of cumulative exposure (62.3% and 56.1%, respectively) and 16 days of exposure (40.0% and 55.5%, respectively) (Figure 5B). Comparatively, vines exposed to medium SLF density had greater amplitudes than those of control vines following 5 days (26.2%), 13 days (35.9%), and 21 days of cumulative exposure (27.2%) (Figure 5B). Contrary to our hypothesis, exposure to SLFs appeared to have an inconsistent effect on trunk radius amplitudes during days in-between infestations, regardless of the length of exposure. For example, vines exposed to high SLF densities only had greater trunk radius amplitudes than control vines between the first and second infestations (days 5-9; 30.3% higher), while vines exposed to low SLF densities had greater (37.0% higher) trunk radius amplitudes between the second and third infestations (days 16-18; Figure 5B).

Aside from the effects of SLF, trunk radius changes followed an expected diurnal pattern that corresponded with meteorological conditions (Figure S3E, F). Throughout the experiment, trunk radii typically increased in the early morning until a peak was reached between 6:30 am and 9:00 am. Thereafter the radius decreased during the midday hours as vines were at peak rates of transpiration until late-afternoon or early evening, typically between 4:30 pm and 10:30 pm, when the trunk shrunk to its minimum radius (Figure S3E, F). The radii increased again throughout the evening and nighttime hours until the following early morning peak was reached, after which the diurnal cycle was repeated. As the experiment progressed, vines exposed to high SLF densities appeared to exhibit a greater contraction during the day than other vines (Figure S3F). Seasonal trunk growth followed the expected trend for grapevines (Figure S4), where growth occurs through the growing season until the beginning of grape ripening (i.e., véraison), after which a finite shrinkage in trunk tissues is detected that is related to bark formation (Van de Wal et al. 2018).

Exposure of vines to SLFs in 2019 did not impact trunk growth the following year, but exposure did affect daily amplitudes in the following spring and summer (May 1 to June 30, 2020), a period beginning at budbreak and ending about two weeks after bloom when vine shoot growth is typically most vigorous (Figure 6). The measurement period exhibited frequent precipitation events, which resulted in large temporary increases in trunk radius for all vines (Figure S5). The final trunk radius measurement recorded at sensor removal (June 30, 2020), interpreted here as the total trunk growth (µm), did not differ between treatments ($p = 0.680$) (Figure 6A), but SLF did significantly affect trunk amplitudes during this two-month period ($p < 0.001$) (Figure 6B). Vines subjected to high SLF densities had the greatest amplitudes and were 41% ($p < 0.001$), 33% ($p = 0.001$), and 32% ($p = 0.001$) greater than the control vines and vines exposed to low and medium SLF densities, respectively, signaling a greater diurnal water use for this time.

4. Discussion

4.1 Reductions in water use due to SLF persist following SLF removal

Sap flow trends presented here illustrate how repeated exposure to a sap-feeding planthopper can reduce grapevine water use, with SLF densities above 8 SLFs shoot^{-1} having the greatest effect. These results expand upon earlier reports of SLF-mediated reductions in gas exchange of upper canopy leaves (Harner et al. 2022) to show that water use in grapevines exposed to SLFs is affected at the whole-plant scale. However, single-leaf gas exchange appeared to be more sensitive than sap flow: for example, exposure of grapevines to 17-21 cumulative days of infestation by medium and high SLF densities in 2019 reduced foliar transpiration by 60.5% and 58.9%, respectively, relative to the control (Harner et al. 2022), while average hourly sap flow rates of these vines were reduced by 9.5% and 32.8%, respectively (Figure 2B). In previous assessments of grapevine water use efficiency under soil water limitation, single-leaf measurements did not always accurately predict whole-plant responses (Tomás et al. 2012), and in some cases suggested opposite physiological trends (Poni et al. 2009). Therefore, these discrepancies could be in part related to the limitations of single-leaf measurements, which targeted only a few sun-exposed and fully expanded leaves in the upper canopy, under optimal evapotranspiration conditions (Harner et al. 2022), while sap flow measurements integrated water use for the whole canopy during the whole day. It is possible that older or shaded leaves responded to SLF differently than younger, fully exposed leaves, resulting in less pronounced effects on whole-vine sap flow than those suggested by single-leaf data.

Contrary to our hypothesis, reductions in sap flow rate in vines exposed to high numbers of SLFs (180 SLFs vine^{-1} on average) consistently persisted during periods following SLF removal, when feeding was not occurring. This was true regardless of the length of SLF exposure, as sap flow rates neither recovered following limited exposure (5 days of cumulative exposure) nor extended exposure (17-21 days of cumulative exposure). This suggests that a recovery of water use to rates comparable to non-infested vines may be limited, at least under the late season growing conditions and high SLF populations imposed in this study. While reductions in grapevine gas exchange and water use due to drought stress can be alleviated following increased water availability (Pou et al. 2012), loss of hydraulic functioning can persist and lead to vine mortality in severe drought scenarios (Gambetta et al. 2020). Drought-mediated stomatal closure in grapevines can be caused by a combination of passive hydraulic cues and active abscisic acid (ABA) signaling, and ABA may prolong stomatal closure during drought recovery to improve long-term plant water status (Tombesi et al. 2015).

Persisting reductions in water use in vines exposed to a high number of SLFs could be a byproduct of a few interacting factors, including ABA-mediated stomatal closure and interference with the integrity of the xylem water column. Stomatal closure is a common response to insect sap-feeding, stimulated by oral secretions containing ABA and other phytohormones (Lin et al. 2022). Recently, ABA was extracted from salivary glands of adult SLFs after feeding on grapevines (Acevedo, 2024) and Islam et al. (2022) documented an upregulation of 68 genes and a downregulation of 34 genes related to ABA in woody stems of Marquette (*Vitis* hybrid) vines exposed heavy adult SLF infestations (13-20 SLFs shoot^{-1}). These included a significant number of pathways involved in stomatal regulation (Islam et al. 2022). Separately, ingestion from both xylem and phloem tissues has been reported for other planthopper species (i.e., Brown planthopper, *Nilaparvata lugens* Stål; Seo et al. 2009) and preliminary evidence suggests that SLF may be doing the same (Shugart H, 2024, personal communication). Thus, we cannot rule out that SLFs are interfering with the physical flow of xylem sap via direct damage to xylem and/or introduction of embolisms into the water column. The phloem plays a critical role in embolism recovery, and irreversible damage to the phloem by

SLF may also impair embolism recovery and prolong reductions in sap flow, as seen in plants subjected to phloem girdling that experienced an impaired capacity to refill embolisms (Nardini et al. 2011). Despite these impacts, it is unclear if the reductions in water use (up to 38% reduction, or from 0.99 L m^{-2} day^{-1} for control vines to 0.61 L m^{-2} day^{-1} for high SLF vines) that we report here in vines under infestation by high SLF populations will negatively affect other important late-season biological processes, like those involved in cold acclimation and preparation for dormancy.

Here, we detailed persistent reductions in water use in vines exposed to high SLF populations that also had altered carbohydrate dynamics, reported previously as a severe reduction in starch storage in belowground root tissues at the end of the season and reduced sugar concentrations in fruit at harvest (Harner et al. 2022). Although the mechanisms underlying stomatal closure and reduced assimilation under heavy SLF infestation remain to be determined, the net effect appears to be a short-term constraint on plant carbon allocation and water use that may benefit feeding SLFs to the detriment of the vine's overall carbon storage, at least within the context of a single infestation season. However, the question remains whether this response will lead to structural, long-term changes in vine physiology and growth, including reproductive growth and yield, or increased susceptibility to future infestations, pathogens, or adverse environmental conditions (Gessler and Zweifel 2024). We did not document a carry-over impact on shoot growth rate following a single infestation year (Harner et al. 2022), but future research should more fully assess other physiological and anatomical traits under variable conditions to determine to what degree this reduction in carbon storage and water use impacts long-term functioning. Interactions between carbon assimilation, carbon storage, and water relations can influence plant decline (Sevanto et al. 2014), and concurrent alterations to carbon and water relations that we measured may be involved in instances of grapevine decline that have been reported in commercial grapevines repeatedly exposed to high SLF populations across multiple seasons (Urban 2020).

4.2 Vines exposed to SLF may rely more on stored tissue water to meet increased water demand

Vines exposed to high densities of adult SLFs appeared to have lower sap flow rates while vines exposed to SLFs in general had greater trunk amplitudes in 2019, relative to non-infested vines, but further study is necessary to confirm this result given the high plant-to-plant variation presented here. In grapevines, the relationship between trunk radius or diameter variations and water status shifts with phenological stage, however, as daily amplitude and other metrics are more sensitive to osmotic stress and water limitation prior to véraison than once fruit ripening begins at véraison (Intrigliolo and Castel 2007; Montoro et al. 2012). Further, as ripening begins, periderm formation typically occurs and trunk tissues shrink as the outer rings of bark tissues dehydrate, a process found in grapevines and other woody species that form ring bark (Van de Wal et al. 2017). This process occurred during the experiment in 2019: a long-term post-véraison contraction phase was observed in all vines, with vines exposed to SLFs generally having greater daily amplitudes compared to non-infested vines during multiple infestations (Figure S4). This occurred alongside a reduction in water use in response to high SLF populations.

The extent of diurnal trunk contraction (i.e., amplitude) can be considered a function of a transpiration (i.e., sap flow), the flow resistance within the xylem, the flow resistance between the bark and xylem, and the water storage capacity of the bark tissues, as previous models have explored (Zweifel et al. 2001; Zweifel et al. 2007). We hypothesized that SLFs would reduce sap flow while also increasing trunk amplitudes, as vines increasingly relied on tissue water storage to meet the combined water use of the vines and SLF under increasing rates of stomatal closure. In other words, SLF would be a ‘leak’ in the whole-plant water system and vines would compensate by increasingly utilizing stored tissue water (Zweifel et al. 2012). The concurrent greater trunk amplitudes and lower water use rates in vines exposed to high SLF infestation densities may suggest that this is the case, if SLFs are removing a biologically relevant volume of water at the high densities applied here (180 SLFs vine^{-1} on average). This may be plausible, given that other planthopper species have been documented to produce up to 30 mg of honeydew per day under ideal conditions (Cao et al. 2013). Other explanations are possible, however: for example, if SLF probing and feeding are potentially damaging phloem tissues, this could likely decrease the flow resistance between bark storage and xylem tissue and increase the diurnal trunk amplitude as water more easily flows between these tissues, especially if sap flow is also reduced (Sevanto et al. 2011). Alternatively, damage to the xylem or embolism formation would increase the flow resistance within the xylem, which could also drive an increase in trunk amplitude if sap flow rates were to remain the same and water flowed along a path of greater tension (Hölttä et al. 2002). This was likely not the case, given the reductions in sap flow we measured (Figures 2, 3). We are limited by having only trunk amplitude data within this study, but future incorporation of inner-bark and xylem-specific radius changes could provide additional insights to what may be contributing to the greater trunk amplitudes documented here.

Under drought and other water-limiting conditions, plants can exhibit concurrent reductions in sap flow and increases in trunk amplitude with decreasing water availability (Steppe et al., 2015a). We did not assess soil or plant water potential, but our weather data suggested that vines had ample water availability. Despite this, by 21 days of cumulative SLF exposure in 2019, the average total water use per day of vines exposed to high SLF populations was about 0.61L m^{-2} of leaf area, compared to about 0.99 L m^{-2} of leaf area for control vines, indicating a 38% lower volume of water transpired under the same environmental conditions (Supplemental Table 3). At the whole plant level, considering the mean total leaf area, this would amount to a difference between 3.24 L day^{-1} (control) and 2.21 L day^{-1} (high SLF). It is important to note that in our study SLFs were forced to infest and feed upon the same vine for a prolonged period, compared to natural infestation scenarios where they feed on more than one plant host and host species. It is possible that the impacts of lower infestation densities may be stronger in these cases if the overall fitness of feeding SLFs is greater than that of the SLFs used in this study. However, the largest number of SLFs recorded on a single vine in commercial vineyards is 426 adult SLF (Leach and Leach, 2020), indicating that the levels of SLFs used in this experiment are indeed reflective of what a vine may experience.

Honeydew excretion, a potential proxy for the volume of sap ingested by vascular-feeding insects, has been measured for different economically important phloem-feeding planthopper species. For example, when feeding on rice (*Oryza sativa* L.), a single brown planthopper or white-backed planthopper (*Sogatella furcifera* Horváth) can excrete up to 30 and 25 mg of honeydew a day, respectively (Cao et al. 2013). Assessment of honeydew excretion of adult SLF would be a key step in determining if SLF can negatively impact the water budget of an infested grapevine, and particularly in cases of infestation by high SLF densities, like those observed previously in Pennsylvania (Leach and Leach, 2020). If so, SLF phloem-feeding may have more severe implications for plant health and functioning during periods of drought or in regions with limited water availability. Indeed, modeling efforts of future SLF range expansion have suggested that SLF can likely establish in many grape- and fruit or nut-producing regions within the United States and worldwide (Wakie et al. 2019; Jones et al. 2023), many of which are semi-arid or arid and use irrigation.

4.3 Exposure to high SLF density had potential carry-over effects on trunk amplitudes

Persistent reductions in sap flow may be due to prolonged stomatal closure or damage to the vasculature that persisted between SLF infestations, but it is unclear what may be driving persistent effects on diurnal trunk amplitudes when differences were present between infestations. While gymnosperms may exhibit different responses to insect feeding than angiosperms like grapevines, Balducci et al. (2021) reported that needle defoliation in black spruce (*Picea mariana* (Mill.) B.S.P.) and fir (*Abies balsamea* (L.) Mill.) by eastern spruce budworm (*Choristoneura fumiferana* Clem.) during needle flush did not affect diurnal contraction or expansion amplitudes but increased the length of contraction and expansion periods, which was interpreted as a reduced capacity for recovery to needle defoliation. We reason that increased contraction amplitudes due to SLFs may signify a reduced capacity of infested grapevines to recover from intensive infestations over the short-term, i.e., within the same season and under our experimental conditions. If the shift in trunk amplitudes reflects a drawdown of tissue water storage, it is possible that the plant is not able to fully replenish tissue water between infestations. While nighttime tissue water replenishment can be inhibited by prolonged drought conditions and low soil water (Steppe et al. 2015a), such conditions were not observed in this study. Interestingly, in October of 2019 multiple fog and rainfall events resulted in rapid tissue expansion events—a phenomenon that has been shown elsewhere to restore depleted water stores (Zweifel et al. 2001; Steppe et al. 2018)—with expansion tending to be greatest in vines exposed to medium and high SLF populations (Figure S6). If SLF probing or feeding is permanently damaging phloem or xylem tissues and the reduction in radial flow resistance is contributing to increased trunk amplitudes, this effect likely persists following removal of SLFs. Knowledge of SLF sap ingestion rates (e.g., proxy rates of sap removal via honeydew quantification), its impacts on radial flow resistance, and how plants respond to SLF phloem-feeding under variable environmental conditions and water availability would provide important context for the results presented here.

Repeated SLF infestations across seasons led to carry-over reductions in seasonal trunk radial growth in woody trees in the following season (Hoover et al. 2023; Dechaine et al. 2023), but in this study we did not detect an effect on trunk radial growth. However, our measurements did not capture the total seasonal growth in 2020, following infestation treatments the previous fall. Nevertheless, they captured a proportion of the season when grapevines exhibit rapid growth rates of new vegetative tissue (Pagay et al. 2016), including trunk growth (Van de Wal et al. 2017). Vines previously exposed to high SLF densities in 2019 had greater diurnal amplitudes relative to the non-infested control in the spring of 2020, but it is unclear what may be causing this effect. It is possible that alteration of water relations and carbon dynamics in the preceding season due to SLFs led to shifts in anatomical traits that could underlie this persistent change in trunk amplitudes, particularly if vascular tissue anatomy was impacted (Gessler and Zweifel 2024). However, an improved understanding of how SLFs impact phloem and xylem tissue integrity and development across multiple seasons will help clarify these reported effects.

5. Conclusion

In this study, we investigated if repeated exposure to adult SLFs would have density- and cumulative effects on grapevine water use. Our results showed that exposure to prolonged exposure to high populations of adult SLFs (180 SLFs plant^{-1}) appears to simultaneously reduce sap flow rates while inducing greater daily trunk amplitudes, indicating a marked shift in plant water relations in response to high populations of SLFs. However, lower densities of SLFs did not have the same impact on sap flow, suggesting that vines may be able to tolerate lower populations of SLFs (i.e., 0-8 SLFs shoot $^{-1}$ or 0-120 SLFs vine^{-1}), possibly by relying on tissue water storage to a greater extent as suggested by the general increase in trunk amplitudes noted here for all vines exposed to SLFs. Importantly, no recovery in water use was measured when SLFs were removed from infested vines, regardless of the length of exposure; instead, the impacts of SLFs appeared to persist, suggesting that frequent infestation by high numbers of adult SLFs may have a cumulative, negative impact on grapevine water relations, at least under conditions like those of this study. These results emphasize the risk that prolonged exposure to high population sizes of adult SLFs has for grapevines and other woody perennials and the implications for both whole-plant water and carbon dynamics. As SLF continues to move into new regions with variable environmental conditions, most notably regions with drier seasonal conditions and a greater use of irrigation, continued evaluation of how SLF impacts water use and carbon dynamics, and the underlying processes and anatomical traits contributing to these themes, is essential to developing improved, targeted methods for SLF management.

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6. References

Acevedo, FE. 2024. The Spotted lanternfly contains high concentrations of plant hormones in its salivary glands: Implications in host plant interactions. J Chem Ecol. https://doi.org/10.1007/s10886-024-01536-4.

Adams HD, Zeppel MJB, Anderegg WRL, Hartmann H, Landhäusser SM, Tissue DT, Huxman TE, Hudson PJ, Franz TE, Allen CD, Anderegg LDL, et al. 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nat Ecol Evol. 1:1285-1291. https://doi.org/10.1038/s41559-017-0248-x.

Anderegg WRL, Hicke JA, Fisher RA, Allen CD, Aukema J, Bentz B, Hood S, Lichstein JW, Macalady AK, McDowell N, Pan Y, et al. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. New Phytol. 208(3):674-683. https://doi.org/10.1111/nph.13477.

Baker JM, Nieber JL. 1989. An analysis of the steady-state heat balance method for measuring sap flow in plants. Agric For Meteorol. 48(1-2):93-109. https://doi.org/10.1016/0168-1923(89)90009-9.

Balducci L, Rozenberg P, Deslauriers A. 2021. Variation of stem radius in response to defoliation in boreal conifers. Front For Glob. 4:874442. https://doi.org/10.3389/ffgc.2021.784442.

Barringer L, Ciafré CM. 2020. Worldwide feeding host plants of spotted lanternfly, with significant additions from North America. Environ Entomol. 49(5):999-1011. https://doi.org/10.1093/ee/nvaa093.

Bradshaw CJA, Leyow B, Bellard C, Roiz D, Albert C, Fournier A, Barbet-Massin M, Salles J-M, Simard F, Courchamp F. 2016. Massive yet grossly underestimated global costs of invasive insects. Nat Commun. 7:12986. https://doi.org /10.1038/ncomms12986.

Cao T-T, Lü J, Lou Y-G, Cheng J-A. 2013. Feeding-induced interactions between two rice planthoppers, *Nilapravata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): Effects of feeding and honeydew extraction. Environ Entomol. 42(6):1281-1291. https://doi.org/10.1603/EN12171.

Choat B, Brodribb TJ, Brodersen CR, Duursma RA, López R, Medlyn BE. 2018. Triggers of tree mortality under drought. Nat. 558:531-539. https://doi.org/10.1038/s41586-018-0240-x.

Cunningham SA, Pullen KR, Colloff MJ. 2009. Whole-tree sap flow is substantially diminished by leaf herbivory. Oecologia. 158:633-640. https://doi.org/10.1007/s00442-008-1170-3.

Deslauriers A, Fournier M-P, Cartenì F, Mackay J. 2019. Phenological shifts in conifer species stressed by spruce budworm defoliation. Tree Phys. 39(4):590-605. https://doi.org/10.1093/treephys/tpy135.

Deslauriers A, Balducci L, Fierravanti A, Bouchard M. 2023. Changes in water status and carbon allocation in conifers subjected to spruce budworm defoliation and consequences for tree mortality and forest management. In: Montoro M, Morin H, Gauthier S, Bergeron Y, editors. Boreal forests in the face of climate change. Cham: Springer International Publishing. P. 249-269.

De Swaef T, De Schepper V, Vandegehucte MW, Steppe K. 2015. Stem diameter variations as a versatile research tool in ecophysiology. Tree Phys. 35(10):1047-1061. https://doi.org/10.1093/treephys/tpv080.

Douglas AE. 2006. Phloem-sap feeding by animals: problems and solutions. J Exp Bot. 57(4):747-754. https://doi.org/10.1093/jxb/erj067.

Epron D, Kamakura M, Azuma W, Dannoura M, Kosugi Y. 2021. Diurnal variations in the thickness of the inner bark of tree trunks in relation to xylem water potential and phloem turgor. Plant-environ Interact. 2(3):112-124. https://doi.org/10.1002/pei3.10045.

Erbilgin N, Zanganeh L, Klutsch JG, Chen S-h, Zhao S, Ishangulyyeva G, Burr SJ, Gaylord M, Hofstetter R, Keefover-Ring K, Raffa KF, et al. 2021. Combined drought and bark beetle attacks deplete non-structural carbohydrates and promote death of mature pine. Plant Cell Environ. 44(12):3866-3881. https://doi.org/10.1111/pce.14197.

Gambetta GA, Herrera JC, Dayer S, Feng Q, Hochberg U, Castellarin SD. 2020. The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. J Exp Bot. 71(16):4658-4676. https://doi.org/10.1093/jxb/eraa245.

Gessler A, Zweifel R. 2024. Beyond source and sink control—toward an integrated approach to understand the carbon balance in plants. New Phytol. https://doi.org/10.1111/nph.19611.

Hajek P, Link RM, Nock CA, Bauhus J, Gebauer T, Gessler A, Kovack K, Messier C, Paquette A, Saurer M, Scherer-Lorenzen M, et al. 2022. Mutually inclusive mechanisms of drought-induced tree mortality. Glob Change Biol. 28(10):3365-3378. https://doi.org/10.1111/gcb.16146.

Harner AD, Leach HL, Briggs L, Centinari M. 2022. Prolonged phloem feeding by the spotted lanternfly, an invasive planthopper, alters resource allocation and inhibits gas exchange in grapevines. Plant Direct. 6(10):e452. https://doi.org/10.1002/pld3.452.

Hölttä T, Vesala T, Perämäki M, Nikinmaa E. 2002. Relationships between embolism, stem water tension, and diameter changes. 215(1):23-38. J Theor Biol. https://doi.org/10.1006/jtbi.2001.2485.

Hoover K, Iavoriska L, Lavely EK, Uyi O, Walsh B, Swackhamer E, Johnson A, Eissenstat DM. 2023. Effects of long-term feeding by spotted lanternfly (Hemiptera: Fulgoridae) on ecophysiology of common hardwood host trees. Environ Entomol. 52(5):888-899. https://doi.org/10.1093/ee/nvad084.

Hultine KR, Nagler PL, Morino K, Bush SE, Burtch KG, Dennison PE, Glenn EP, Ehleringer JR. 2010. Sap flux-scaled transpiration by tamarisk (*Tamarix* spp.) before, during and after episodic defoliation by the saltcedar leaf beetle (*Diorhabda carinulata*). Agric For Meteorol. 150(11):1467-1475. https://doi.org/10.1016/j.agrformet.2010.07.009.

Intrigliolo DS, Castel JR. 2007. Evaluation of grapevine water status form trunk diameter variations. Irrig Sci. 26:49-59. https://doi.org/10.1007/s00271-007-0071-2.

Islam MT, Kudla-Williams C, Kar S, Londo JP, Centinari M, Rosa C. 2022. Deciphering genome-wide transcriptomic changes in grapevines heavily infested by spotted lanternflies. Front Insect Sci. 2:971221. https://doi.org/10.3389/finsc.2022.971221.

Johnson MW, Daane K, Groves R, Backus E, Son Y, Morgan D, Lynn-Patterson K. 2006. Spatial population dynamics and overwintering biology of the glassy-winged sharpshooter in California's San Joaquin Valley. CDFA Pierce's Dis Sym Proc. 12-15.

Jones C, Skrip MM, Seliger BJ, Jones S, Wake T, Takeuchi Y, Petras V, Petrasova A, Meentemeyer RK. 2022. Spotted lanternfly predicted to establish in Calfornia by 2033 without preventative management. Comm Biol. 5:558. https://doi.org/10.1038/s42003-022-03447-0.

Knüsel S, Peters RL, Haeni M, Wilhelm M, Zweifel R. 2021. Processing and extraction of seasonal tree physiological parameters from stem radius time series. Forests. 12(6):765. https://doi.org/10.3390/f12060765.

Lavely E, Iavoriska L, Uyi O, Eissenstat DM, Walsh B, Primka EJ, Harper J, Hoover K. 2022. Impacts of short-term feeding by spotted lanternfly (*Lycorma delicatula*) on ecophysiology of hardwood trees in a common garden. Front Ins Sci. 2:1080124. https://doi.org/10.3389/finsc.2022.1080124.

Leach H, Leach A. 2020. Seasonal phenology and activity of spotted lanternfly (*Lycorma delicatula*) in eastern US vineyards. J Pest Sci. 93:1215-1224. https://doi.org/10.1007/s10340-020-01233-7.

Lin P-A, Chen Y, Ponce G, Acevedo FE, Lynch JP, Anderson CT, Ali JG, Felton GW. 2022. Stomata-mediated interactions between plants, herbivores, and the environment. Trends Plant Sci. 27(3):287-300.

Lops C, Pinto PA. 2005. Easy and accurate estimation of grapevine leaf area with simple mathematical models. Vitis. 44(2):55-61.

Marini RP, Schupp JR, Baugher TA, Crassweller R.2019. Relationships between fruit weight and diameter at 60 days after bloom and at harvest for three apple cultivars. HortSci. 54(1):86-91. https://doi.org/10.21273/hortsci13591-18.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytol. 178(4):719-739. https://doi.org/10.1111/j.1469-8137.2008.02436.x.

Mobley KN, Marini RP. 1990. Gas exchange characteristics of apple and peach leaves infested by European red mite and twospotted spider mite. J Amer Soc Hort Sci. 115(5):757-761.

Montoro A, Fereres E, López-Urrea R, Mañas F, López-Fuster P. 2012. Sensitivity of trunk diameter fluctuations in *Vitis vinifera* L. Tempranillo and Cabernet Sauvignon cultivars. Am J Enol Vitic. 63:85-93. https://doi.org/10.5344/ajev.2011.11010.

Nardini A, Gullo MAL, Salleo S. 2011. Refilling embolized xylem conduits: Is it a matter of phloem unloading? Plant Sci. 180:604-611. https://doi.org/10.1016/j.plantsci.2010.12.011.

Pagay V, Zufferey V, Lakso AN. 2016. The influence of water stress on grapevine (*Vitis vinifera* L.) shoots in a cool, humid climate: growth, gas exchange and hydraulics. Funct Plant Biol. 43(9):827-837. https://doi.org/10.1071/FP16017.

Peltier DMP, Carbone MS, McIntire CD, Robertson N, Thompson RA, Malone S, LeMoine J, Richardson AD, McDowell NG, Adams HD, Pockman WT, et al. 2023. Carbon starvation following a decade of experimental drought consumes old reserves in *Pinus edulis*. New Phytol. 240(1):92-104. https://doi.org/10.1111/nph.19119.

Poni S, Bernizzoni F, Civardi S, Gatti M, Porro D, Camin F. 2009. Performance and water-use efficiency (single-leaf vs. whole-canopy) of well-watered and half-stressed split-root Lambrusco grapevines grown in Po Valley (Italy). Agric Ecosyst Environ. 129(1-3):97-106. https://doi.org/10.1016/j.agee.2008.07.009.

Pou A, Medrano H, Tomàs M, Martorell S, Ribas-Carbó M, Flexas J. 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour. Plant Soil. 359:335-349. https://doi.org/10.1007/s11104-012-1206-7.

Schaffer B, Mason LJ. 1990. Effects of scale insect herbivory and shading on net gas exchange and growth of a subtropical tree species (*Guaiacum sanctum* L.). Oecologia. 84:468-473. https://doi.org/10.1007/BF00328162.

Seo BY, Kwon Y-H, Jung JK, Kim G-H. 2009. Electrical penetration graphic waveforms in relation to the actual positions of the stylet tips of *Nilaparvata lugens* in rice tissue. J Asia Pac Entomol. 12:89-95.

Sevanto S, Hölttä T, Holbrook NM. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant Cell Environ. 34(4):690-703. https://doi.org/10.1111/j.1365-3040.2011.02275.x.

Sevanto S, McDowell NG, Dickman LT, Pangle R, Pockman WT. 2013. How do trees die? A test of the hydraulic failure and carob starvation hypotheses. Plant Cell Environ. 37(1):153-161. https://doi.org/10.1111/pce.12141.

Steppe K, Sterck F, Deslauriers A. 2015a. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends Plant Sci. 20(6):335-343. https://doi.org/10.1016/j.tplants.2015.03.015.

Steppe K, Vandegehuchte MW, Tognetti R, Mencuccini M. 2015b. Sap flow as a key trait in the understanding of plant hydraulic functioning. Tree Phys. 35(4):341-345.

Steppe K, Vandegehuchte MW, Van de Wal BAE, Hoste P, Guyot A, Lovelock CE, Lockington DA. 2018. Direct uptake of canopy rainwater causes turgor-driven growth spurts in the mangrove *Avicennia marinia*. Tree Phys. 38(7):979-991.

Sun Y, Guo H, Yuan L, Wei J, Zhang W, Ge F. 2015. Plant stomatal closure improves aphid feeding under elevated CO_2. Glob Change Biol. 21(7):2739-2748. https://doi.org/10.1111/gcb.12858.

Tomás M, Medrano H, Pou A, Escalona JM, Martorell S, Ribas-Carbó M, Flexas J. 2012. Water-use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole-plant level. Aust J Grape Wine Res. 18(2):164-172. https://doi.org/10.1111/j.1755-0238.2012.00184.x.

Tombesi S, Nardini A, Frioni T, Soccolini M, Zadra C, Farinelli D, Poni S, Pallioti A. 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci Rep. 5:12449. https://doi.org/10.1038/srep12449.

Urban, JM. 2020. Perspective: shedding light on spotted lanternfly impacts in the USA. Pest Manag Sci. 76(1):10-17. https://doi.org/10.1002/ps.5619.

Van de Wal BAE, Leroux O, Steppe K. 2018. Post-veraison irreversible stem shrinkage in grapevine (*Vitis vinifera*) is caused by periderm formation. Tree Phys. 38(5):745-754. https://doi.org/10.1093/treephys/tpx158.

Wakie TT, Neven LG, Yee WL, Lu Z. 2020. The establishment risk of *Lycorma delicatula* (Hemiptera: Fulgoridae) in the United States and globally. J Econ Entomol. 113(1):306-314. https://doi.org/10.1093/jee/toz259.

Wolf T. 2008. Wine grape production guide for eastern North America. Ithaca (NY): NRAES.

Yan Y, Wang Y-C, Feng C-C, Wan P-HM, Chang KT-T. 2017. Potential distributional changes of invasive crop pest species associated with global climate change. Appl Geogr. 82:83-92. https://doi.org/10.1016/j.apgeog.2017.03.011.

Zweifel R, Item H, Häsler R. 2001. Link between diurnal stem radius changes and tree water relations. Tree Phys. 21(12-13):869-877. https://doi.org/10.1093/treephys/21.12-13.869.

Zweifel R, Steppe K, Sterck FJ. 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiology field data with a hydraulic plant model. J Exp Bot. 58(8):2113-2131. https://doi.org/10.1093/jxb/erm050.

Zweifel R, Bangerter S, Rigling A, Sterck FJ. 2012. Pine and mistletoes: how to live with a leak in the water flow and storage system? J Exp Bot. 63(7):2565-2578. https://doi.org/10.1093/jxb/err432.

Zweifel R, Drew DM, Schweingruber F, Downes GM. 2014. Xylem as the main origin of stem radius changes in *Eucalyptus*. Funct Plant Biol. 41(5):520-534. https://doi.org/10.1071/FP13240.

Figure Captions

Figure 1. Concept diagram depicting hypothesized density- and time-dependent effects of SLF infestations on diurnal plant water use. This experiment hypothesized that sap flow and trunk amplitude (green box) would respond differently to a short period of exposure (5-7 days) (A) and extended cumulative (>7 days) exposure (B). In scenarios of short exposure (A), reductions in sap flow and increased trunk amplitudes (solid lines) are hypothesized to occur for all vines exposed to SLFs with the overall impact increasing with SLF density. Physiological activity is expected to recover when SLFs are removed. Under extended cumulative exposure (e.g., cumulative repeated short exposures; > 7 days total exposure) (B), reductions in water use would again occur in in all SLF-infested vines, but recovery in water use would no longer occur following removal of SLFs between SLF exposures. Figure created with BioRender.com.

Figure 2. (A) Mean hourly sap flow rate (mL m^{-2} hr^{-1}) averaged across each day for four infestations (i.e., days with SLF) and the time between infestation (i.e., days with no SLF), from August 26 to September 28, 2019; (B) relative change (%) between mean sap flow rates for vines subjected to SLFs relative to the non-infested vines for the same four infestations and the time between infestations. Shaded vertical areas indicate time between infestations (i.e., days with no SLF) in which SLF populations were temporarily removed before reinfestation occurred. Within each time point, different stacked letters in (A) refer to statistical differences ($p < 0.05$) between vines assigned to the zero (control; 0 SLFs $shoot^{-1}$), low (4 SLFs $shoot^{-1}$), medium (8 SLFs $shoot^{-1}$), and high (12 SLFs $shoot^{-1}$) SLF density treatments, respectively, determined by simple effects *t*-tests using PROC GLIMMIX with the SLICEDIFF option. Standard error bars were also determined using SLICEDIFF.

Figure 3. Mean hourly sap flow rate (mL m-2 hr-1) averaged across each day for the first infestation (A; Days 1-7), second infestation (C; Days 14-28) and third infestation (E; Days 35-43) and the time in between (B and D; Days 8-13 and 29-34, respectively) when no infestations occurred, from August 25 to October 6, 2020. Dashed lines represent linear regression models constructed for each infestation and the time in between infestations and inset r2 values are the coefficient of determination for each regression model. Standard error bars indicate the standard error of the mean hourly sap flow rate for each vine. Each point refers to a single vine from a group of eight experimental vines selected for analysis of sap flow rates in 2020.

Figure 4. Coefficient of determination (r2) for linear regressions performed on daily means of hourly sap flow rates throughout the experimental period from August 25 to October 6, 2020, when SLFs were present on the vines (white background; Days 1-7, 14-28, and 35-43) and when vines were not infested (vertical grey background; Days 8-13 and 29-34). Vertical bars with diagonally hashed lines indicate days with statistically significant linear regression models ($p < 0.05$).

Figure 5. (A) Average daily trunk amplitudes (µm) averaged across each day for four infestations (i.e., days with SLF) and the time between infestation (i.e., days with no SLF), from August 26 to September 28, 2019; (B) relative change (%) between mean amplitudes for vines subjected to SLFs relative to the non-infested vines for the same four infestations and the time in between infestations. Shaded vertical areas indicate time between infestations (i.e., days with no

SLF) in which SLF populations were temporarily removed before reinfestation occurred. Within each time point, different stacked letters in (A) refer to statistical differences ($p < 0.05$) between vines assigned to the zero (control; 0 SLFs shoot^{-1}), low (4 SLFs shoot^{-1}), medium (8 SLFs shoot^{-1}), and high (12 SLFs shoot^{-1}) SLF density treatments, respectively, determined by simple effects *t*-tests using PROC GLIMMIX with the SLICEDIFF option. Standard error bars were also determined using SLICEDIFF.

Figure 6. Total growth (mm) (A) and average daily amplitude (mm) (B) measured in the spring and early summer (May 1 – June 30) of 2020 for non-infested (control; 0 SLFs shoot^{-1}) and vines previously exposed to low (4 SLFs shoot^{-1}), medium (8 SLFs shoot^{-1}), and high (12 SLFs shoot^{-1}) SLF densities in 2019. Standard error bars indicate the standard error of the mean for each treatment. Within each panel, mean values with different letters indicate statistically significant differences between treatment means determined using a Tukey's multiple comparisons test ($p < 0.05$).

Supplemental Materials

Figure S1. Vineyard daily average temperatures (°C; solid black line), PAR (μmol m^{-2} sec^{-1}; dotted red line), and rainfall (mm; black vertical bars) for the experimental periods in 2019 (A) and 2020 (B).

Figure S2. Mean hourly sap flow rate (mL m^{-2} hr^{-1}) averaged across each day for four infestations (i.e., days with SLF; A, C, E, and G) and the time between infestation (i.e., days with no SLF; B, D, F), from August 26 to September 28, 2019 for grapevines exposed to zero (control; 0 SLFs $shoot^{-1}$), low (4 SLFs $shoot^{-1}$), medium (8 SLFs $shoot^{-1}$), and high (12 SLFs $shoot^{-1}$) SLF density treatments, respectively. The data from this series of graphs corresponds with Figure 2A.

Figure S3. (A, B) Diurnal trends for air temperature (°C) and photosynthetically active radiation (PAR, mmol m^{-2} s^{-1}) measured at the experimental vineyard; (C,D) hourly sap flow rates (mL m^{-2} hr^{-1}) and (E,F) continuous trunk radial changes (µm) measured for Riesling grapevines exposed to zero (control; 0 SLFs $shoot^{-1}$), low (4 SLFs $shoot^{-1}$), medium (8 SLFs $shoot^{-1}$), or high (12 SLFs $shoot^{-1}$) infestation densities of adult SLFs during two infestations in 2019. Panels A, C, and E show trends from August 26 to August 30, when the first infestation occurred (1-5 days of cumulative SLF exposure) and panels B, D, and F show trends from September 23 to September 27, 2019, when the fourth infestation occurred (17-21 days of cumulative SLF exposure). For sap flow and trunk radius data, black, red, blue, and green lines represent the means per control, low, medium, and high SLF infestation density treatments, respectively, (black, control; red, low; blue, medium; and green, high) and shaded areas represent the standard error of the mean for each line. Gaps in data in panels E and F represent brief periods when dendrometer sensors were offline and not transmitting data.

Figure S4. Seasonal stem radius changes (SRC; mm) from July 2 – November 15 in 2019 for non-infested vines (control; 0 SLFs shoot^{-1}; A) and vines exposed to low (B; 4 SLFs shoot^{-1}), medium (C; 8 SLFs shoot^{-1}), and high (D; 12 SLFs shoot^{-1}) adult SLF densities. Lines represent mean values while shaded areas indicate the standard error per treatment.

Figure S5. (A) Average daily temperature (°C) and cumulative rainfall (mm) and (B) continuous trunk radius changes (SRC, mm) during the late spring and early summer (May 1 – June 30) of 2020 for non-infested (control; 0 SLFs shoot^{-1}) and vines previously exposed to low (4 SLFs shoot^{-1}), medium (8 SLFs shoot^{-1}), and high (12 SLFs shoot^{-1}) SLF densities in 2019. Lines in B represent mean values per SLF treatment while shaded areas indicate the standard error of the mean per treatment.

Figure S6. Changes in trunk radius (μm) for non-infested grapevines (control; 0 SLFs shoot^{-1}) and grapevines subjected to low (4 SLF*s* shoot^{-1}), medium (8 SLF*s* shoot^{-1}), and high (12 SLF*s* shoot^{-1}) SLF infestation density from October 14-18, 2019 (28-32 days of SLF exposure), when multiple rain and fog events occurred. Lines represent mean values per treatment while shaded areas represent the standard error of the mean per treatment.

Supplemental Table 1. Mean average sap flow rate (mL m^{-2} hour^{-1}) per infestation and the days in between for Riesling vines exposed to SLFs in 2019 from August 26 to September 27. Data was analyzed using a mixed model in PROC GLIMMIX and since there was a significant interaction, differences between treatments were analyzed using simple effects *t*-tests by the

SLICEDIFF option. Different letters between treatments indicate significant differences between treatments ($p < 0.05$).

Supplemental Table 2. Mean trunk amplitude (µm) per infestation and the days in between for Riesling vines exposed to SLFs in 2019 from August 26 to September 27. Data was analyzed using a mixed model in PROC GLIMMIX and since there was a significant interaction, differences between treatments were analyzed using simple effects *t*-tests by the SLICEDIFF option. Different letters between treatments indicate significant differences between treatments ($p < 0.05$).

Supplemental Table 3. Mean daily total sap flow (mL m^{-2} day^{-1}) per infestation for Riesling vines exposed to SLF infestation in 2019, from August 26 to September 27. Data presented was not statistically analyzed and instead computed only as a numerical reference for total vine water use per day.