Supplemental Data for:
Harner AD, Rowles TK, Kar S, Briggs L and Centinari M. 2025. Feeding by adult spotted lanternfly affects carbon allocation postinfestation in young grapevines. Am J Enol Vitic 76:0760014. DOI: 10.5344/ajev.2025.25002

Supplemental Figure 1 Examples of 3-yr-old container-grown Cabernet franc grapevines netted with insect exclusion netting (AgFabric, WellCo Industries, Inc.). The image was taken on 25 Aug 2022. Grapevines were exposed to zero spotted lanternflies (SLFs) (Control; 0 SLFs/vine; n = 10) or high numbers of adult SLFs (SLF; 15 to 20 SLFs/shoot, or 45 to 60 SLFs/vine; n = 10) from 25 Aug to 30 Sept 2022.

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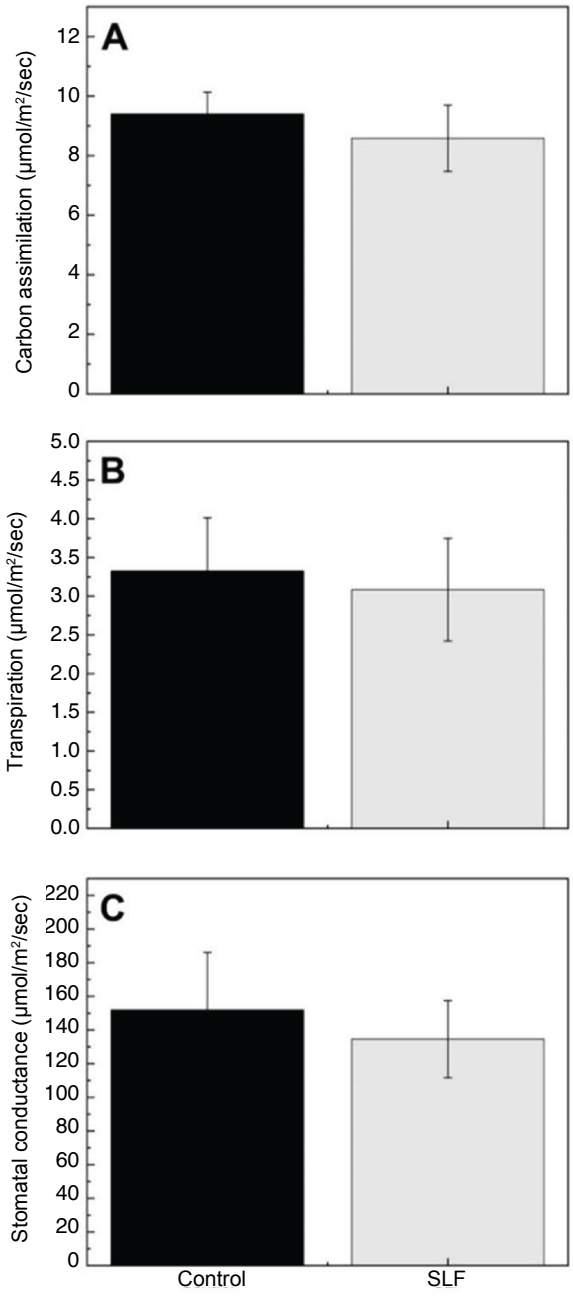

Supplemental Figure 2 Single-leaf carbon assimilation, transpiration, and stomatal conductance (measured on 29 Sept 2022) of 3-yr-old, container-grown Cabernet franc grapevines exposed to zero spotted lanternflies (SLFs) (Control; 0 SLFs/vine; n = 10) or high numbers of adult SLFs (SLF; 15 to 20 SLFs/shoot, or 45 to 60 SLFs/vine; n = 10) from 25 Aug to 30 Sept 2022, a day before application of $^{13}CO_2$.

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Appendix 1 Method for analysis of ^{13}C content in grapevine tissues, modified from previously published protocols.

Grapevines were fed with $^{13}CO_2$, using a method adapted from Morinaga et al. (2003) and Frioni et al. (2018). Six vines per treatment were randomly selected for pulse labeling and the remaining four vines were used as unlabeled controls. A main shoot on each vine was randomly selected and enclosed in a 30-L (88.2 × 76.5 cm) inert plastic bag, covering ~55 to 60% of the total shoot (i.e., from the shoot apex to the lower portion, including lateral shoots). Application of $^{13}CO_2$ occurred in the greenhouse on 30 Sept 2022 immediately after spotted lanternflies (SLFs) were manually removed from infested vines. Vines from each treatment were randomly paired, such that one control and one SLF vine were pulsed concurrently, beginning at 1100 hr and continuing until 1500 hr. For each shoot, 5 mL of 85% lactic acid (Sigma-Aldrich) was added to a sealed 50-mL centrifuge vial containing 342.8 mg $Na^{13}CO_2$ (99 atomic %^{13}C; Sigma-Aldrich), releasing $^{13}CO_2$ into the vial headspace. $^{13}CO_2$ was pumped into the labeling bag and circulated for 30 min using a hand-held suction pump attached via tubing to the vial and the labeling bag. After labeling, the bags were removed and the shoots were exposed to ambient air. Measurement of ^{13}C allocation from previous work showed that ^{13}C can be translocated among shoot tissues up to 7 days after application in actively growing vines (Frioni et al. 2018). Therefore, we waited 12 days after SLF removal and labeling to collect the labeled shoots.

On 12 Oct 2022, the vegetative tissues within each labeling bag (e.g., lateral shoot apices less than 5-cm long, stems and petioles combined, and leaves) were sampled separately from tissues of the same shoot that were outside of the bag, to account for the presence/absence of $^{13}CO_2$-enriched atmosphere between the two groups of tissues. Only the $^{13}CO_2$-pulsed shoot was sampled for analysis. On the same day, a sample of woody roots between 0.5 to 3.0 mm diameter (representing <0.4 to 1.9% of the total root biomass) was also collected throughout the rooting zone using a trowel, soil knife, and pruners. The roots were washed with deionized water before drying. To assess the effect of SLFs on root system biomass, root systems were destructively harvested the spring following budbreak. Under typical conditions in our region, there is generally little new root growth (Comas et al. 2005), confirmed by visual assessment during the sampling process.

All tissue samples were dried at 60°C until reaching constant mass, and dry biomass was measured using a benchtop scale (Ranger 3000, Ohaus Corp.). Tissues were milled (<1-mm mesh; UDY Cyclone Mill, UDY Corporation) and mailed to the Cornell University Stable Isotope Laboratory in Ithaca, NY, where 2-mg subsamples were used for analysis of ^{13}C. For each tissue, ^{13}C atomic excess (%) and excess ^{13}C content (mg) were determined using calculations outlined in previous work (Morinaga et al. 2003).

Appendix 2 Method for extraction and analysis of soluble sugars and starch in grapevine tissues, and modifications made to previously published protocols.

The digestion method was modified to include twice the concentration of enzymes, since a preliminary test indicated that this modification yielded less variability in starch concentrations between replicates. To determine the concentration of the reducing sugars, a modified version of previously outlined protocols (Nelson 1944, Somogyi 1952), adapted by Marais et al. (1966), was used; primary modifications included steps that improved the completion of the enzymatic reactions, based on when the measured absorbance at 520 nm of each subsample and standard reached a steady plateau. These included increasing the heating time of the diluted soluble sugar extract subsamples from 10 to 25 min using a water bath set to 100°C, then placing subsamples in the dark for 30 min prior to measuring absorbance at 520 nm with a benchtop spectrophotometer (Model UV-1600PC, VWR). Standards with dextrose concentrations of 0, 30, 60, 90, 120, and 150 µg/mL were analyzed with every replicate using the same protocol, and absorbances were used to create a linear equation for determination of the concentration of reducing sugars based on sample absorbance at 520 nm. Starch concentration was determined by subtracting the concentration of reducing sugars in the enzymatically digested subsample from the reducing sugar concentration of the undigested subsample. The undigested tissue sample was then used to calculate the soluble sugars in the tissue. Content of starch, soluble sugars, and total nonstructural carbohydrates (TNC) were determined for both stems and the whole root system. These values were determined by multiplying the starch, soluble sugars, and TNC concentrations by the dry biomass for each respective tissue.