# Effects of Foliar Phylloxera (Hemiptera: Phylloxeridae) Infestations on Wine Grape Photosynthesis, Yield, and Fruit Quality[1]

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**Abstract** Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae), is an important pest that forms galls on the foliage and roots of *Vitis* species. The effects of grape phylloxera on grape root have been studied extensively. This study investigated the effects of the foliar form of grape phylloxera, which is a persistent pest of North American native *Vitis* species and cold-hardy hybrid wine grapes between *Vitis vinifera* L. and *Vitis riparia* Michx. For a susceptible variety, 'Frontenac', in commercial Minnesota vineyards, there were reductions in cluster weight due to foliar phylloxera infestations in 2017 (one site; $P = 0.05$) and 2018 (three sites; nonsignificant). Reductions in cluster weight can be economically important to growers. For four grape varieties (susceptible and resistant) evaluated in the greenhouse, there were numerical reductions in photosynthetic rate and localized photosystem II efficiency in 1 of 2 yr. Resistant varieties had less of a reduction on conductance due to grape phylloxera infestation than susceptible varieties in 1 of 2 yr. The degree to which this occurs in resistant varieties may be related to the formation of fewer adaxial stomata; however, this hypothesis requires additional research. These results add important information to better understand how foliar phylloxera injury affects wine grape photosynthesis and yield. The results serve as a foundation for investigating variety-specific responses to foliar phylloxera through the use of advanced phenotyping technologies. This knowledge will lead to a better understanding of the effect of the pest on the diversity of cold-hardy grape varieties grown in the Midwestern United States.

**Key Words** grape phylloxera, leaf-galling, grape yield, photosynthesis, resistant variety

Grape phylloxera, *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae), attacks the foliage of *Vitis riparia* Michx., a grape species native to the upper Midwest and Eastern United States, and many important cold-hardy hybrid wine grape varieties (Stevenson 1969, Yin et al. 2019). Grape phylloxera can infest both the roots and foliage of grapevines with root infestations better studied due to the 19th century outbreak on *Vitis vinifera* L. (Granett et al. 2001). However, grape phylloxera has been more of a problem on foliage (hereafter referred to as "foliar phylloxera") of several widely planted cold-hardy hybrid wine grape varieties (e.g.,

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‘Frontenac,’ ’Frontenac blanc,’ ‘Frontenac gris,’ and ‘La Crescent’) in areas with endemic grape phylloxera (Smiley and Cochran 2016). There have not been many recent studies on the effect of foliar phylloxera infestations on photosynthetic capacity, grape yield, or juice quality (Granett et al. 2001, Yin et al. 2019). In addition, studies on these effects have mostly been limited to one variety, making it difficult to quantify the overall effects of phylloxera on hybrid wine grape varieties. Currently, economic thresholds have not been established for foliar phylloxera (Yin et al. 2019).

We are aware of only three studies that have examined the effects of foliar phylloxera infestation on grape yield and quality. Schvester (1959), a French researcher using Seyve-Villard 18-315 (‘Villard noir,’ a French hybrid), determined that vines treated to control phylloxera had a 15–20% higher yield and 20% higher soluble solids. In a 3-yr experiment using ‘Seyval’ (syn. ‘Seyval blanc,’ a French American hybrid), McLeod (1990), via research in Ohio, found that vines artificially infested at prebloom or postbloom had significantly reduced cluster weights and berries per cluster in at least 1 yr. The authors also found that prebloom infestations on ‘Seyval’ resulted in lower soluble solids in 2 of 3 yr. In Canada, Stevenson (1969) did not find differences in soluble solid content or wine sensory quality for a postbloom treatment for foliar phylloxera with endosulfan, compared to untreated French American hybrids ‘Maréchal Foch,’ Seibel 7053 (‘Chancellor’), and Seibel 5279 (‘Aurore’). Current recommendations for hybrid grape growers to control foliar phylloxera are prebloom (mostly) and postbloom sprays (Yin et al. 2019).

It is known that galled leaf tissues tend to have a reduced photosynthetic rate and an increased stomata conductance (McLeod 1990, Nabity et al. 2013, Rilling and Steffan 1978). The photosynthetic rate of infested ‘Seyval’ leaves was lower than that of uninfested leaves at 2–4 weeks after infestation (McLeod 1990). McLeod (1990) also found that adjacent, uninfested leaves of infested vines had lower photosynthetic rates than that of uninfested vines, but this reduction became nonsignificant 4 weeks after infestation. Gall formation was found to “reprogram” the adaxial leaf surface forming stomata in increasing density with an increased proximity to the gall (Nabity et al. 2013). Stomatal conductance of galled tissues was significantly higher than adjacent ungalled tissues in ‘Frontenac’ (Nabity et al. 2013). However, it is not known if or how infestation differentially affects the photosynthetic rate and stomata conductance of susceptible and resistant varieties.

Photosynthesis is most commonly measured by the rate at which atmospheric $CO_2$ is absorbed per unit leaf area, but with the advancement in high-throughput phenotyping technologies, photosynthesis can also be measured by the energy fixed through fluorescence imaging. As a photon excites a chlorophyll molecule, there are three main de-excitation pathways, as follows: photochemistry (induction of a stable charge separation used for photosynthesis), emission as heat, and emission as chlorophyll a (Chl a) fluorescence (Kalaji et al. 2017). Chl a fluorescence is inversely proportional to the chemically fixed energy and, thus, can be used to monitor plant abiotic and microbial stresses (Kalaji et al. 2017). Pulse-amplitude-modulation (PAM) imaging provides actinic illumination and saturation pulses that drive photosynthesis and convert energy at photosystem II (PS II) and captures them nondestructively through fluorescent cameras. PAM and other imaging fluorometers have been used to measure the effects of fungal infection and insect gall formation on effective PS II quantum yield [Y(II)] and nonphotochemical fluorescence quenching in tree species and cabbage (Aldea et al. 2006, Hajiboland and Amirazad 2010).

In this study, we pursued three objectives to better understand the effects of foliar grape phylloxera feeding injury under Minnesota production systems, including (a) conduct field studies to examine the effect of foliar phylloxera infestation on yield and juice quality of a widely grown cold-hardy hybrid grape variety, 'Frontenac', (b) investigate the variety-specific photosynthetic responses of two resistant and two susceptible varieties in a greenhouse setting, and (c) investigate the responses of 27 selected individuals from a biparental population with variable resistance levels to foliar phylloxera in a greenhouse setting. For objectives (b) and (c), the photosynthetic responses were assessed by the traditional method to quantify the rate and by the imaging method to quantify the energy.

## Materials and Methods

**Experiment 1.** Minnesota vineyards with 'Frontenac' vines were selected for field trials in 2017 and 2018. We relied on natural phylloxera infestations for all field trials. To establish a phylloxera-free (uninfested) treatment, the systemic insecticide Movento® (active ingredient: spirotetramat; Bayer Corp., Whippany, NJ) was used to treat vine foliage (Johnson et al. 2009).

Three field sites were selected each year, with a total of four unique sites used in 2 yr. Two sites (Melrose, MN and Prior Lake, MN) used the Vertical Shoot Position (VSP) trellis system with 7.3-m panels (distance between trellis posts) consisting of three vines per panel. A third site (Red Wing, MN) used the Geneva Double Curtain (GDC) trellis system with 6.1-m panels consisting of four to six vines per panel. In 2017, due to a low level of infestation at the Melrose site and management practices at the Prior Lake site, only the Red Wing site was included. The fourth site (Welch, MN) was added in place of the Melrose site in 2018, and it also used the VSP system. Each panel served as the unit of the treatment. For the VSP systems, harvest samples were collected from each panel, whereas for the GDC system, samples were collected from each side of the split canopy and averaged to represent each panel.

In 2017, following recommended practices, two sprays of Movento (prebloom and postbloom) were applied by the grower to every other panel for a total of three treated and three untreated panels at 584 ml/ha/spray by using a tractor-driven air blast sprayer. In 2018, to determine if a single spray of Movento (prebloom) was sufficient to control phylloxera, the experimental design included an additional treatment of one spray. This procedure resulted in a completely randomized block design of three treatments with each treatment replicated five times. Panels were sprayed by the researcher using a $CO_2$ backpack sprayer at 35 psi (R&D Sprayers, Opelousas, LA) and a 0.9- or 1.5-m boom (depending on the grape canopy) fitted with two or three XR8002 flat fan nozzles (TeeJet®, Glendale Heights, IL). Sprays were applied in the mornings at a wind speed of $<9.7$ km/h with a walk speed of 4.8 km/h. In each year, the timing for the first spray or single spray treatment was immediately before bloom (prebloom), and the second spray was 1 mo later (postbloom) to evaluate the effect of prebloom infestations (0-spray versus 1-spray treatments), postbloom infestations (1-spray versus 2-spray treatments), and infestations at both pre- and postbloom (0-spray versus 2-spray treatments). All other fungicides or herbicides were applied as normal by the grower.

Each field site was visited twice in 2017 and four times in 2018 to determine the optimal timing for observing variation in phylloxera severity of different treatments where a visual 0–7 score (Clark et al. 2018) was given to each panel. Briefly, an average score was given to the young shoots of each panel (the insect infests young leaves) where 0 = 0% of the leaf was infested, 1 =1–9% infested, 2 = 10–19%, 3 = 20–29%, 4 = 30–39%, 5 = 40–49%, 6 = 50–59%, and 7 ≥ 60%. About 1.5 mo before harvest in 2017 only, the cluster number per panel was counted and the cluster density was scored on a 1 to 9 scale defined as 1 = berries loosely distributed to 9 = densely distributed as per the International Organisation of Vine and Wine OIV 204 (OIV 2009). At harvest maturity (according to the grower's parameters), cluster weight within 1 m of the center of each panel was measured (hereafter referred to as "1-m cluster weight"). One-hundred berry weight also was obtained for each panel. In 2017, the berries were hand-macerated in zip-lock bags and filtered through a fine metal sieve to extract fermentable juice. An alternative juicing method was used in 2018 leading to highly variable results, and the data were not used. Total titratable acidity (g/L), pH, and soluble solids content (°Brix) of the juice were each measured using a 916 Ti Touch® autotitrator (Metrohm, Herisau, Switzerland), an Acumet AR15® pH meter (Fischer Scientific, Fair Lawn, NJ), and a PA 201® digital refractometer (Misco, Solon, OH), respectively. Specifically, to obtain titratable acidity, a 0.1 mol/L NaOH solution was used to titrate each juice sample diluted in 1:4 deionized water, and tartaric acid was used as a standard.

Paired *t* tests were used to analyze the 2017 data by using the *t.test* function in R (R-Core-Team 2019). Analysis of variance (ANOVA), fitting a linear model of treatment and replication effects, was used to analyze the 2018 data for each site. Each ANOVA model was checked to verify it met the assumption of normally distributed residuals. If there was a significant treatment effect, treatment means were separated using the *Tukey HSD* function of *multcompView* package in R (R-Core-Team 2019, Graves et al. 2019).

**Experiment 2.** Three photosynthesis-related traits were measured on two treatments (phylloxera infested and uninfested control) of five replications/plants of 'Edelweiss,' 'Frontenac,' MN1264, and MN1246 in the greenhouse in 2020. In 2019, the design varied slightly with only 'Frontenac' and MN1246 as uninfested controls. 'Edelweiss' is a *Vitis labrusca*-derived variety known to be resistant to phylloxera. MN1264 and MN1246, two advanced hybrid genotypes of multiple *Vitis* species, were the resistant and susceptible parent, respectively, of a mapping population that was used to fine map genetic resistance to phylloxera (Yin 2020). Briefly, this population is composed of ∼1,000 full-sib seedlings that segregated for phylloxera susceptibility, for which a major quantitative trait locus on chromosome 14 (Clark et al. 2018) was narrowed to a genetic region of a few candidate resistance genes.

From May to June 2019, plants were propagated from 1- or 2-node cuttings of field-grown vines at the University of Minnesota Horticultural Research Center, Excelsior, MN. Cuttings were rooted using Hormex® powder (0.3% indole-3-butyric acid) in perlite under a daytime misting rate of 6 s every 8 min for about 1 mo. Rooted cuttings were transplanted into Sungrow® Professional Growing Mix (SS#8-F2) with 4.3 g/L Osmocote® Plus slow-release fertilizer (15-9-12) and maintained in the greenhouse with supplementary 16-h lighting at ~24°C for about 1 mo. Plants were maintained and fertilized every other week with Peters® Professional Peat Lite Special 20-10-20.

To infest plants, a large active gall was attached to the second mature leaf (the second youngest nontender leaf) of each plant by using an alligator hair clip, and plants were maintained in a greenhouse at ~24°C. The large galls were taken from a phylloxera colony reared on 'Frontenac' and MN1246 plants in a greenhouse originating from a single gall of naturally occurring phylloxera in Minnesota vineyards. The plants in the infested treatment were kept in a neighboring greenhouse to the uninfested plants with similar lighting and temperature conditions in 2019 and at the opposite end of the same greenhouse in 2020. After the completion of the experiment in August 2019, the plants were pruned to two to four nodes to be used again in 2020. To ensure there was no evidence of foliar phylloxera, the infested plants were stored at 4°C for ~3 mo, whereas the uninfested plants were pruned another time and maintained at supplementary 16-h lighting at ~24°C. Before the start of the 2020 experiment, to minimize the treatment effect in 2019, plants were randomized within each variety of each treatment.

Photosynthetic rate, as a function of absorbed $CO_2$, and total conductance to water vapor (boundary layer conductance plus stomata conductance, hereafter referred to as conductance) were measured on each plant at infestation and weekly after infestation for 4 weeks (2019) and 6 weeks (2020) by using a LI-6400XT® system (LiCor, Lincoln, NE). A longer measurement period (6 weeks) was used in 2020 to capture the infestation effects. The $CO_2$ mixer was fully monitored at 400 μmol/mol; relative humidity and temperature were ambient; the flow rate to sample cell was 500 μmol/s; and quantum flux was 1,500 $\mu mol/m^2/s$, representative of a typical sunny morning. Measurements were taken in the greenhouse with ambient lighting by clamping on the first mature/last tender leaf of each plant. After 1.5 min, photosynthetic rate and conductance data were taken.

Y(II) was measured on each plant at infestation and weekly after infestation for 4 weeks (2019; no measurement at week 0) and 6 weeks (2020) by using IMAGING-PAM® Chlorophyll Fluorometer (Walz, Effeltrich, Germany). The MINI version was used in 2019 and MAXI version was used in 2020 to improve image resolutions. To ensure the uniformity of measurement conditions, plants were imaged in a dark room and dark adapted for >15 min before imaging. The fluorescent image was taken on the first mature/last tender leaf of each plant flattened on a nonfluorescent background. In 2019, the light intensity parameter was set to one with blue light-emitting diodes and a standard working distance using default parameters: (ML3/MF8 = AL0) where measuring light intensity at 3, measuring light frequency at 8, and actinic light intensity 0). In 2020, an aperture of 1.7 and light intensity of 3 were used, for which the focus and zoom were manually adjusted for each image. The Y(II) value of each image was visualized using the Walz ImagingWin software.

ANOVA was conducted for photosynthetic rate and conductance fitting a linear model of week and replication effects for each genotype ('Edelweiss,' 'Frontenac,' MN1264, and MN1246) and each treatment (infested and uninfested with phylloxera). Residual normality and equal variances were examined on the model by using the QQ-plot and Scale-Location plot from the *plot.lm* function in R. To visualize the results, each trait was plotted using *ggplot2* with standard errors plotted by week using *geom_errorbar* (Wickham 2016); the value at each week was compared to that of week 0 by using *t.test* function in R with unequal variances.

Because the comparison of Y(II) values on a whole-leaf basis showed no significant results, we investigated the localized effects of galled versus ungalled areas per leaf. Effective Y(II) values of 3 galled and 3 ungalled circular areas with a diameter of ~25 pixels of each leaf for 2 leaves/plants of each genotype at week 4 each year were compared using *t.test* with unequal variances. To further examine the dynamic of Y(II) around a galled area, we also took a ~40-pixel transect across 3 galled versus 3 ungalled areas on a 'Frontenac' leaf in 2020.

**Experiment 3.** Five replications of each of 27 selected individuals of GE1783 ($n = \sim1{,}023$) representing different haplotypes based on the parent chromosomal contribution, resistant or susceptible, at 4.805 Mbp on chromosome 14 accordingly to the fine mapping data (Yin 2020) were similarly infested with phylloxera in 2019. These plants were in a randomized complete block design and were used to fine map the phylloxera resistance quantitative trait locus (Yin 2020). Photosynthetic rate and conductance were measured at weeks 2 and 4 after infestation by using LiCor as in experiment 2. Y(II) was measured at week 4 using PAM MINI as in experiment 2. Measurements of all plants were performed on the same day except at week 4 in 2019, and Y(II) was measured over 2 days. Statistical analysis included an ANOVA, by fitting a linear model of haplotype effect, number of individuals within each haplotype, and replication effects. Pearson correlations between photosynthetic rate/conductance/Y(II) and phylloxera severity rating at week 2 and week 4 were calculated using the *rcorr* function of the *Hmisc* package (Harrell 2019).

## Results

**Experiment 1.** Foliar phylloxera infestations as measured by phylloxera ratings at ~2 mo after the first spray (at véraison and ~2 mo before harvest) were significantly lower in Movento spray treatments. This optimal timing for evaluation was chosen for observing the variation in phylloxera severity 2 mo after the spray treatments based on ANOVA results. Specifically, the mean phylloxera rating of the 0-spray treatment was the highest of all site-years at Red Wing in 2017, and the rating decreased significantly from 6.6 to 0.5 in the 2-spray treatment ($t = 40.2$; df = 2; $P \leq 0.001$; Table 1). In 2018, the field sites had an overall lower phylloxera incidence as reflected in the 0-spray treatment data. Despite this result, there were significant differences among treatments at Prior Lake ($F = 31$; df = 2, 8; $P \leq 0.001$ from ANOVA) and Welch ($F = 13$; df = 2, 8; $P \leq 0.01$) but not at Red Wing, where there was very low incidence (Table 1). Specifically, at Prior Lake, there was a change of rating by 2.1 units and at Welch a change of 1.4 units. There were no significant differences in rating scores between the 1-spray and 2-spray treatments.

At Red Wing in 2017, the 1-m cluster weight (yield estimate) increased in the 2-spray treatment from the 0-spray treatment by 1,298 g/m ($t = -4.1$; df = 2; $P = 0.055$; Table 1). In 2018, no significant differences in the 1-m cluster weight were observed among the treatments at each site, even though there were trends for higher yields due to the spray treatments, specifically at the Welch site ($F = 0.60$; df = 2, 7; $P = 0.58$). The 1-m cluster weight increased in the 2-spray treatments from 0-spray treatments by 450 g/m at Welch (Table 1). Also, the 1-m cluster weight increased numerically in the 2-spray treatments from 1-spray treatments at all 3 sites in 2018.

**Table 1. The effect of Movento® spray treatments on phylloxera rating, fruit yield, and juice quality traits on the hybrid wine grape 'Frontenac' at Red Wing, MN, in 2017 (a paired *t* test) and at three sites in 2018 (separate ANOVA and Tukey's HSD per site). Means are shown with ± standard errors.***

| | 2017 Red Wing | | 2018 Prior Lake | | |
|---|---|---|---|---|---|
| | **0 spray** | **2 spray** | **0 spray** | **1 spray** | **2 pray** |
| Phylloxera rating** | 6.6 ± 0.1**a** | 0.5 ± 0.1**b** | 3.3 ± 0.3**a** | 1.2 ± 0.2**b** | 1.3 ± 0.3**b** |
| Cluster number** | 198 ± 6 | 226 ± 19 | - | - | - |
| Cluster density** | 2.8 ± 0.2 | 3.1 ± 0.1 | - | - | - |
| 1-m cluster weight (g) | 1,600 ± 132**a***** | 2,899 ± 246**b** | 744 ± 217 | 503 ± 49 | 749 ± 111 |
| 100-berry weight (g) | 115 ± 2 | 118 ± 4 | 89 ± 4 | 85 ± 5 | 93 ± 1 |
| Soluble solids (°Brix) | 16.9 ± 0.4 | 18.3 ± 0.4 | - | - | - |
| pH | 3.27 ± 0.02 | 3.29 ± 0.03 | - | - | - |
| Titratable acidity (g/L) | 12.61 ± 0.15 | 12.67 ± 0.59 | - | - | - |

*Bolded letters show significant differences between treatments of paired *t* tests ($\alpha = 0.05$).

**Traits were measured ~2 mo before harvest; other traits were measured at harvest.

***Means are significant at $\alpha = 0.10$. All other mean comparisons are based on Tukey's honestly significant difference (HSD), and ANOVA fitting a linear model ($\alpha$=0.05); no letters indicate no significant differences.

It increased 188 g/m at Red Wing ($t = -1.1$; df = 8; $P = 0.30$), 246 g/m at Prior Lake ($t = -2.0$; df = 5; $P = 0.094$), and 49 g/m at Welch ($t = -0.11$; df = 7; $P = 0.91$).

Although not significant, at Red Wing in 2017 (the GDC trellis system), the number of clusters per panel also increased by 29 ($t = -2.0$; df = 2; $P = 0.18$) and the cluster density increased by 0.3 score ($t = -1$; df = 2; $P = 0.42$) in the 2-spray treatment (Table 1). Juice quality data were collected only at Red Wing in 2017. Soluble solids content increased in the 2-spray treatment compared to the 0-spray treatment by 1.4 °Brix ($t = -2.9$; df = 2; $P = 0.10$; Table 1). No significant differences were observed between the treatments for juice pH or titratable acidity.

**Experiment 2.** Under controlled greenhouse conditions, photosynthetic rate decreased significantly after infestation for the susceptible variety 'Frontenac' in 2019 ($t = 3.5$; df = 6.8; $P \leq 0.05$; week 0 compared with infested at week 4) and the resistant genotype MN1264 in 2020 ($t = 4.0$; df = 5.1; $P \leq 0.05$; week 0 compared with infested at week 6), whereas this reduction was not observed in the corresponding uninfested 'Frontenac' ($t = 0.8$; df = 6.9; $P = 0.45$) or MN1264 ($t = -1.6$; df = 3.3; $P = 0.21$) (Fig. 1). There were decreasing trends at weeks 1 to 4 for the resistant variety 'Edelweiss' in 2019 and at weeks 5 to 6 for the susceptible genotype MN1246 in 2020 (Fig. 1). For these genotypes in the other years, no clear trends were observed.

**Table 1. Extended.**

| 2018 Red Wing | | | 2018 Welch | | |
|---|---|---|---|---|---|
| **0 spray** | **1 pray** | **2 spray** | **0 spray** | **1 spray** | **2 spray** |
| 0.7 ± 0.4 | 0.7 ± 0.1 | 0 ± 0.0 | 1.5 ± 0.4**a** | 0.2 ± 0.1**b** | 0.1 ± 0.1**b** |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| 529 ± 44 | 442 ± 114 | 630 ± 123 | 895 ± 388 | 1,297 ± 241 | 1,345 ± 355 |
| 132 ± 4 | 133 ± 6 | 131 ± 3 | 104 ± 11 | 122 ± 7 | 114 ± 4 |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| - | - | - | - | - | - |

The only significant observation for conductance to water vapor was made for resistant 'Edelweiss' in 2019 for which there was a decrease due to infestations ($t = 3.59$; df $= 4.3$; $P \leq 0.05$; Fig. 2). There were no clear trends for conductance of other genotypes in 2019. In 2020, there were numeric increases for susceptible 'Frontenac' (no statistical tests) and at weeks 0 to 5 for susceptible MN1246, whereas there was a decreasing trend for resistant MN1264 and no change for resistant 'Edelweiss' (Fig. 2).

When comparing the effective Y(II) of galled and ungalled areas, we found that galled areas had lower Y(II) values than ungalled areas for all varieties (Fig. 3). Significant or near-significant reductions associated with gall formation were observed in susceptible 'Frontenac' in 2019 ($t = -4.7$; df $= 6.5$; $P \leq 0.01$) and 'Edelweiss' in 2019 ($t = -2.7$; df $= 4.2$; $P = 0.054$). For 'Frontenac' in 2020 and for the susceptible MN1246 in both 2019 and 2020, there were numeric reductions. There were no clear trends in MN1264 in 2019 and 2020. Looking at the transect across an area, we observed a dip in Y(II) for a galled area, whereas we did not observe a clear dip for an ungalled area (Fig. 4).

**Experiment 3.** There was a significant haplotype effect for conductance at week 2 ($F = 4.63$; df $= 1, 24$; $P \leq 0.05$; 27 individuals tested), whereas individuals with phylloxera-resistant haplotypes had a 0.023 mol/m$^2$/s higher conductance to water vapor than individuals with susceptible haplotypes. There were no significant haplotype effects for other traits measured. Conductance correlated positively ($r =$

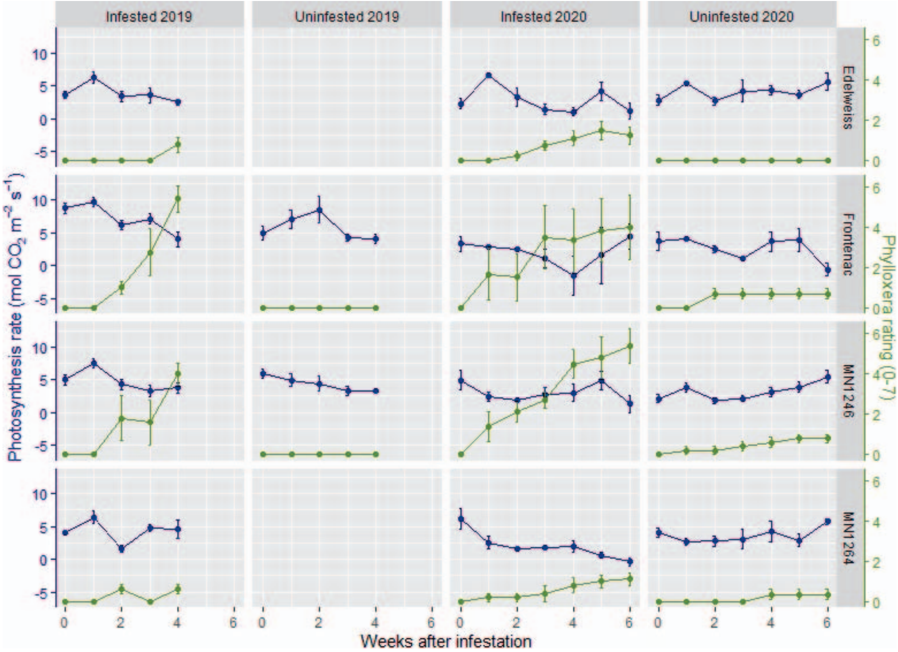

**Fig. 1 Photosynthetic rate (blue) and foliar phylloxera rating (green) of infested and uninfested 'Edelweiss,' 'Frontenac,' MN1246, and MN1264 at each week after infestation in 2019 and 2020. Significant differences are reported comparing that of week 0 to week *n* ($n = 1–6$) by using *t* tests; ns, nonsignificant; $n = 5$. Error bars, standard errors.**

0.24; $P \leq 0.05$) with gall ratings at week 4; however, there were no significant correlations for other traits measured (data not shown).

## Discussion

This study demonstrated that naturally occurring foliar phylloxera infestations in the Midwestern United States may lead to some level of yield reduction, as measured by 1-m cluster weight, for the cold-hardy, hybrid wine grape 'Frontenac.' The level of yield reduction, however, was inconsistent across years. Under controlled greenhouse infestations, by using a few resistant and susceptible varieties in addition to 'Frontenac,' we found reductions in photosynthesis rate and Y(II), and likely a variety-specific response on conductance. This study, although not comprehensive, serves as a starting point for additional multiyear field studies to investigate the effects of folia phylloxera under a broader range of environmental conditions. The results also highlight the possibility of a variety-specific response to grape phylloxera infestations and the potential use of high-throughput phenotyping technologies to achieve this goal.

With the hybrid wine grape 'Frontenac' and natural phylloxera populations, we confirmed a significant reduction ($P = 0.055$) in 1-m cluster weight of foliar

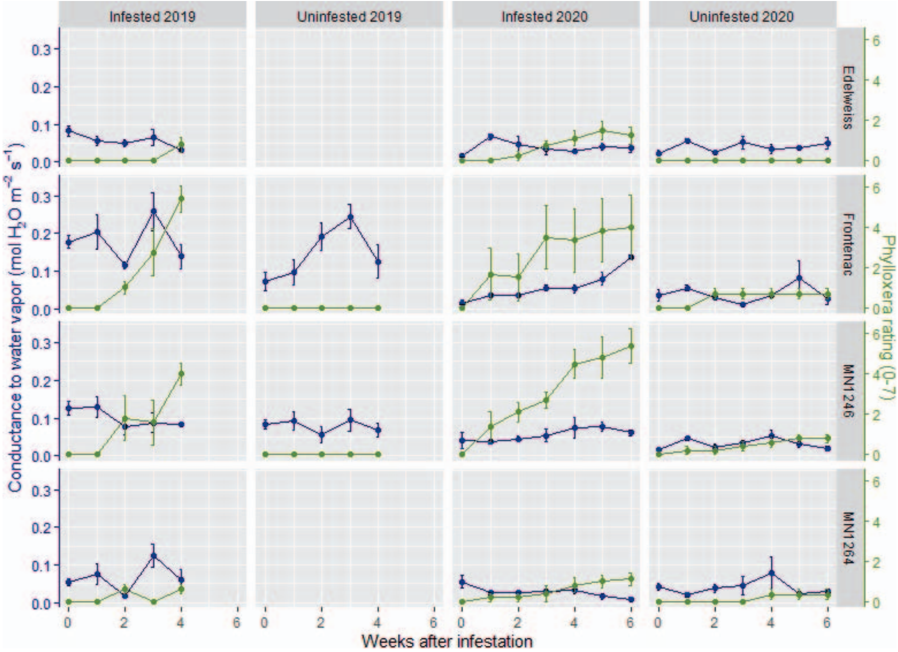

**Fig. 2 Conductance to water vapor (blue) and foliar phylloxera rating (green) of infested and uninfested 'Edelweiss,' 'Frontenac,' MN1246, and MN1264 at each week after foliar phylloxera infestation in 2019 and 2020. Significant differences are reported comparing that of week 0 to week *n* (*n* = 1–6) by using *t* tests; ns, nonsignificant; *n* = 5. Error bars, standard errors.**

phylloxera infestation at Red Wing, MN, in 2017 and a numerical reduction at Welch, MN, in 2018; these results may be of economic importance to grape growers. Two sprays of Movento cost $107/acre but can result in an 84% increase in 1-m cluster weight that is equivalent to a potential $2,275/acre increase in profit, assuming that phylloxera infestations were as high as that in Red Wing in 2017. The profit estimate also assumes a current price for Minnesota 'Frontenac' grapes at $0.82/lb and an average yield for Minnesota grapes at 3,303 lb/acre (Clark et al. 2019). We also observed numeric reductions in the number of clusters per panel, but this was only evaluated in one site year. Of particular interest at the Welch site in 2018, there was only a 1.4 difference in the phylloxera scores between treated and untreated plots, and yet we observed a 450 g difference in 1-m cluster weight. At the other two sites in 2018, we did not observe differences in 1-m cluster weight between the 0-spray and 1-spray prebloom treated vines. These results agree with McLeod (1990), in which prebloom phylloxera infestations led to some reduction in 1-m cluster weight, indicating a variable phylloxera response each year and across sites, because of either phylloxera pressure or environmental conditions.

A second postbloom spray did not result in a decrease in phylloxera severity rating but showed an increasing trend in cluster yield in 2018. Prebloom sprays of Movento

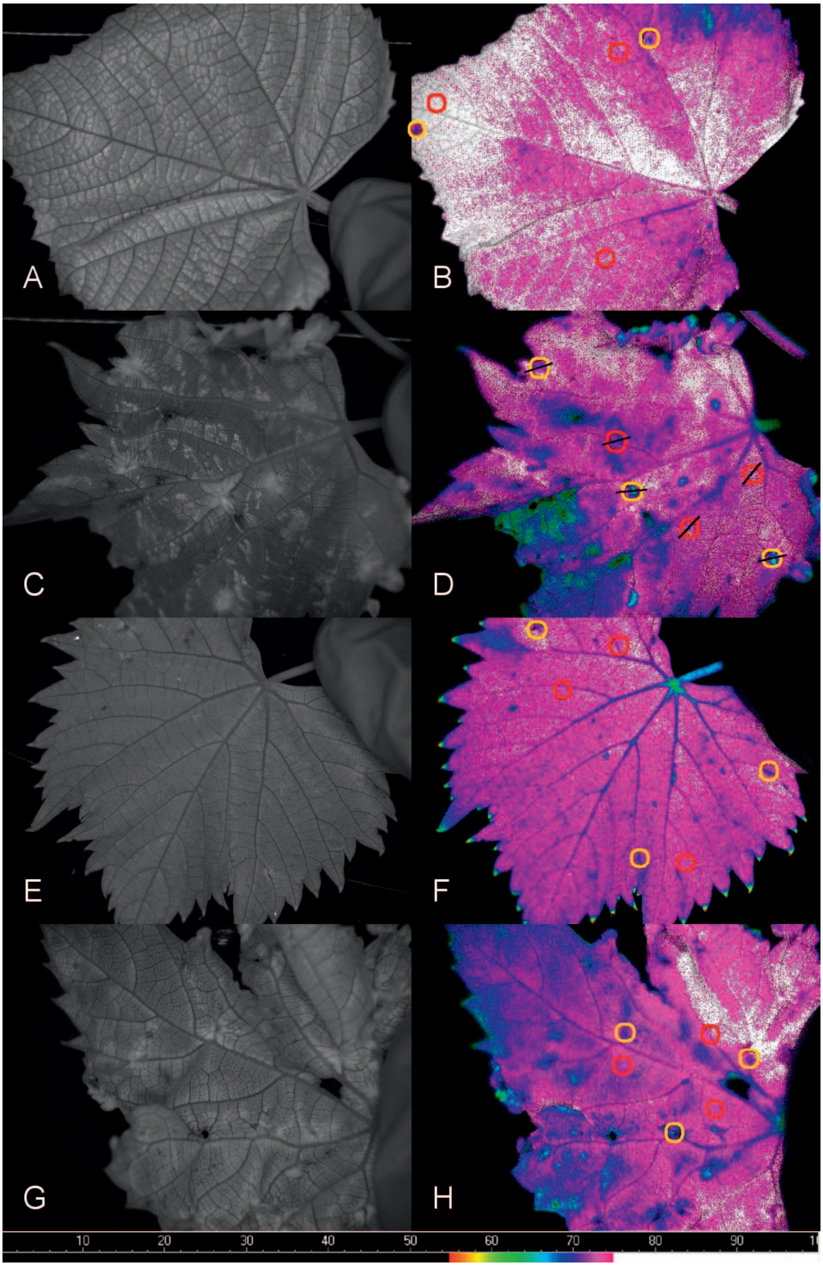

**Fig. 3** Near-infrared gray-scale (left panel) and corresponding effective photosystem II quantum yield images ([Y(II); right panel] of phylloxera infested ‘Edelweiss’ (A and B), ‘Frontenac’ (C and D), MN1264 (E and F), and MN1246 (G and H) after 4 weeks measured by PAM-MAXI in 2020. Scale for Y(II) images: the lower the value, the more stressed the leaf tissue; the higher the value, the less stressed. For Y(II)

**Fig. 4 Effective photosystem II quantum yield at a 40-pixel transect across three galled and three ungalled areas of an infested 'Frontenac' leaf after 4 weeks in 2020.**

on 'Frontenac' resulted a significant difference in phylloxera severity. An additional postbloom spray did not reduce phylloxera severity any further. If phylloxera pressure at the site is low (rating of <1) and if the site has a spray history (e.g., the Red Wing site that was sprayed in 2017 had low phylloxera pressure in 2018), the grower might not need to spray. Growers should scout for signs of galls early in the season and perhaps use spot-spraying and other integrated pest management strategies as outlined in Yin et al. (2019). This procedure would result in economic savings and reduced environmental risk (Radcliffe et al. 2009). However, to maximize yield, the 2-spray treatment seemed to outperform the 1-spray treatment numerically at all 3 sites in 2018. For example, the additional spray resulted in a 49% increase in 1-m cluster weight at Prior Lake in 2018. In other words, prebloom infestations seemed to be important in both phylloxera severity development and yield reductions, whereas postbloom infestations (vines already sprayed prebloom) appeared to be important in yield reductions. More site-years and studies on additional varieties are needed to further investigate this effect on yield.

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**comparison analysis: three galled (yellow circles) and three ungalled ~25-pixel diameter areas (red circles) per leaf; for Y(II) dynamic analysis: ~40-pixel black transects across three galled and three ungalled areas per leaf.**

Many factors may a play role in the variability of some of the phylloxera effects observed. Precipitation at Melrose, MN, in 2017 was below 81.3 cm (32 inches), which lower than the other 2 sites, providing one reason why we may have observed low phylloxera pressure (Minnesota Department of Natural Resources). This hypothesis agrees with McLeod (1990) that foliar phylloxera infestation was low in dry years. A better understanding on the favorable conditions for foliar gall formation would allow for better timing of measurements and the precise definition of an economic threshold (McLeod 1990). Another confounding factor is spray management at different vineyards. In 2017, a fungicide was included with the Movento treatment at the Prior Lake site, whereas the Melrose site was sprayed with Danitol 2.4 EC the previous year. Both sites had confounded results and were not included in 2017. At Prior Lake in 2018, we cleaned the clusters of damaged berries before weighing, which was not done at other site-years, and likely contributed to the less significant differences among treatments at that site.

Phylloxera infestations may lead to reductions in photosynthetic rate on both resistant and susceptible varieties. However, this trend was only observed in 1 of 2 yr of data. A $\sim$5 mol $CO_2$ $m^{-2}s^{-1}$ reduction in photosynthesis rate due to infestation (a healthy plant has a rate of 9 ± 1 mol $CO_2$ $m^{-2}s^{-1}$) was observed in susceptible varieties 'Frontenac' in 2019 ($P \leq 0.05$) and MN1246 in 2020, as well as resistant varieties 'Edelweiss' in 2019 and MN1264 in 2020 ($P \leq 0.05$). Nabity et al. (2013) also observed a reduction in photosynthetic rate in infested 'Frontenac.' The 'Frontenac' plants in 2020 had poor growth, providing one plausible explanation for why we did not observe the same trend in 2020. Under the greenhouse conditions, 1 mo after infestations, infested 'Frontenac' had an average score of about 4.5 and uninfested 'Frontenac' had a score of 0.5, which is comparable to that in the field at the same timing (data not shown), where 0-spray (infested) 'Frontenac' had a score of 3 and the 1-spray and 2-spray treatments (uninfested) had a score of 0.5. Despite all the variation due to the machine measurements and plant health, the data imply that the effect of phylloxera infestations on photosynthesis was detrimental that even low levels of infestations in the resistant varieties had reductions in photosynthetic rate, whereas similar infestations did not result in such a severe effect on yield. Stevenson (1969) observed a reduction in leaf number per shoot for phylloxera infested treatments. Thus, the reduction in photosynthetic rate might be due to a reduction in total photosynthetic leaf area.

Although there might be less of an effect of phylloxera infestations on conductance to water vapor in resistance varieties, we have only 1 of 2 yr of data to support this conclusion. For the susceptible genotypes 'Frontenac' and MN1246 infested in 2020, we saw an increasing (yet nonsignificant) trend of conductance after infestations, of which the resistant genotypes had no such trend or an opposite trend. It could be that the increase in stomatal conductance due to gall formation (Nabity et al. 2013) was more pronounced for susceptible genotypes. If this is true, it suggests resistant plants have some fitness advantages and that there is the need to breed for such plants. However, this trend was not observed in both years, and limited conclusions can be drawn. Although we found a positive correlation of conductance and gall rating at week 4 (resistant plants had lower conductance), there was a significant haplotype effect for conductance at week 2 (resistance haplotype had higher conductance). That said, more research is needed regarding the degree to which conductance is affected by infestations on susceptible and resistant varieties.

For effective Y(II), there were no significant effects due to infestations at the whole-leaf level (data not shown), but Y(II) was numerically lower for galled areas than that of ungalled areas of the leaf in both susceptible and resistant varieties, and more often in susceptible varieties. The ~25-pixel area may have been too coarse to capture significant differences between galled and ungalled areas, and more refined image segmentation is needed. Aldea et al. (2006) found lower PS II operating efficiency [an equivalent to Y(II) but under light-adapted conditions] surrounding galled areas formed by midge and wasp species. They also found increased nonphotochemical quenching and lower leaf temperatures surrounding galls, suggesting that the reduction in PS II efficiency was not due to stomatal closure but increased transpiration rates, which we did not measure here. Also, whether Y(II) has a more variety-specific response to phylloxera infestations needs further investigation.

In summary, our results suggest (a) foliar phylloxera infestations may have an economic effect on wine grape yield with the popular variety 'Frontenac'; (b) prebloom infestations were likely more important in leading to high grape phylloxera severity, whereas both prebloom and postbloom infestations might have an effect on yield; (c) photosynthetic rate was affected by infestations of both susceptible and resistant grape varieties, whereas yield, conductance, and Y(II) were less sensitive to infestations, which might have a variety-specific response; and (d) additional studies are needed, with more growing seasons, more varieties, and more traits to fully understand the pest's potential effects in this perennial system. How yield is affected and whether photosynthesis rate, conductance, and/or Y(II) play a role need to be better understood. The response of crop yield to foliar phylloxera infestations might be more related to the latter two traits than to photosynthetic rate as previously thought, assuming yield, like conductance and Y(II), also has a variety-specific response. Studying the impact of foliar phylloxera on crop yield of resistant varieties may help answer this question. In addition, stomata density, transpiration rate, and other yield components such as number of berries per cluster are worth investigating to better understand the mechanism through which yield was affected. Increased knowledge of the effects of infestations on the diversity of grape varieties grown in the Midwest Region will help develop management guidelines to control this pest and understand the significance of breeding resistant plants.

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