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Research Article

Spatial Roguing Reduces the Incidence of Leafroll Disease and Curtails its Spread in a Finger Lakes 'Cabernet Franc' Vineyard

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Abstract: Leafroll is one of the most economically important viral diseases of grapevines worldwide. Grapevine leafroll-associated virus 1 (GLRaV-1) and grapevine leafroll-associated virus 3 (GLRaV-3) are prevalent in New York vineyards in which low-density grape mealybug populations reside. A five-year experiment was performed in a commercial 'Cabernet franc' vineyard in the Finger Lakes region of New York to test the influence of spatial roguing, i.e., the elimination of virus-infected vines and their two immediate within-row neighbors on each side, on the annual incidence of GLRaV-1 and GLRaV-3. In a second treatment, spatial roguing was combined with insecticides. Vines eliminated in both spatial roguing treatments were replaced by clean vines derived from virus-tested stocks. The objective of this study was to reduce temporal virus incidence to less than 1% over two consecutive years and limit virus spread. In both spatial roguing treatments, virus incidence was reduced from 5% in 2016 to less than 1% in 2020-2021. Among vines in the insecticide-free, non-rogued control treatment, virus incidence increased from 5% to 16% from 2016 to 2021. Insecticides applied in 2016-2021 helped significantly reduce grape mealybug populations to near zero annually, while populations in the untreated control vines were 57 to 257-fold higher during the same period. However, insecticides contributed relatively little to limit the number of newly infected vines. Together, these findings highlight the salient contribution of roguing to an overall leafroll disease management response in a vineyard with low disease incidence and low grape mealybug abundance. To our knowledge, this is the first report on the effectiveness of spatial roguing at reducing the annual incidence of leafroll disease in a vineyard.

Key words: clean vines, grape mealybug, insecticides, leafroll, roguing, virus

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Introduction

Leafroll is one of the most damaging viral diseases of grapevines worldwide. It reduces vine vigor and fruit yield, delays fruit ripening, and alters wine sensory attributes by drastically limiting post-véraison leaf photosynthesis and altering the berry maturation process, particularly anthocyanin biosynthesis and sugar metabolism (Maree et al. 2013, Naidu et al. 2014, Naidu et al. 2015, Mannini and Digiaro 2017, Song et al. 2021). In the Finger Lakes region of New York, a delayed accumulation of juice soluble solids by about three weeks, and reduced yield by up to 25% was documented in *Vitis vinifera* 'Cabernet franc' vineyards (Martinson et al. 2008). The economic loss of leafroll disease is estimated to range from $25,400 to $40,000 per hectare over a 25-year lifespan of a 'Cabernet franc' vineyard in New York (Atallah et al. 2012).

Six viruses have been identified in leafroll diseased vines (Fuchs 2020). These viruses are introduced to vineyards in infected planting stocks. Some of them, i.e., grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-3, and GLRaV-4, are also transmitted from infected to healthy vines by several species of mealybugs and soft scale insects (Daane et al. 2012, Naidu et al. 2014, Naidu et al. 2015, Herrbach et al. 2017, Fuchs 2020). No insect vector is known for GLRaV-2 and GLRaV-7 (Naidu et al. 2015, Herrbach et al. 2017), and GLRaV-13 is likely transmitted by mealybugs and soft scale insects, given its distant relationship to GLRaV-1 (Ito and Nakaune 2016). Among the six viruses associated with leafroll disease, GLRaV-3 is prevalent in most diseased vineyards worldwide (Almeida et al. 2013, Maree et al. 2013, Naidu et al. 2014, Naidu et al. 2015, Song et al. 2021). GLRaV-3 and to a lesser extent GLRaV-1 are widespread in vineyards in the Finger Lakes region of New York (Fuchs et al. 2009).

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Transmission of leafroll viruses by mealybugs is non-circulative and non-propagative (Almeida et al. 2013, Maree et al. 2013, Naidu et al. 2015, Herrbach et al. 2017, Song et al. 2021). First instars of the vine mealybug (*Planococcus ficus*) acquire and transmit GLRaV-3 within less than an hour and are more effective vectors than adults (Tsai et al. 2008). Similarly, first instars of the apple mealybug (*Phenacoccus aceris*) and cottony grape scale (*Pulvinaria vitis*) are more efficient vectors of GLRaV-1 than adults (Le Maguet et al. 2012, Hommay et al. 2021). In addition, a single instar of the vine mealybug carrying GLRaV-3 can initiate infection (Tsai et al. 2008). In vineyards in the Finger Lakes region of New York, the grape mealybug (*Pseudococcus maritimus*) is an important vector of GLRaV-1 and GLRaV-3, although it is not a direct pest of grapevines and its population density is typically low (Fuchs et al. 2009, Wallingford et al. 2015).

Spread dynamics of GLRaV-1 in a diseased vineyard in France revealed that the two adjacent vines to a vine infected with GLRaV-1 are more likely to become infected over time than their counterparts located across the row, suggesting predominant within-row spread of GLRaV-1 and a spatial dependence for secondary spread (Hommay et al. 2020). This work on spatial trends of GLRaV-1 confirmed similar trends of GLRaV-3 spread previously reported in vineyards of California (Arnold et al. 2017), Spain (Cabaleiro and Segura 1997), Australia (Habili and Nutter 1997) and New Zealand (Charles et al. 2009). No association was found between the spatial distribution of vines infected by GLRaV-1 and density of soft scales (Hommay et al. 2020). In contrast, spread dynamics of GLRaV-3 in vineyards of California and New Zealand are primarily influenced by virus incidence and mealybug population density (Blaisdell et al. 2016, Bell et al. 2018, Cooper et al. 2018).

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Leafroll disease can be effectively managed in the vineyard by reducing the number of infected vines and controlling mealybug vector populations (Almeida et al. 2013, Maliogka et al. 2015, Naidu et al. 2015, Herrbach et al. 2017). For example, the elimination of diseased vines (known as roguing) and their replacement with clean vines derived from virus-tested stocks (nursery vines that produce rootstock cuttings or scion budwood and test negative for economically relevant viruses, including leafroll-associated viruses) reduced the incidence of GLRaV-3 and limited its secondary spread in vineyards of California (MacDonald et al. 2021), South Africa (Pietersen et al. 2013) and New Zealand (Bell et al. 2017, Bell et al. 2018). In these studies, only diseased vines, not any neighbor vines, were eliminated to successfully manage leafroll disease (Pietersen et al. 2013, Bell et al. 2017, Bell et al. 2018, MacDonald et al. 2021).

To refine roguing, a model of disease spread in relation to bioeconomic factors was developed (Atallah et al. 2015). This model incorporated spatial parameters of leafroll disease spread. It predicted that roguing diseased vines and neighboring vines, a strategy referred to as spatial roguing, is more effective at reducing the level of virus inoculum in a diseased vineyard than roguing only diseased vines, with the best strategy consisting of eliminating diseased vines and two immediate within-row neighboring vines on each side, regardless of their disease status (Atallah et al. 2015). This spatial roguing strategy was inspired by the fact that (i) mealybug crawlers are more efficient vector of leafroll viruses than adults (Tsai et al. 2008, Le Maguet et al. 2012), (ii) crawlers are more likely to move along rows than between rows (Daane et al. 2012, Almeida et al. 2013, Herrbach et al. 2017), (iii) leafroll spread predominantly occurs at a short spatial scale (Almeida et al. 2013, Herrbach et al. 2017), (iv) a healthy-looking vine that is adjacent to a diseased vine may be infected without exhibiting disease symptoms (Bell et al.

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2018), and (v) disease symptoms are only apparent at least one year after inoculation by viruliferous mealybugs (Almeida et al. 2013, Blaisdell et al. 2016). The effectiveness of spatial roguing based on the removal of diseased vines and their two neighbor vines on each side, as proposed by Atallah et al. (2015), has yet to be validated in a vineyard anywhere in the world.

Here were report a six-year study designed to test the effectiveness of spatial roguing and the application of insecticides, either separately or in combination, to reduce the incidence of leafroll viruses and limit their secondary spread in a commercial 'Cabernet franc' vineyard in the Finger Lakes region of New York for which both the virus incidence and the residing grape mealybug population were low.

Materials and Methods

Vineyard selection. Several vineyards affected by leafroll disease were inspected in the Finger Lakes region of New York to select a vineyard study site. We used five criteria to determine which of these vineyards would become the single study site: (i) relative ease with which to identify diseased vines based on visual assessment of typical disease symptoms on a red-fruited *Vitis vinifera* cultivar, i.e., reddening of leaf blades, cupping, and poor fruit ripening, (ii) overall low to moderate leafroll disease prevalence (1-25%), as previously recommended Atallah et al. 2012; Ricketts et al. 2015), (iii) confirmed presence of the grape mealybug, (iv) suspected occurrence of virus spread, and (v) willingness of the vineyard manager to host this long-term study and actively contribute to the research.

Treatments and replications. The following four treatments were applied to select vine panels in the 'Cabernet franc' vineyard study site in 2016-2021: (1) spatial roguing only, (2)

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148 spatial roguing in combination with insecticide applications targeting grape mealybugs, (3)
149 insecticide applications only (no spatial roguing), and (4) no spatial roguing and no insecticide
150 intervention (the untreated control). Spatial roguing consisted of removing diseased vines and
151 two within-row adjacent vines on each side, and replacing them with clean vines, as modeled by
152 Atallah et al. (2015). For example, if an isolated vine was found infected with GLRaV-1 and/or
153 GLRaV-3, five vines, including the infected one and two on each side of it were removed. Vines
154 neighboring an infected vine were eliminated regardless of whether they displayed disease
155 symptoms or were infected by one of two of the target viruses. If three adjacent vines were found
156 infected with GLRaV-1 and/or GLRaV-3, seven vines, including the three infected vines and two
157 on each side of the first and third infected vines, were removed.

158 Each treatment included seven panels of three vines across five rows for a total of 105
159 vines per replicate. Treatments were replicated five times and assigned to vine panels throughout
160 the vineyard study site using a randomized complete block experimental design. A treatment was
161 deemed successful in managing leafroll disease if virus incidence was less than 1% per annum
162 over two consecutive years.

163 **Spatial roguing strategy.** To implement roguing, infected vines were flagged in the fall
164 of 2016, removed the following spring and clean vines were established without fallow or
165 herbicide application on the removed vines because the grape mealybug does not overwinter on
166 remnant roots in the soil (Daane et al. 2012). The same roguing approach was implemented from
167 2017 to 2020. Clean vines used as replants consisted of Cabernet franc vines grafted on
168 rootstocks 3309C or 101-14 MGt. These vines were supplied by nurseries and produced with

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cuttings and budwood derived from vine stocks that tested negative for viruses of economic relevance, including all major leafroll viruses.

Insecticide treatments. Insecticides selected for this study were Lorsban® Advanced (chlorpyrifos) and Movento® (spirotetramat). The contact insecticide chlorpyrifos was applied at label rate of 2.3 L per hectare in April after bud break when the first instar mealybugs that hatched from eggs laid the previous late summer or early fall acquire GLRaV-1 and GLRaV-3 in Finger Lakes vineyards (Fuchs et al. 2015). The systemic insecticide spirotetramat was applied at a 0.46 L per hectare rate in mid-June and mid-July when the summer generation mealybug crawlers acquire GLRaV-1 and GLRaV-3 in Finger Lakes vineyards (Fuchs et al. 2015). Spirotetramat applications included a non-ionic surfactant adjuvant (LI 700 at 0.25% v:v). Insecticides were applied in 467 liters of water per hectare. Because chlorpyrifos was banned in 2021 from use on grapevines, there was no spring application that year.

To assess the efficacy of insecticides at reducing mealybug vector populations, grape mealybug surveys were carried out annually from mid-August to early-September throughout the vineyard study site. Trunks and older canes of vines within a treatment plot were visually inspected for 15 minutes once per year to record solitary females, females that started laying eggs, and egg masses with viable eggs and/or crawlers, but no female. Trunks and older canes were targeted because grape mealybugs are rarely observed in grape clusters or on vine leaves in vineyards in New York, including in the ‘Cabernet franc’ vineyard study site. Surveys were also purposely timed late in the summer after females initiated laying eggs, which primarily occurs on trunks and older canes, and when crawlers hatching from egg masses tend to stay on or near egg masses until the following spring in New York (Fuchs et al. 2015). Observers did not spend a set

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191 amount of time per vine; rather, they moved from vine to vine by checking loose bark that could
192 be pulled back from the cambium where mealybugs tend to congregate. This approach was
193 consistent from year to year. Mealybug records were combined into grape mealybug counts, and
194 the number of grape mealybugs per minute of count was tallied annually from 2016 to 2021.

195 **Virus testing.** Prior to the onset of the study, the occurrence of leafroll viruses in the
196 'Cabernet franc' vineyard study site in 2015 was assessed by testing a sub-set of vines for
197 GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-4 by double sandwich antibody (DAS) enzyme-
198 linked immunosorbent assay (ELISA) using specific antibodies from Bioreba AG (Reinach,
199 Switzerland). Compositive leaf samples from 4-vine panels, every other vine panel, were
200 processed and tested for leafroll viruses by following the manufacturer's recommendations. Each
201 sample was tested in duplicate. A vine sample was considered positive if the average of its mean
202 absorbance values was at least three times the average of the healthy control samples.

203 To determine the efficacy of spatial roguing in combination or not with insecticides at
204 reducing the number of infected vines, leaf samples were collected annually from every vine in
205 the 'Cabernet franc' vineyard study site in early September from 2017 to 2021. Collected leaves
206 were tested for GLRaV-1 and GLRaV-3 in the laboratory by DAS-ELISA. In addition, some
207 vines were also assayed by reverse transcription (RT) polymerase chain reaction (PCR) using
208 specific primers for GLRaV-1 and GLRaV-3, as previously described (Fuchs et al. 2009). The
209 18S ribosomal gene was used as a housekeeping gene. The RT-PCR reaction products were
210 resolved by electrophoresis in 1.5% agarose gels in 40 mM Tris-acetate and 10 mM EDTA, pH
211 8.0; stained with GelRed®; and subsequently visualized under UV light (Fuchs et al. 2009). A
212 vine sample was considered positive if its total RNA yielded an RT-PCR product of the expected

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size. RT-PCR was important to ascertain the presence of GLRaV-1 in select vines because false positives were occasionally obtained for GLRaV-1 in DAS-ELISA when leaf tissue from approximately 1-5% of the young, 2-3-year old vines was tested. Through this testing procedure, the spatial distribution of infected vines was mapped within the vineyard study area from 2017 to 2021.

To assess the efficacy of the different treatments at reducing virus incidence, the number of leaf samples that tested positive for GLRaV-1 and/or GLRaV-3 in DAS-ELISA and/or RT-PCR was tallied every year for each treatment group. Then the cumulative number of infected vines was determined annually for each treatment group. To determine how the different treatments affected virus spread, the cumulative number of infected vines was assessed over time.

Statistical analyses. Virus incidence for a given treatment was expressed as the number of infected vines, as shown by DAS-ELISA and/or RT-PCR, divided by the total number of vines tested in the replicate plot and analyzed using a linear mixed model. Binomial roguing (yes or no) and insecticide (treated or not) status, year and interactions were treated as fixed effects and experimental block was treated as a random effect. A linear mixed model was also used for assessing treatment effects on mealybug counts per minute. Normality and constant variance of model residuals were assessed visually and mealybug counts per minute were log transformed to meet model normality and constant variance assumptions.

The relationship between the virus status of each vine in non-rogued treatments (untreated control plots and insecticides only plots) and its two within-row neighbor vines, one

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vine and two vines away in either direction, was assessed using a mixed effects logistic regression. The dependent variable was the virus status (yes or no) of each vine. Year, virus status of the vine on either side of the focal vine, virus status of two vines away on either side of the focal vine, and their interactions were modeled as fixed effects, and experimental blocks was modeled as a random effect.

All statistical analyses were conducted using R software version 3.02 (R Core Team. 2013). Linear mixed effect models were run using lmer function within the lme4 package (Bates et al. 2014). Mixed effects logistic regression was run using glmer function within the lme4 package.

Estimating the economic cost of spatial roguing. The economic cost of spatial roguing was estimated based on the outcomes of our study and the following five assumptions: (i) no production in years 1-3 for replants following spatial roguing, (ii) half production of replants in year 4, (iii) full production of replants in year 5, and (iv) a targeted production of 1.2 tons per hectare, a typical production of a 'Cabernet franc' vineyard in the Finger Lakes of New York (Davis et al. 2020), and (v) an average income of $2,000 per ton of 'Cabernet franc', a common sales value in New York (Davis et al. 2020). Based on these assumptions, the number of vines eliminated in the spatial roguing only treatment in 2017-2020 was used to estimate the annual reduction in fruit yield per hectare from 2017 to 2021 and make predictions for 2022 and 2023. These estimates were then used to calculate an annual lost revenue from 2017-2023. Next, the estimated and predicted losses due to spatial roguing were analyzed and compared to estimated losses caused by a strategy of no intervention to manage leafroll disease. The latter losses were

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255 previously calculated for a 'Cabernet franc' vineyard in the Finger Lakes of New York (Atallah
256 et al. 2012).

257 **Results**

258 **Vineyard selection and treatments.** Several leafroll-diseased vineyards in the Finger
259 Lakes region of New York were considered for this study. We eventually selected a 3-hectare
260 commercial 'Cabernet franc' vineyard planted in 1999. 'Cabernet franc' vines were on the
261 rootstock 3309C. This vineyard (42° 38' 35.30" N. 76° 48' 27.28" W) met the five selection
262 criteria described above. Notably, the grape mealybug was observed in the vines during spring
263 and summer of 2015. In addition, a general virus survey undertaken in 2015 revealed an overall
264 virus prevalence of 15% (29 of 192) with single infections by GLRaV-1 (13%, 25 of 192) and
265 GLRaV-3 (1%, 2 of 192), and mixed infections by GLRaV-1 and GLRaV-3 (1%, 2 of 192), as
266 shown by DAS-ELISA. A 15% disease prevalence was within the desired low to moderate range
267 of disease incidence (1-25%) previously defined for vineyards in New York (Atallah et al. 2012),
268 and California and Washington State (Ricketts et al. 2015). Furthermore, the vineyard manager
269 suspected the occurrence of virus spread in the 'Cabernet franc' vineyard, given a temporal
270 increase of diseased vines based on visual assessments of typical leafroll symptoms. Information
271 on the spatial distribution of infected vines throughout the 'Cabernet' franc' vineyard study site
272 was then used in 2016 to select an approximate 2-hectare experimental area within the 3-hectare
273 vineyard. The four treatments were randomly assigned within the experimental vineyard area.

274 **Implementation of roguing.** The 2015 leafroll virus survey indicated a prevalence of
275 GLRaV-1 and the presence of GLRaV-3 to a much lesser extent in the 'Cabernet franc' vineyard

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study site. In 2016, every vine (n=2,054) within the 2-hectare study vineyard area was tested for GLRaV-1 and GLRaV-3 by DAS-ELISA and/or RT-PCR. This allowed us to precisely map the location of infected vines since the initial 2015 virus surveys only targeted a sub-set of vines in the entire vineyard block. Based on the spatial distribution of vines that tested positive for GLRaV-1 and/or GLRaV-3 in 2016, infected vines and two within-row adjacent vines on each side were removed in May 2017 (spring in the Northern Hemisphere) and replaced with healthy vines. The same approach was implemented from 2017 to 2020. Roguing was based on laboratory-based diagnostic assays because visual inspections of infected vines was reliable for GLRaV-3 but more challenging for GLRaV-1. Indeed, in most years of the study, foliar symptoms of GLRaV-1 were extremely mild and easily confused with potassium deficiency or other biotic factors. For example, only half (51%, 63 of 124) of the vines infected with GLRaV-1 showed leafroll symptoms in 2021 and symptoms were very mild, while most (93%, 14 of the 15) of the vines infected with GLRaV-3 in the 'Cabernet franc' vineyard study site exhibited strong leafroll symptoms that year.

The cumulative number of infected vines that were removed in the two spatial roguing treatments (spatial roguing only and spatial roguing in combination with insecticides) decreased from 205 in 2017 to just five in 2020 (Table 1). This result represented 20.5% and 0.5% of the total number of vines in the spatial roguing and spatial roguing plus insecticide treatments in 2017 and 2020, respectively. More precisely, the number of infected vines eliminated from 2017 to 2020 decreased from 49 to one, respectively; and the number of neighboring vines eliminated during the same period decreased from 156 to four, respectively (Table 1). A decrease in the number of infected vines was confirmed in the two spatial roguing treatments in 2021 (Table 1).

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Efficacy of insecticides at reducing grape mealybug populations. A feature of this study from its outset was a low abundance of mealybugs found throughout the 'Cabernet franc' vineyard study site, particularly in the vines in both treatments in which insecticides were applied, as illustrated by less than 0.1 mealybug counted per minute between 2016 and 2021 (Figure 1). Low mealybug counts are consistent with previous observations in other New York vineyards (Fuchs et al. 2015, Wallingford et al. 2015). Nevertheless, the combined applications of a contact insecticide (Lorsban® Advanced, chlorpyrifos) and a systemic insecticide (Movento®, spirotetramat) from 2015 to 2020 (and only spirotetramat in 2021) was effective at maintaining low mealybug populations in the vines ($F_{1,92} = 219.8$, $P < 0.001$). In contrast, the grape mealybug population was 32- to 257-fold higher in untreated control vine panels compared with insecticide treated vine panels over the six-year period, though overall numbers tended to decline over time; hence there was a significant interaction between insecticide treatment and year ($F_{5,92} = 6.2$, $P < 0.001$) (Figure 1). Mealybug populations were generally unaffected by roguing treatment ($F_{1,92} = 2.4$, $P = 0.13$), as expected, nor was there an interaction between roguing and insecticide ($F_{1,92} = 0.9$, $P = 0.35$) (Figure 2).

Efficacy of spatial roguing at reducing virus incidence and limiting virus spread. Spatial roguing was applied from 2017 to 2020 in vine panels subjected to spatial roguing only and spatial roguing combined with insecticides. Results showed that the spatial roguing treatment (with no insecticides) significantly reduced virus incidence ($F_{1,76} = 135.1$, $P < 0.001$). The cumulative number of vines infected by GLRaV-1 and/or GLRaV-3 decreased from 4.2% in 2017 (21 of 500 vines) to no infections detected in 2020 (0%, 0 of 500 vines) and very few (0.6%, 4 of 500 vines) in 2021 (Figure 2). Achieving less than 1% virus incidence over two

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consecutive years satisfied our criterion for a successful leafroll disease management response. There was also a roguing by year interaction ($F_{4,76} = 57.9$, $P < 0.001$) indicated by an increasing difference in the proportion of infected vines between replicate plots that were rogued or not (Figure 2).

Spatial roguing in combination with insecticides also resulted in reduced virus incidence from 5.6% (26 of 500 vines) in 2017 to 0.2% (1 of 500 vines) in 2020 and 0.6% (3 of 500 vines) in 2021 (Figure 2), although insecticides did not significantly contribute to this decline in virus incidence ($F_{1,76} = 0.35$, $P = 0.5$, Figure 2). Together, both spatial roguing treatments were effective at reducing the number of infected vines to nearly zero over the course of five growing seasons, however, spatial roguing was the dominant factor in reducing virus incidence and limiting virus spread with insecticides contributing relatively little additional reduction. Similar to the spatial roguing treatment only, however, results obtained with the spatial roguing plus insecticide treatment satisfied our criterion for successful management of leafroll disease. By contrast, virus incidence in the untreated control panels increased from 5.2% (26 of 500 vines) in 2017 to 15.6% (78 of 500 vines) in 2021. Similarly, in the non-rogued vine panels treated with insecticides, virus incidence increased from 5.8% (29 of 500 vines) in 2017 to 13.4% (67 of 500 vines) in 2021 (Figure 2).

Virus status of a focal vine and its neighbor vines. The relationship between the virus status of each vine (a focal vine) and its two within-row neighbor vines was analyzed within untreated control plots and plots treated with insecticides only from 2017 to 2021. Based on Atallah et al (2015) we predicted that the probability of infection of neighbor vines, one vine and two vines away from a focal vine, increases when the focal vine is infected. A significant

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positive association was found both for the immediate adjacent vines ($\chi 2 = 40.3$, $P < 0.001$) and vines two positions away ($\chi 2 = 15.6$, $P < 0.001$). The overall odds of adjacent vines being infected if the focal vine was positive for GLRaV-1 and/or GLRaV-3 was 2.9 times greater than the odds if the focal vine was uninfected. Similarly, the odds for the vines two positions away being infected if the focal vine was infected was 1.5 times greater than the odds if the focal vine was uninfected.

There was also a significant effect of year ($\chi 2 = 26.6$, $P < 0.001$) possibly due to an annual variation in virus incidence, being especially low in 2018. In addition, a significant interaction between year and virus status of immediate adjacent vines ($\chi 2 = 20.4$, $P < 0.001$) was found due to variation in the strength of the relationship among years. Specifically, the association between the virus status of a focal vine and the immediate adjacent vines was statistically significant in three (2017, 2019 and 2020) out of the five years of the study. The interaction between year and virus status of vines two positions away from the focal vine was statistically insignificant throughout the five years of the study ($\chi 2 = 7.3$, $P = 0.12$), although some trends among years were apparent. Overall, these data supported spatial roguing and the removal of two vines adjacent to an infected vine.

Cost estimates of spatial roguing. Given the number of vines eliminated in the 'Cabernet franc' vineyard study site due to the spatial roguing only treatment in 2017-2020, a reduction in fruit yield ranging from 4-28% was estimated in 2017-2022 (Table 2). Based on a targeted production of 1.2 tons per hectare and anticipated income of $2,000 per ton (Davis et al. 2020), accrued revenue losses ranged from $2,135 per hectare in 2017 to $4,211 per hectare in 2019. The cumulative lost revenues reached $14,136 per hectare by 2023 when fruit production

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was predicted to return to normal with no cost penalty incurred (Table 2). Our estimates of the economic impact of spatial roguing were substantially lower than a scenario of no intervention for which costs were previously calculated to range from $25,000 to $41,00 per hectare over a 25-year lifespan of a 'Cabernet franc' vineyard in New York (Atallah et al. 2012).

Discussion

Leafroll disease remains a major concern to the grape and wine industries in New York and beyond. Our study confirmed roguing as a salient contributing factor to a successful leafroll disease management response, in agreement with previous studies (Pietersen et al. 2013, Bell et al. 2017, Bell et al. 2018, MacDonald et al. 2021). Roguing aims to reduce sources of virus inoculum in a diseased vineyard. Here, spatial roguing was applied by removing diseased vines and the two within-row neighboring vines on each side, regardless of their disease status. Spatial roguing and spatial roguing in combination with the application of insecticides in 2017-2020 drastically reduced the incidence of GLRaV-1 and GLRaV-3 to close to zero in 2020-2021 in a diseased vineyard with virus incidence of approximately 5% in 2016 and a low-density grape mealybug population (1-3 mealybugs counted per minute). This is the first validation ever of spatial roguing to manage leafroll disease in a vineyard. Nonspatial roguing was previously applied in vineyards of California (MacDonald et al. 2021), South Africa (Pietersen et al. 2013) and New Zealand (Bell et al. 2017, Bell et al. 2018). Interestingly, in their study on GLRaV-3, Bell et al. (2018) noted that 'Mapping virus spread annually showed within-row vines immediately either side of an infected vine (the so-called 'first' vines) were most at risk of vector mediated transmission, but a temporal decline in ('first' vine) infections was observed'. The

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385 authors concluded their study on the need to adopt a multi-tactic response targeting virus and
386 vector populations annually for a successful leafroll disease management (Bell et al. 2018).

387 The concept of spatial roguing for leafroll disease management was developed from
388 predictive modelling analyses of disease spread and bioeconomic factors (Atallah et al. 2015).
389 Predictive models suggested that spatial roguing targeting symptomatic vines and their four
390 immediate neighbor vines, two on each side, would be of statistically significant greater
391 economic value than spatial roguing targeting symptomatic vines and the two immediate
392 neighbor vines, one on each side. Simulations further predicted that a nonspatial strategy
393 targeting only diseased vines is less effective and more costly than spatial roguing, a strategy that
394 was anticipated to never reach high infection rates and never achieve high yield reductions
395 (Atallah et al. 2015). It would be interesting to validate these predictions by experimentally
396 comparing the efficacy of the two spatial roguing approaches and a nonspatial roguing approach
397 as a response to leafroll disease management. Of similar interest would be a study to test whether
398 a sequential roguing strategy based first, for instance, on the implementation of a spatial roguing
399 approach to drastically reduce sources of virus inoculum and then of a nonspatial roguing
400 approach to limit secondary spread would be of value. If carried out in different vineyards with
401 distinct disease prevalence, rate of spread, mealybug species and abundance, such research
402 would inform the best approach for leafroll disease management both from a biological and
403 economical perspective.

404 Spatial roguing significantly reduced the annual incidence of GLRaV-1 and GLRaV-3 in
405 the ‘Cabernet franc’ vineyard study site (Figure 2). A temporal decline of infected vines was
406 noticeable in vine panels subjected to the spatial roguing treatment, reducing the sources of virus

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inoculum accessible to the vectors. In contrast, newly infected vines in untreated vine panels continued to increase in number every year, thereby failing to reduce the source of virus inoculum present at the onset of this study in 2015. This outcome exacerbated vector-mediated virus transmission among vines in the untreated vine panels (Figure 2). If disease incidence is allowed to increase to the points where the virus inoculum is very high, leafroll disease becomes very difficult to manage effectively, as previously documented and discussed (Almeida et al. 2013, Blaisdell et al. 2016, Bell et al. 2018, Cooper et al. 2018). Our analysis of the relationship between the virus status of a focal vine and its first and second nearest within-row neighbors supported spatial roguing to manage leafroll disease. For spatial roguing to achieve a near zero incidence of GLRaV-1 and GLRaV-3 in the fifth and sixth year of our study, it was necessary to eliminate diseased vines yearly. In other words, continuous efforts were required every year to reduce sources of virus inoculum and limit virus spread effectively.

In our study, spatial roguing relied on the elimination of infected vines that were identified by virus diagnostic assays performed in the laboratory rather than on visual inspections of vines for disease symptoms. This approach enabled us to identify symptomatic vines and infected vines that had yet to exhibit disease symptoms. This strategy to identify infected vines may be challenging for growers to implement in the absence of a rapid, sensitive, and inexpensive tool for on-site diagnostics. Such a diagnostic assay was recently developed for grapevine red blotch virus (Romero Romero et al. 2019) and subsequently adopted by growers in Northern California (Fuchs, personal observations). It would be advantageous to devise a similar assay for GLRaV-1 and eventually for GLRaV-3. In the meantime, growers dealing with leafroll disease in vineyards of red cultivars and willing to adopt spatial roguing should identify infected

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429 vines to be eliminated by visual assessment of typical disease symptoms and confirmatory
430 diagnostic testing.

431 GLRaV-1 was the predominant leafroll virus in the 'Cabernet franc' vineyard study site
432 although GLRaV-3 was also present. Therefore, our study is the first to report on roguing with
433 GLRaV-1 as the prime target. Earlier reports on roguing exclusively focused on GLRaV-3
434 (Pietersen et al. 2013, Bell et al. 2017, Bell et al. 2018, MacDonald et al. 2021). In general,
435 symptoms attributable to GLRaV-1 are milder on red-berried wine grape cultivars than those of
436 GLRaV-3 (Naidu et al. 2015), as observed in this study, although no difference in symptom
437 severity between the two viruses was apparent in other New York vineyards (Fuchs, personal
438 observations). Here, it was challenging to exclusively rely on visual symptom assessment to
439 identify diseased vines, particularly those infected with GLRaV-1. Interestingly, visual
440 assessment of foliar symptoms in 44 vineyards of red wine grape cultivars in New Zealand and
441 South Africa was reliable to identify vines infected with GLRaV-3 for roguing. Visual
442 assessment scores were in strong agreement with DAS-ELISA (99.9%) (Bell et al. 2017). Based
443 on these findings, assuming GLRaV-3 was the primary agent of leafroll in the 'Cabernet franc'
444 vineyard study site and symptoms were more apparent, the identification of diseased vines by
445 visual monitoring of disease symptoms, instead of laboratory tests, may have been easier and
446 roguing may have achieved the same level of disease control without annual virus testing of
447 every vine, as documented in similar studies in California (MacDonald et al. 2021), South Africa
448 (Pietersen et al. 2013) and New Zealand (Bell et al. 2017, Bell et al. 2018). Nonetheless, relying
449 exclusively on visual assessments to identify vines infected with GLRaV-3 would overlook
450 infected, asymptomatic vines.

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The limited contribution of insecticides at reducing virus incidence and spread documented in our study was unexpected. It may be explained by some level of aerial movement of viruliferous mealybugs (Almeida et al. 2013, Herrbach et al. 2017) from surrounding diseased vineyard parcels into our vineyard study area, including into the insecticide-treated vine plots. These relocating insects may not have been optimally targeted by our insecticide applications, maybe due to a late season dispersal. This hypothesis seems plausible because vines that became infected in the spatial roguing treatments were predominantly located at the edge of the vineyard study area, suggesting some level of aerial movement of viruliferous mealybugs. Alternatively, the grape mealybugs in the 'Cabernet franc' vineyard study site (Figure 1), despite a low abundance, were highly effective at transmitting viruses, particularly GLRaV-1; this hypothesis however would be at odds with previous observations (Fuchs et al. 2015, Wallingford et al. 2015). Limited information is available on attributes of mealybug-mediated GLRaV-1 spread although lower transmission rates were obtained for GLRaV-1 compared with GLRaV-3 when mixed infected vines were used as donors in controlled transmission assays with the apple mealybug (Le Maguet et al. 2012). However, since the virus titer was not determined in the infected vines used as donor plants in this study, it is challenging to reasonably speculate on a root cause of the differential transmission rates with regards to specific viruses. Another more plausible explanation for the rate of virus spread observed in the 'Cabernet franc' study site despite low-density grape mealybugs could be the timing of spirotetramat applications that may have provided incomplete or delayed mortality, as described for the pink hibiscus mealybug (Ganjisaffar et al. 2019), thus enabling some viruliferous crawlers to transmit viruses before the advent of full insecticide toxicity. Alternatively, our insect vector survey method may have

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underestimated mealybug abundance. This may provide some context to the rate of secondary virus spread observed, assuming the actual population density of grape mealybugs was higher than the one estimated.

Our results obtained in a vineyard with low disease incidence and low mealybug abundance were consistent with studies in some but not all the vineyards monitored in California (MacDonald et al. 2021) and New Zealand (Bell et al. 2018) for which GLRaV-3 incidence and spread were significantly reduced by roguing symptomatic vines only. When vineyards with higher disease incidence and higher mealybug populations were studied, the combination of roguing and insecticide applications was needed for effective leafroll disease control (Pietersen et al. 2017, Bell et al. 2018, MacDonald et al. 2021). For example, a California study based on grower-driven data collected over five years on spatial and temporal trends in grape mealybug abundance and incidence of GLRaV-3 reported the effectiveness of roguing in combination with insecticides at achieving disease control when initial disease incidence was 1-20%, with the effect of roguing only or insecticide applications only at best slightly decreasing disease incidence. Roguing was critical when disease incidence was less than 1% and more than 20% (MacDonald et al. 2021). In the South African study performed in 34 commercial vineyard parcels on close to 78 hectares, roguing was practiced alongside the application of contact and systemic insecticides to limit populations of the vine mealybug, the major mealybug vector of GLRaV-3 in the country. Remarkably, the incidence of GLRaV-3 was successfully reduced from nearly 100% to 0.027% over 10 years (Pietersen et al. 2013). In New Zealand, where the citrophilus mealybug (*Pseudococcus calceolariae*) is a major mealybug vector of GLRaV-3, 12% of the vines monitored in 13 commercial vineyard blocks were rogued in 2009; by 2015,

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when the study concluded, the necessity for roguing was reduced to just 1.4% (Bell et al. 2018). From the findings of the California, South Africa, and New Zealand studies, it would seem reasonable to anticipate a reduction in virus incidence and spread by spatial roguing in combination with insecticide applications if the mealybug population and disease prevalence would have been higher in our 'Cabernet franc' vineyard study site. Together, it seems that the selection of roguing (spatial and/or nonspatial) or the combination of roguing and insecticide applications needs to be specifically tailored to vineyards based on disease prevalence, rate of spread, mealybug species and abundance, as previously reported (Bell et al. 2018, MacDonald et al. 2021).

A model of roguing was recently developed to predict the efficacy of roguing in relation to mealybug populations (Bell et al. 2021). This model suggests that the outcomes of roguing are dependent on vector density. At a low mealybug vector density (six mealybugs per 100 leaves inspected), roguing sustained a low GLRaV-3 incidence, incurred the least need for planting replacement vines, and resulted in low annual costs. At median (26 mealybugs per 100 leaves) and high (75 mealybugs per 100 leaves) mealybug vector densities, roguing was much less effective, and the costs incurred were higher at controlling GLRaV-3. These authors concluded that achieving economic sustainability relies on integrating efficient roguing with effective vector management (Bell et al. 2021). It seems that New York conditions correspond to the low mealybug density situation described by Bell et al. (2021) with even lower mealybug counts (this study, Fuchs et al. 2015, Wallingford et al. 2015) than those reported in New Zealand, thus in agreement with the simulations (Bell et al. 2021).

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Roguing adds to the overall cost of vineyard maintenance (Atallah et al. 2012, Ricketts et al. 2015, Bell et al. 2021). This disease management strategy requires eliminating diseased vines, purchasing clean replants, establishing clean replants, maintaining clean replants that require more water and fertilizers than mature vines in some viticultural regions, and dealing with a temporary loss of production due to the vine removal and the lag time for young vines to fully produce. Revenue losses directly related to spatial roguing were estimated in our study at $14,136 per hectare over six years (Table 2). These estimates agreed with earlier predictions and underscored the economic value of spatial roguing, despite added costs relative to the costs of maintaining a healthy vineyard, particularly when considering a scenario of no intervention for which $25,400-$40,000 losses per hectare were calculated over a 25-year lifespan of a 'Cabernet franc' vineyard in New York (Atallah et al. 2012). Similarly, Atallah et al. (2015) predicted that spatial roguing increases expected revenues of a vineyard with moderate disease incidence by 18% compared to a strategy of no disease control, regardless of its age, while nonspatial roguing is only of value in young vineyards with low to moderate disease incidence. The cost/benefit analysis of roguing may need to be evaluated by individual vineyard owners who are willing to adopt this leafroll disease management strategy.

Critical to the success of roguing is the health status of the replants. Replants should be sourced from nursery vine stocks (scions and rootstocks) that have been extensively tested for viruses, including leafroll viruses, and shown to be clean. To this end, it is appropriate to recognize the efforts of the USDA-APHIS-sponsored National Clean Plant Network program (Anonymous 2021) in support of the sustainable production and maintenance of clean vine

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stocks in foundation vineyards and the distribution of clean material to nurseries and growers (Fuchs et al. 2021, Gergerich et al. 2015).

Conclusions

A six-year experiment in a commercial 'Cabernet franc' vineyard with low disease prevalence and low-density grape mealybug populations in the Finger Lakes region of New York showed that spatial roguing and the combination of spatial roguing and insecticides significantly reduced the percentage of vines primarily infected with GLRaV-1 and secondarily with GLRaV-3 from 5% in 2016 to less than 1% in 2020 and 2021. By comparison, virus incidence among vines in the untreated control vine panels where roguing was not implemented and no insecticides were applied increased from 5 to 16% during the same period. Insecticides applied in 2015-2020 maintained low numbers of grape mealybug in the vines, but in and of itself, the use of insecticides did not substantially contribute to reducing the number of newly infected vines. Our study is the first to demonstrate the effectiveness of spatial roguing at reducing the incidence of leafroll disease and limiting its spread. It is also the first to implement roguing for the management of GLRaV-1. Our findings confirmed roguing as a salient contributor to an overall leafroll disease management response that should be integrated, as previously documented by studies on GLRaV-3 (Pietersen et al. 2013, Bell et al. 2018, Bell et al. 2021, MacDonald et al. 2021). It will be interesting to see whether our results on spatial roguing are reproducible in other vineyards of New York, and in vineyards of other grape-growing regions of the world, including in California, where many more mealybug species, including the vine mealybug, are of concern and reside at higher populations.

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Table 1. Number of infected and neighbor vines eliminated from 2017 to 2020 in a commercial 'Cabernet franc' vineyard study site affected by leafroll disease, and number of infected vines found in 2021.

	2017		2018		2019		2020		2021	
	Infected[1]	Neighbor[2]	Infected	Neighbor	Infected	Neighbor	Infected	Neighbor	Infected	Neighbor
Spatial roguing	21 (4.2%)[3]	72 (14.4%)	11 (2.2%)	32 (6.4%)	13 (4.6%)	38 (7.6%)	0 (0%)	0 (0%)	3 (0.6%)	na
Spatial roguing and insecticides	28 (5.6%)	84 (16.8%)	17 (3.4%)	35 (7.0%)	12 (2.4%)	34 (6.8%)	1 (0.2%)	4 (0.8%)	3 (0.6%)	na
Yearly total	49 (9.8%)	156 (31.2)	28 (5.6%)	67 (13.4%)	25 (5%)	73 (14.6%)	1 (0.2%)	4 (0.8%)	6 (0.6%)	na
Cumulative yearly total	205 (20.5%)		95 (9.5%)		98 (9.8%)		5 (0.5%)		6 (0.6%)	

[1]Infected vines were determined by DAS-ELISA and/or RT-PCR with specific reagents to grapevine leafroll-associated virus 1 and grapevine leafroll-associated virus 3 (Fuchs et al. 2019).

[2]Neighbor vines were adjacent to infected vines, two on each side within a vineyard row.

[3]Percentages were determined by dividing the number of vines eliminated by the total number of vines for a given treatment (N =500).

[4]na: not applicable. Note that the 2021 totals consider only infected vines and no neighbor vines because roguing was not implemented when the study concluded.

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Table 2. Estimates of the economic cost of spatial roguing of vines infected with grapevine leafroll-associated virus 1 and/or grapevine leafroll-associated virus 3 in a 'Cabernet franc' vineyard study site in the Finger Lakes region of New York.

		Unproductive vines			Cumulative
Year	Vines rogued (N)[a]	Yearly (%)	Cumulative (%)	Yield reduction (%)	Lost revenue ($)/ha
2017	72	0.144	0.144	14	2,135
2018	32	0.064	0.208	21	5,219
2019	38	0.076	0.284	28	9,430
2020	0	0	0.284	21	12,573
2021	na	0	0	11	14,174
2022	na	0	0	4	14,736
2023	na	0	0	0	14,736

[a]Spatial roguing was applied from 2016 to 2020. It consisted of the removal of infected vines and two neighbor vines on each side, regardless of their infectious status.
na: not applicable

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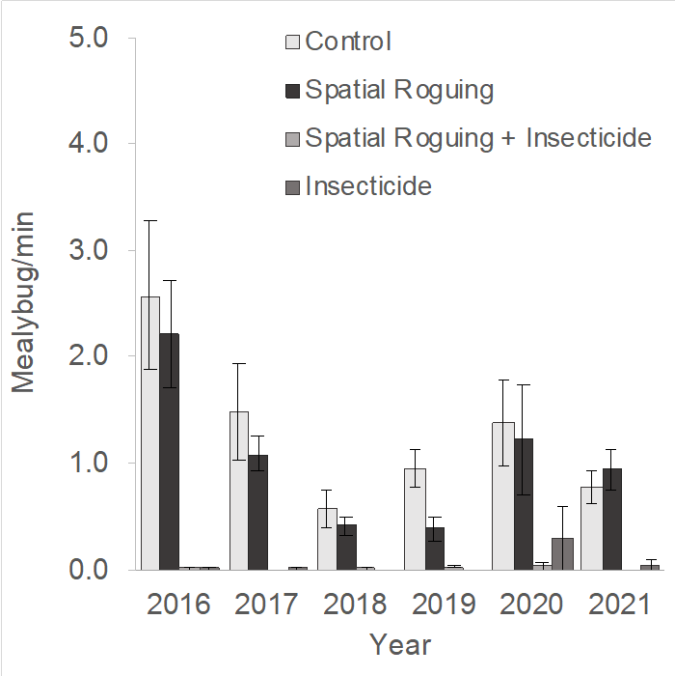

Figure 1. Yearly grape mealybug counts (± standard error) in vine panels subjected to spatial roguing, insecticide applications, a combination of spatial roguing and insecticides, and untreated controls from 2016 to 2021 in a commercial Cabernet franc vineyard study site affected by grapevine leafroll disease. Mealybugs were monitored in replicate vine panels for 15 minutes.

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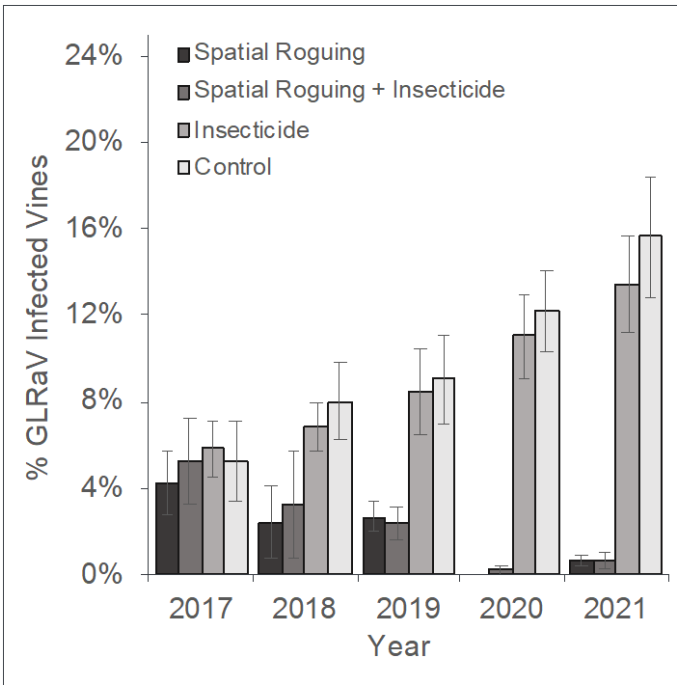

Figure 2. Cumulative mean incidence (± standard error) of grapevine leafroll-associated virus 1 and grapevine leafroll-associated virus 3 in vine panels subjected to spatial roguing, insecticide applications, a combination of spatial roguing and insecticides, and untreated controls from 2017 to 2021 in a commercial Cabernet franc vineyard study site affected by leafroll disease.