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Best Practices for Monitoring Visual Symptoms of Grapevine Red Blotch Disease in Black-Fruited Winegrape Cultivars

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Abstract

Background and goals

Emerging evidence suggests that removing infected vines is a critical strategy for decreasing vineyard inoculum and mitigating grapevine red blotch disease (GRBD). However, this approach is limited by the uncertainty of GRBD symptom recognition and the variable timing of symptom appearance. Our objective was to describe the nuances in symptom development through space and time that can limit visual symptom recognition and effective management of GRBD.

Methods and key findings

From 2021 to 2022, our team visually assessed and mapped GRBD symptoms across 12 vineyard blocks in Napa Valley, CA, where disease incidence ranged from 0.5 to 78.1%. We observed previously unreported features of GRBD expression, such as symptoms restricted to one part of the canopy, differing symptom appearance on opposite sides of the canopy, and late season onset of initial symptoms. For three sites, we quantified the within-season, spatiotemporal trends in symptom development. Two of the sites had significant increases in GRBD symptom incidence every two weeks from 25 Aug to 13 Oct 2022.

Conclusions and significance

This is the first study to quantify the progressive, within-season increase in GRBD incidence from veraison (modified Eichhorn-Lorenz [E-L] stage 35) through the beginning of leaf fall (modified E-L 43). We emphasize the importance of correct timing for GRBD symptom evaluation. If visual symptoms are quantified and mapped prior to peak expression, then diseased vines may be overlooked and disease incidence underestimated. Observation of variation in symptom expression can increase practitioners' confidence in discerning virus symptoms, and diagnostic tools can reinforce visual assessments. Selecting an optimal symptom mapping date and marking the correct vines for removal are critical for effective GRBD management.

Key words: grapevine red blotch disease (GRBD), grapevine red blotch virus (GRBV), loop-mediated isothermal amplification, red blotch disease visual symptoms diagnostics

Introduction

Grapevine red blotch disease (GRBD) has emerged in the last decade as an important viral disease of grapevine in North America (Cieniewicz et al. 2020). Grapevine red blotch virus (GRBV) is the causal agent of GRBD (Yepes et al. 2018) and is the representative member of genus *Grablovirus* in the family *Geminiviridae* (Varsani et al. 2017). GRBV is widely distributed across vineyards in North America from the dissemination of infected plant material (Krenz et al. 2014), with secondary spread documented in northern California and southern Oregon vineyards (Cieniewicz et al. 2017, KC et al. 2022). The virus can be transmitted in the vineyard by the three-cornered alfalfa hopper, *Spissitilus festinus* (Hemiptera: Membracidae) (Flasco et al. 2023). Economic effects of GRBV include reduced yields (Bowen et al. 2020) and diminished fruit and wine quality. GRBD alters sugar, acid, and phenolic accumulation in grape berries (Blanco-Ulate et al. 2017, Girardello et al. 2019, Pereira et al. 2021), leading to lower ethanol concentration, higher acidity, and less color in resultant wines (Girardello et al. 2019). Physiological effects such as lowered photosynthesis and carbon assimilation, and decreased winter hardiness, can have long-term effects on grapevine health (Martínez-Lüscher et al. 2019, Bowen et al. 2020). There is no cure or known cultural practice that can alleviate the disease, and economic losses can reach up to $68,548 USD/ha over the lifespan of the vineyard (Ricketts et al. 2017).

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Despite the detrimental effects of GRBD, initial identification of the causal agent in 2011 (Krenz et al. 2012, Al Rwahnih et al. 2013) was hampered by confusion with disease symptoms caused by grapevine leafroll-associated viruses (GLRaV), which have historically caused the most significant economic losses in grapevines worldwide (Maree et al. 2013). Grapevine leafroll disease (GLD) results in a wide range of deleterious effects on fruit and wine quality (Guidoni et al. 2000, Lee et al. 2009, Blaisdell et al. 2016), with symptoms similar in appearance to GRBD, including reddening of the leaf blade in black-fruited cultivars. Although GRBV has been present in plant material for decades (Al Rwahnih et al. 2015), the overlapping symptomology presented difficulties for detecting and visually separating disease symptoms from GLD (Calvi 2011, Krenz et al. 2014). Other common causes of red leaf symptoms in the vineyard, such as spider mite feeding damage and nutritional disorders, can also confound accurate GRBD symptom identification (Sudarshana et al. 2015). Furthermore, the severity and onset of GRBD symptoms varies among grapevine cultivars and growing seasons (Sudarshana et al. 2015), adding to the challenge of identifying symptomatic vines.

GRBD is a chronic infection, and current disease management practices follow those established for *GLRaV*-3, including mapping the location of diseased vines and removing them to reduce vineyard inoculum (Bell et al. 2017, KC et al. 2022). Given the uncertainty of visual symptom identification, disease management requires an investment to train vineyard personnel on symptom recognition, and a heavy reliance on diagnostic assays (Hobbs et al. 2022). However, the costs and labor associated with commercial diagnostic assays make it economically prohibitive to test all symptomatic vines in a block, and symptom-based assessment remains a reliable and practical strategy for trained personnel to monitor GRBD incidence and rates of spread (KC et al. 2022). In many cases, the winegrape industry makes costly virus management decisions based principally on visual symptom diagnosis, because once identified, diseased vines must be removed, replanted, and reestablished, incurring a further investment of skill and resources (Hobbs et al. 2023). Therefore, ensuring proper identification is key for effective GRBD management.

This report is part of a larger, ongoing study of GRBV detection and disease ecology to improve management efforts by the winegrape industry. We visually assessed and mapped GRBD symptoms at 12 vineyards across five American Viticultural Areas (AVA) in Napa Valley, California, from 2021 to 2022. Here, our objective is to describe spatiotemporal nuances in symptom development that can limit the visual recognition and effective management of GRBV. First, we characterize GRBD symptom progression within the canopy and describe symptom restriction to one or more parts of the canopy, symptom appearance on opposite sides of the canopy, and late season symptom onset. None of these symptom variations have been previously described, although there are general depictions of GRBD symptoms in the literature (Al Rwahnih et al. 2013, Sudarshana et al. 2015). Second, we quantify the spatiotemporal trends in GRBD symptom expression. Whereas previous studies have quantified seasonal variation in GRBV detection and diagnosis (Setiono et al. 2018, DeShields and KC 2023), there has been no similarly detailed report of within-season variability in symptom expression. By increasing the availability of technical information and directing attention to these trends in symptom expression, we aim to improve practitioner ability to identify and remove infected vines as part of a GRBD management strategy.

Materials and Methods

Study sites

A total of 12 vineyard blocks were selected from the Oak Knoll AVA to the St. Helena AVA in Napa Valley, CA. Study blocks ranged in age, size, cultivar, and rootstock (Table 1), and locations were anonymized by AVA. Prior to the initiation of the study, GRBV was confirmed as the predominant

Table 1 Descriptions of study blocks mapped for grapevine red blotch disease incidence in 2021 and 2022. AVA, American Viticultural Area.

Site name	AVA	Year planted	Scion	Rootstock	Hectares mapped
Oakville River East	Oakville	2012	Cabernet Sauvignon clone 685	039-16	1.3
Oakville River West	Oakville	2007	Cabernet Sauvignon clone 169	101-14	1.6
Oakville West	Oakville	2015	Cabernet Sauvignon clone 169	110R/St. George	1.9
Oakville West 2	Oakville	1974	Cabernet Sauvignon masal selection	St. George	2.9
Rutherford Central 2	Rutherford	2013	Cabernet Sauvignon clone 15	101-14	1.4
Rutherford West	Rutherford	1994	Cabernet Sauvignon clone 29	1103P	0.7
Rutherford West 2	Rutherford	2012	Cabernet Sauvignon clone 7	3309	1.9
St. Helena	St. Helena	2014/2016[a]	Malbec clone 596	St. George	1.2
St. Helena 2	St. Helena	2000	Cabernet Sauvignon clone 4	101-14	0.5
Wooden Valley	Wild Horse Valley	2015	Cabernet Sauvignon clone 7	St. George	1.6
Yountville	Yountville	2010	Cabernet Sauvignon clone 337	101-14	1.0[b]
Yountville West	Yountville	1999	Cabernet Sauvignon masal selection	3309C	2.8

[a]Planted in 2014 and grafted in 2016.
[b]Total block size is 4.2 ha, but a smaller portion was mapped.

virus in each of the blocks as a prerequisite for inclusion in the study. Annual removal of symptomatic vines was the primary method of GRBD mitigation, and this was practiced at all study sites except St. Helena, St. Helena 2, Wooden Valley, and Rutherford West. Vineyards used both conventional and organic management practices and there were no insecticides applied that specifically targeted the vector during the study period.

Mapping symptomatic vines for GRBD

We visually assessed and mapped GRBD symptoms in seven and 12 blocks in 2021 and 2022, respectively (including the original seven blocks from 2021 with an additional five blocks in 2022). The mapping personnel had 10 years or more of experience with the visual diagnosis of GRBD. We created a georeferenced grid of vines for each study site (ArcGIS Pro ver. 3.03, ESRI, Inc.), and when printed, these maps were used to mark the location of symptomatic vines by walking every row and recording the presence or absence of GRBD symptoms for each vine. Additionally, we recorded observations of symptom expression within the canopy or on opposite sides of the canopy. Data were manually transferred from scanned hardcopy maps to the vine database, and ArcGIS was used to build digital maps displaying symptomatic vine data. From 2 Sept to 25 Oct in both 2021 and 2022, GRBD symptoms were mapped or surveyed (Table 2). Disease incidence at each site was calculated as the percentage of symptomatic vines per total number of vines surveyed.

Spatial and temporal development of symptoms

We quantified the spatiotemporal development of disease symptoms in 2021 and the temporal development of disease symptoms in 2022 for a subset of three blocks: Oakville West (OW), Oakville River East (ORE), and Wooden Valley (WV). In 2021, GRBD symptoms were mapped on two consecutive dates from mid-September to the end of October. Frequent site visits to monitor symptom progression were used to select the mapping date at each site. At OW and ORE, additional symptoms were recorded during site visits on 10 and 15 Oct, respectively, but blocks were not fully mapped. In 2022, the same blocks (OW, WV, and ORE) were used to quantify temporal changes in GRBD incidence. GRBD symptoms were surveyed in a 20-row subsection of each block that included the area of highest symptom aggregation. Blocks were surveyed every two weeks from 25 Aug to 26 Oct; a total of five survey points.

Diagnostic assays

To confirm our visual assessment of GRBD symptoms, we tested a subset of surveyed vines from all sites using loop-mediated isothermal amplification (LAMP) for GRBV—a point of use, colorimetric, DNA-based assay—following Romero Romero et al. (2019). Primers were custom synthesized by IDT DNA Technologies, and the Warm Start Colorimetric LAMP 2X was sourced from New England BioLabs. Positive controls consisted of diluted GRBV DNA obtained from AL&L Crop Solutions. Negative controls consisted of leaf petiole material that had previously tested negative for GRBV.

Sampling design

Petiole tissue was predominantly analyzed from mid-September to late October. Six petiole samples from leaves were collected from basal sections of shoots (Setiono et al. 2018, DeShields and KC 2023). For a set of 56 vines with senescing canopies, we collected 15-cm sections from the basal portions of canes. Three petioles or three canes were processed and tested with the LAMP assay and the remaining three kept, if needed, for retesting. Sampled vines were selected from the following categories: 1) visually negative for GRBD symptoms,

Table 2 For each study site, the number of vines visually assessed for grapevine red blotch disease (GRBD) is listed, followed by the disease incidence in parentheses.

Site name	Vines evaluated for GRBD (% incidence of symptomatic vines)		Dates of symptom evaluation (vine-by-vine mapping)	
	2021	2022[a]	2021	2022
Oakville River East	3614 (2.7)	3611 (8.64)	30 Sept, 22 Oct	19 Oct, 24 Oct
Oakville River West	5294 (18.1)	4330 (25.8)	30 Sept	4 Oct
Oakville West	2148 (7.7)	2281 (9.5)	24 Sept, 15 Oct	6 Oct
Rutherford West 2	7944 (4.7)	7546 (8.1)	22 Oct	7 Oct
St. Helena	5192 (61.4)	5197 (78.1)	2 Sept, 27 Sept	22 Sept
Yountville	3522 (3.0)	3522 (12.1)	15 Oct	4 Oct
Wooden Valley	4180 (39.8)	4177 (51.1)	20 Sept, 13 Oct	21 Sept, 12 Oct
Oakville West 2	---	3889 (0.51)	---	11 Oct
St. Helena 2	---	6113 (18.4)	---	18 Oct
Rutherford Central 2	---	3820 (5.8)	---	10 Oct
Rutherford West	---	1432 (0.8)	---	25 Oct
Yountville West	---	4550 (1.1)	---	14 Oct
Total vines evaluated	31,894	50,468		

[a]The 2022 disease incidence listed in parentheses includes the incidence from 2021.

2) visually positive for GRBD symptoms, and 3) questionable symptoms ("Q vines"). Following the symptom assessments in 2021, we digitized all observations to create a georeferenced map of each study site (ArcGIS Pro ver. 3.03). We used these maps to select vines to be sampled using the LAMP assay. We randomly selected 10 vines from the visually positive category. In the visually negative category, we randomly selected asymptomatic vines located within one to four vines of selected symptomatic vines (up to 48 samples per vineyard site). Finally, we randomly selected asymptomatic vines with no symptomatic neighboring vines (n = 8) and sampled six to eight vine locations where yellow panel traps were deployed for insect monitoring. The total samples per vineyard in 2021 ranged from 51 to 72. In 2022, we sampled fewer vines overall and randomly selected between 16 and 38 vines per block in the visually negative and visually positive categories (Supplemental Table 1). The Q vine category included vines with symptoms that confounded GRBD diagnosis such as nutrient deficiency, leafhopper or spider mite feeding damage, physical damage from girdling, or other red leaf virus symptoms (e.g., GLD). Given the ease and point of use nature of the LAMP assay, we were able to test all vines with uncertain symptoms and confidently assign vines as visually positive or negative for GRBD.

Statistical analysis

To compare temporal changes in the number of symptomatic vines across the five survey periods in 2022, we analyzed each site independently (SPSS ver. 28, SPSS, Inc.). Raw data were analyzed and tested for normality using the Shapiro-Wilk test. Data for most weeks at each site were not normally distributed. Square root transformation did not satisfy normality assumptions for repeated measures of analysis of variance (ANOVA); therefore, Friedmans' test for nonparametric repeated measures was used to test overall effects of sampling date for each site. The Wilcoxon signed-rank test was used for pairwise comparisons between consecutive dates, with additional pairwise comparisons between nonconsecutive dates at the OW site. The Holm's sequential Bonferroni adjustment was applied to all post-hoc tests.

Results

Disease incidence

In 2022, GRBD visual incidence across all blocks ranged from 0.5 to 78.1% (Table 2). The lowest GRBD incidence (0.5%) was at Oakville West 2, where symptomatic vines have historically been removed on an annual basis. The St. Helena and Wooden Valley sites, where the disease has not been actively managed, had the highest GRBD incidence, 78 and 51%, respectively.

Verification of GRBD visual assessment with diagnostic assays

We tested a total of 746 vines for GRBV across the 12 sites over the two study years, using the LAMP assay. For vines in the visually negative category, all asymptomatic petiole tissue tested negative for GRBV. For vines in the visually positive category, the LAMP assay confirmed 99% of our visual ratings (Table 3), which reinforced our confidence in our visual symptom assessments.

The LAMP assay was an important diagnostic tool, particularly during the first year of the study because we had no prior experience with symptomology at these sites. As such, in 2021, we tested 620 of the visually rated vines (1.9%). By the second year, we were more familiar with symptomology at the sites; we rated 18,574 more vines (Table 2) but tested fewer (0.2%) (Supplemental Table 1). Across both years, confounding red leaf symptoms made it difficult to confidently assess visual symptoms in 80 vines. Of these, 29 vines tested positive for GRBV.

Characterization of GRBD symptoms

GRBD symptoms have been characterized as diffuse red blotches on the leaf blade upon symptom onset. When symptoms advance, blotches coalesce and basal leaves may turn entirely red, including the primary veins (Al Rwahnih et al. 2013, Sudarshana et al. 2015). Concurrently, leaf blades in the upper canopy develop the diffuse red blotches characteristic of initial disease onset (Figure 1).

Restricted symptoms

It is common to find GRBD symptoms throughout a vine, however, we also observed that symptoms can be limited in distribution to one or several spurs, shoots, or leaves while the rest of the canopy remains asymptomatic (Figure 2). In 2021, we tested symptomatic tissue from 29 vines with restricted symptoms, and in 28 of the 29 vines, symptomatic petiole tissue tested positive for GRBV using the LAMP assay.

Canopy orientation

Our visual assessment strategy considered how row orientation may affect the appearance of GRBD symptoms. From ripening to the beginning of leaf fall (modified Eichhorn-Lorenz [E-L] stage 35 to 43) (Coombe 1995), we observed that symptoms had a different appearance on opposite sides of the canopy for a block with east-west row orientation. On the south-facing side of the canopy, higher exposure to solar radiation resulted in leaf chlorosis and GRBD leaf symptoms that were less distinct and harder to visually distinguish. Conversely, on the less-exposed, north-facing side of the canopy, leaves retained their green hue and GRBD symptoms appeared darker, more defined, and more visible (Figure 3).

Table 3 Comparison of the visual ratings for grapevine red blotch disease and loop-mediated isothermal amplification (LAMP) assay results for grapevine red blotch virus (GRBV) detection. Petiole samples were taken from mid-September to late October in 2021 and 2022.

	Visually positive	Visually negative
LAMP GRBV-positive	240	0
LAMP GRBV-negative	3	423

Spatiotemporal trends in symptom expression

We observed that GRBD symptoms develop inconsistently, both within and between blocks. The appearance of initial symptoms varied temporally by site, and we selected a mapping date for each site to optimize visual symptom assessments (Table 2). Despite this, we still observed vines with very faint symptoms in the same block and on the same date as vines with more advanced symptoms (Figure 4). The onset of symptoms—faint blotches with limited distribution (Figure 5)—continued postharvest until just prior to leaf senescence.

Temporal trends in three blocks (ORE, OW, and WV) were tracked more closely, showing an increase in symptom development from mid-September (E-L 37) to mid-October 2021 (E-L 41) (Figure 6), regardless of overall disease incidence (Table 2). In 2022, Friedman's test confirmed overall significant effects of sampling date at all sites ($p < 0.001$; Supplemental Table 2) as GRBD symptoms continued to develop from late August through October.

Symptoms at OW developed sooner than at ORE and WV. In 2021, symptoms were initially mapped on 14 Sept, and by the following mapping date (nine days postharvest; 24 Sept 2021), symptom incidence had increased by more than 2.5-fold. Late symptom onset was minimal at this site, with only four newly symptomatic vines identified on 15 Oct 2021 (Figure 6), and no newly symptomatic vines identified after 13 Oct 2022 (four weeks postharvest) (Figure 7). The trend toward earlier symptom development was also seen in 2022, when 73% of the total symptomatic vines displayed GRBD symptoms by the week of 25 Aug. We did not observe significant increases in symptomatic vines over each consecutive survey period (Figure 7). Rather, symptoms increased gradually with significant differences in symptom incidence between the first week and later weeks of the survey (Supplemental Table 3).

The WV site had the greatest number of symptomatic vines (1419), although symptoms developed later than OW. The first mapping date was 20 Sept 2021 (10 days postharvest), and an additional 244 (15% of total) symptomatic vines were recorded on 13 Oct 2021. Early senescence in 2021 prevented us from assessing symptoms again that season. In 2022, pairwise comparisons showed significant increases in the number of symptomatic vines between four consecutive survey periods (Supplemental Table 2). The block was harvested on 31 Aug, and the peak period of symptom development was between 29 Sept and 13 Oct (Figure 7). Again, early senescence in 2022 limited the effectiveness of symptom tracking after 13 Oct, with fewer symptomatic vines captured on 26 Oct than on the previous sampling date.

ORE was the last of these sites to develop symptoms in both years. In 2021, 77% of the total symptomatic vines were recorded on the first mapping date (30 Sept), with an additional 23% mapped on 22 Oct (Figure 6), only three days after harvest. In 2022, pairwise comparisons showed significant increases in the number of symptomatic vines between each survey point (Supplemental Table 2). The period of peak symptom development was around 13 Oct (Figure 7), just before harvest on 15 Oct. Late onset of symptoms was common at this site, with an additional 17 vines (14% of total symptomatic) marked on 26 Oct 2022. This exemplifies the number of symptomatic vines that would have been underestimated if symptoms were mapped at an earlier date (around harvest).

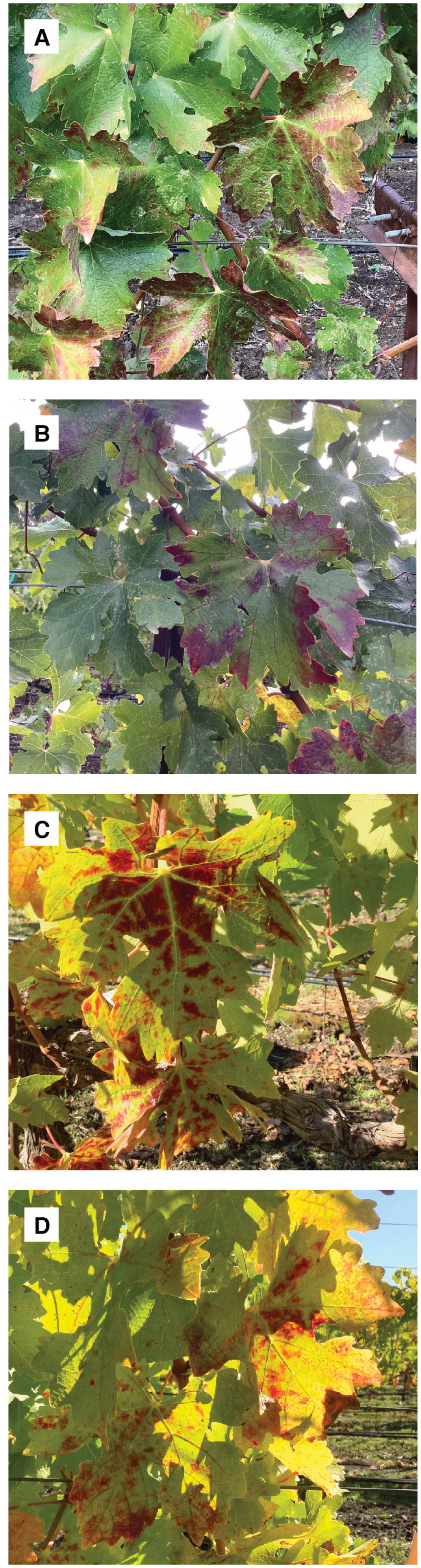

Figure 1 Grapevine red blotch disease symptom variability captured within the grapevine canopy at Oakville River East on 22 Oct 2021 (**A**, **B**) and Yountvillle on 29 Oct 2021 (**C**, **D**). **A**, **C**: Advanced symptom expression results when diffuse red blotches coalesce on basal leaf blades. **B**, **D**: Concurrent with advanced basal leaf symptoms, leaves in the upper canopy may have red, diffuse blotches, characteristic of the initial onset of virus symptoms.

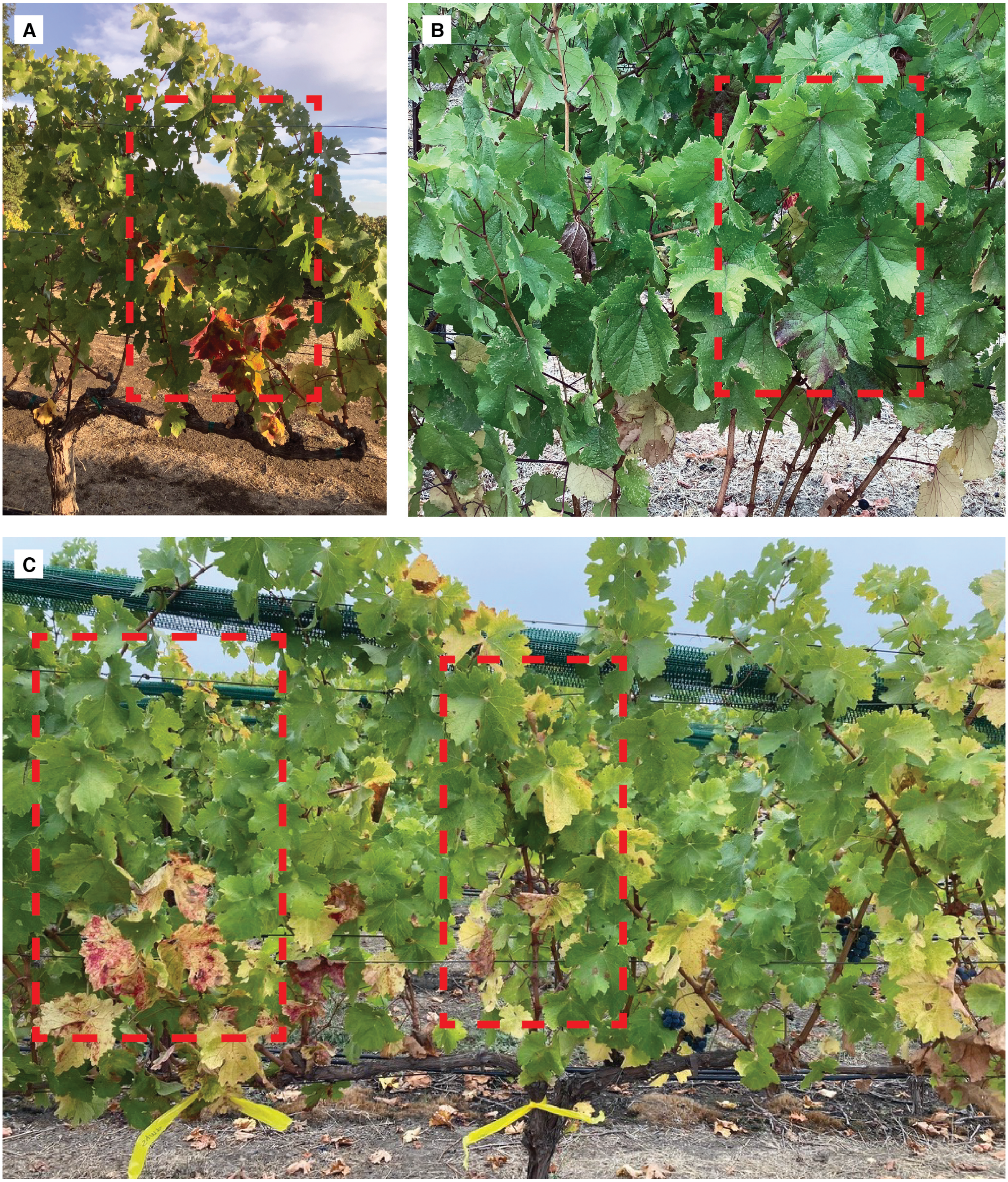

Figure 2 Grapevines with red blotch disease symptoms restricted to one or several canes, while the majority of the canopy is asymptomatic. (**A**) Oakville River East on 22 Oct 2021, (**B**) St. Helena on 17 Sept 2021, and (**C**) Yountville on 10 Nov 2022. Symptoms are highlighted with dashed red outline. Petiole samples from symptomatic canes tested positive for grapevine red blotch virus using the loop-mediated isothermal amplification (LAMP) assay.

Discussion

This report describes spatiotemporal nuances in symptom development that can limit the visual recognition and effective management of GRBV. We evaluated symptoms across 12 sites with a wide variation in disease incidence, which was mainly driven by differences in the annual removal of symptomatic vines. We observed extensive variation in GRBD symptom expression, which may be explained by rootstock, cultivar, site, and season (Girardello et al. 2019, Vondras et al. 2021). This study focuses on the within-season variation of symptom expression. Practitioners who rely on visual symptom mapping to assess disease incidence should consider the following factors when managing GRBD. First, symptoms can advance over the period from veraison to senescence, with more severe symptoms in the lower part of the canopy and milder symptoms in the upper canopy. Second, initial symptom onset will persist from veraison to leaf fall. In our study blocks, we observed a peak period of symptom development followed by the continual onset of initial symptoms until just prior to leaf fall. For the three sites (OW, WV, and ORE) where we quantified symptom incidence over time, we demonstrate that disease incidence will be underestimated if visual symptoms are mapped before the peak period of expression. Third, repeated symptom observations at initial symptom onset, during the peak period of expression, and during the late onset of symptoms will not only help practitioners accurately quantify disease incidence, but will also aid in selecting an optimal mapping date. Repeated symptom observations require a time investment, but visits can be optimized by surveying vines in a subsection of the vineyard with the highest area of symptom aggregation ("hotspot").

Practitioners are also encouraged to develop strategies and protocols for visual assessment because GRBD symptoms may not always be characteristic or consistent. For instance, row orientation can affect GRBD symptom appearance because canopy exposure to sunlight influences the definition and hue of leaf symptoms, especially for east-west oriented rows. Additionally, symptoms may have restricted distribution within the vine, with apparent symptoms on only one or a few shoots or leaves. One potential explanation for symptom restriction is functional sectorality: grapevine trunk

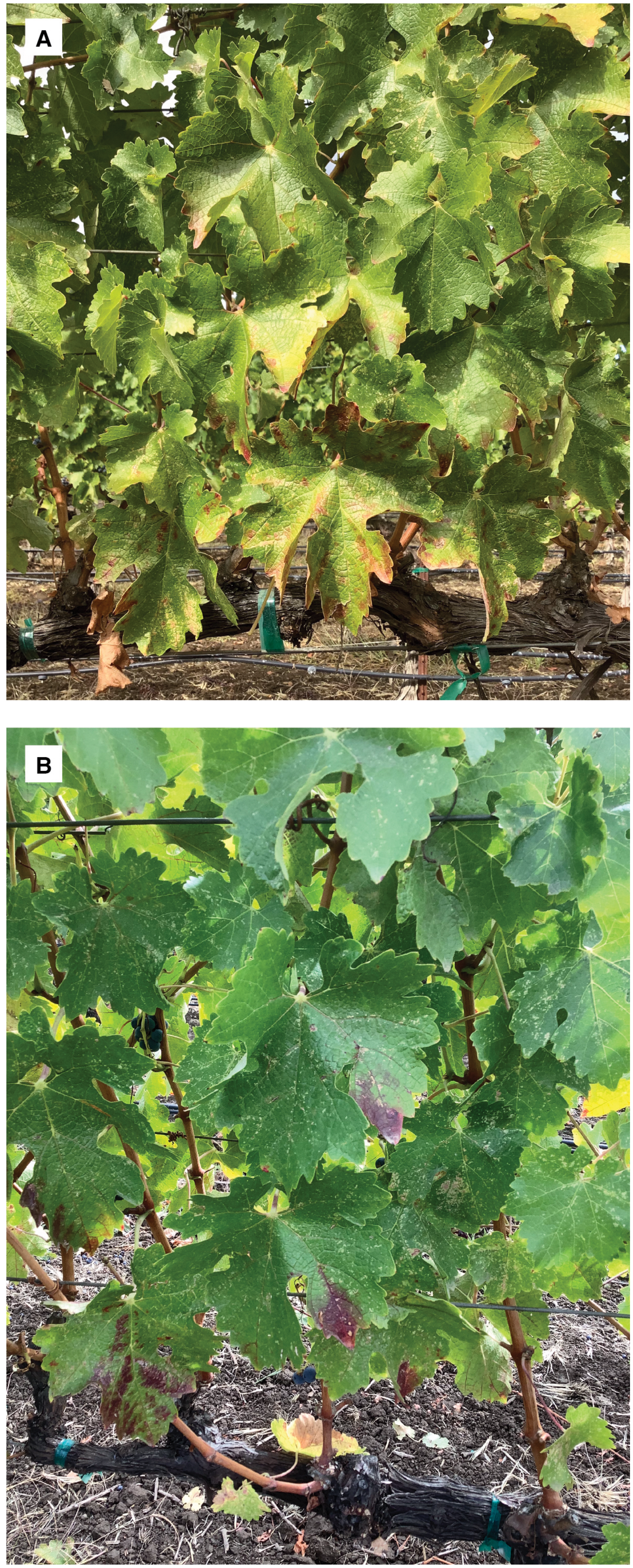

Figure 3 Exposure to solar radiation affected grapevine red blotch disease (GRBD) symptoms at Oakville River East on 22 Oct 2021. Leaves on south facing side of the canopy are chlorotic, making GRBD symptoms more difficult to distinguish (**A**), whereas leaves on the north-facing side of the canopy retain a darker green color and symptoms are more distinct (**B**).

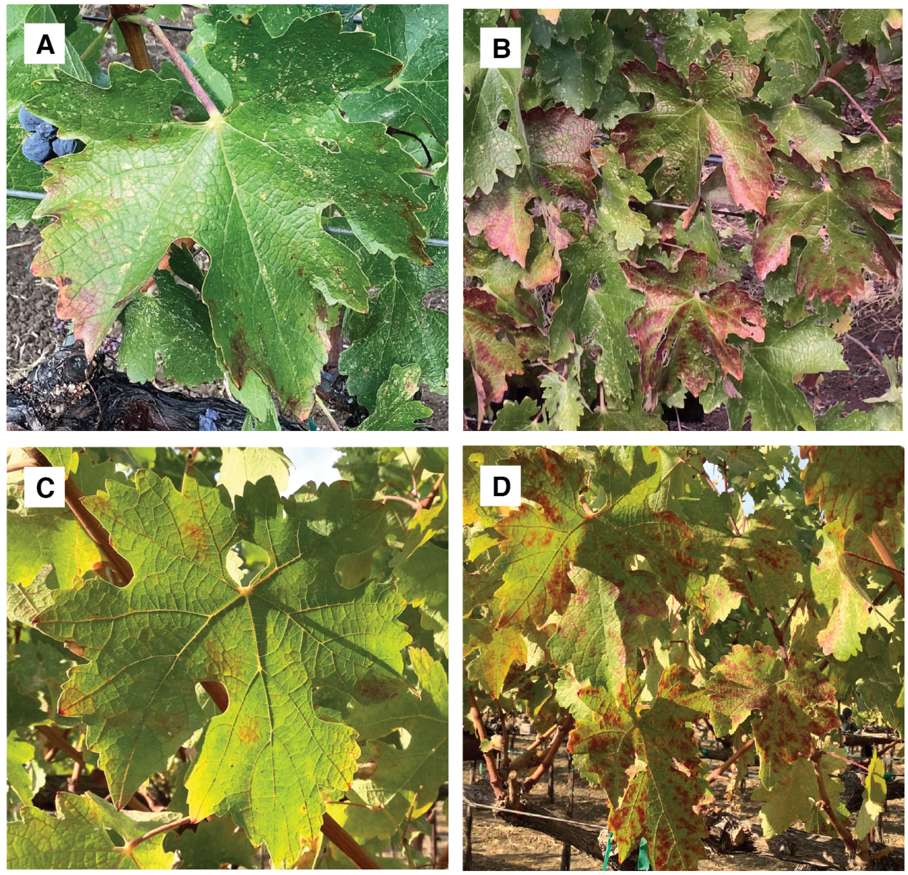

Figure 4 Temporal differences in the development of grapevine red blotch disease (GRBD) symptoms on the lower half of the canopy were observed at the Oakville River East site on 22 Oct 2021 (**A**, **B**) and at the Wooden Valley site on 5 Oct 2021 (**C**, **D**). Subtle and diffuse symptoms on vines that were newly symptomatic (**A**, **B**) were found on the same date that other vines had more distinctive and severe GRBD symptoms (**C**, **D**).

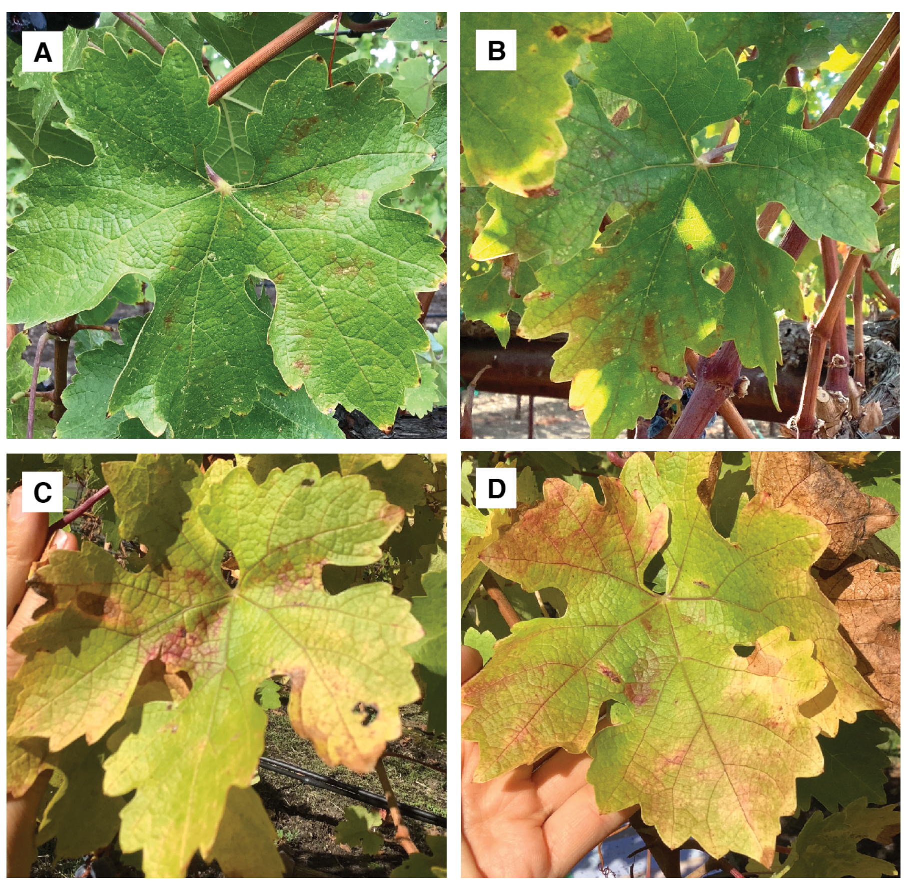

Figure 5 Faint grapevine red blotch disease symptoms may be overlooked and difficult to visually diagnose, such as symptoms at Oakville River East on 22 Oct 2021 (**A**), Wooden Valley on 8 Nov 2021 (**B**), and the Yountville site on 29 Oct 2021 (**C**, **D**).

xylem, and likely phloem by association, functions in discrete sections that supply to specific and limited portions of the grapevine canopy (McElrone et al. 2021). Sectored vascular function has been observed for *Xylella fastidiosa*, which is the causal agent of Pierce's disease (Stevenson et al. 2004), and could be explored for GRBV (a phloem-limited virus). Careful attention to detail—reinforced with diagnostic assays—is necessary to avoid disregarding or overlooking vines with symptoms that are restricted or develop late in the season.

Symptoms of GRBD can be difficult to distinguish from GLD, nutritional abnormalities, and insect feeding damage. However, we developed expertise from the repeated practice of visually assessing vines, reinforced by the LAMP assay, an in-house diagnostic tool. The high level of accuracy (99%) between our visual diagnosis and LAMP results in this report should be taken within the context that the study blocks were specifically selected for the predominance of GRBD and the low incidence of other viruses, such as leafroll and corky bark, which may have overlapping symptomology. High accuracy levels (92 to 100%) between visual diagnosis and GRBV PCR results have also been reported for blocks where GRBV is the primary viral infection (Cieniewicz et al. 2017), while lower accuracy levels are reported in vineyards with co-infections, confounding symptoms (Adiputra et al. 2018), and young vineyards with limited symptom expression (KC et al. 2022).

Further study

Recent studies have documented the effectiveness of visual symptom diagnosis for guiding vine removal strategies and controlling GRBD spread (KC et al. 2022). However, the contribution of asymptomatic infections to the spread of GRBV is currently unknown. In this study, the LAMP assay

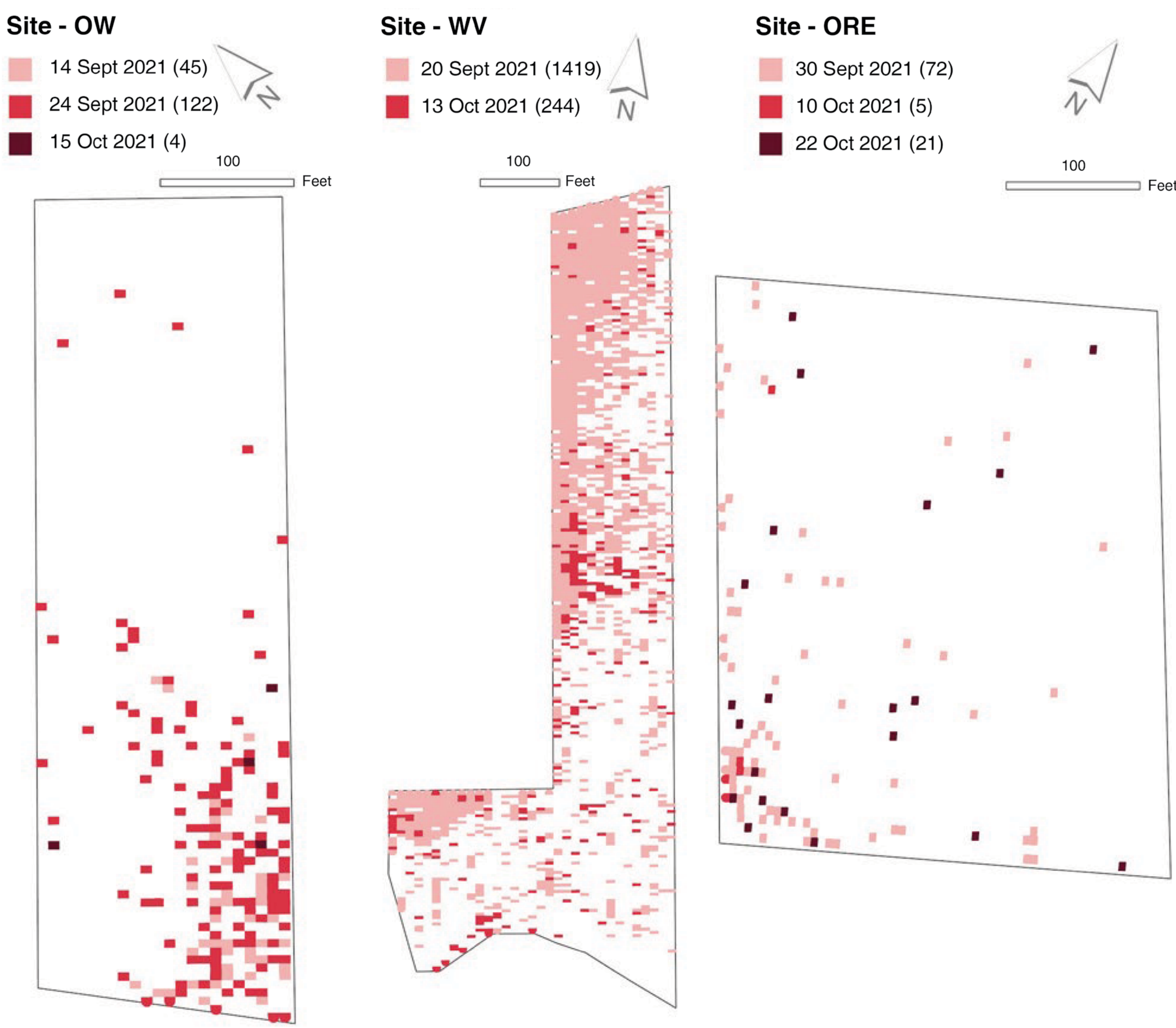

Figure 6 Spatiotemporal development of grapevine red blotch disease symptoms at Oakville West (OW), Wooden Valley (WV), and Oakville River East (ORE). In the legend, the number of symptomatic vines is listed next to the date in parentheses. For the ORE and OW blocks, symptoms included on 10 Oct and 14 Sept 2021, respectively, are observations from periodic symptom monitoring, but sites were not fully mapped on those dates.

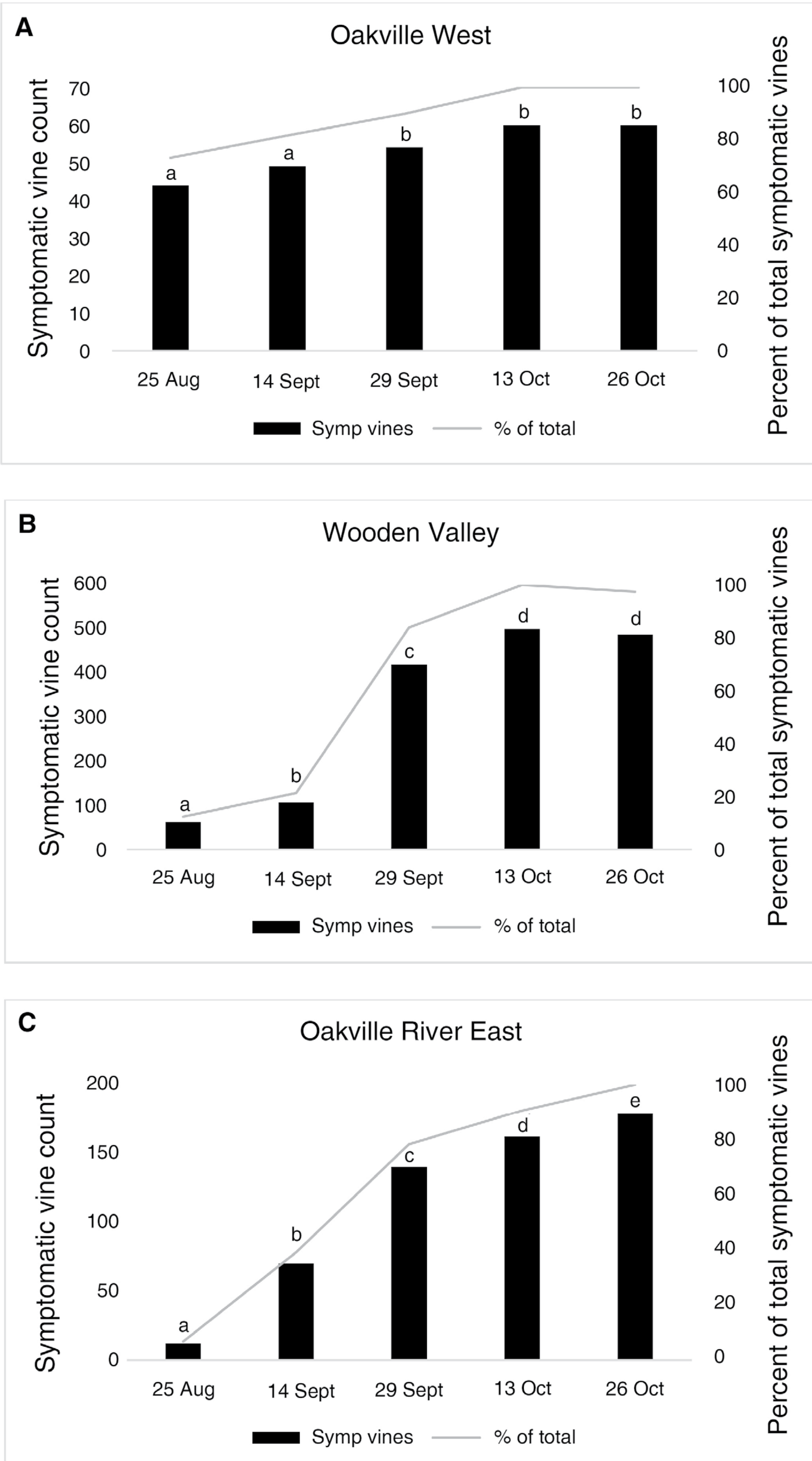

Figure 7 Temporal grapevine red blotch disease symptom development at three sites in 2022. The number of symptomatic vines (y-axis) and the percentage of the total number of symptomatic vines (secondary y-axis) is depicted across five consecutive time periods. The percentage of the symptomatic vines is calculated as (number of symptomatic vines at given time period/total number of symptomatic vines counted at the site) × 100. Significant differences between time periods are denoted by lowercase letters.

detected only three out of 746 (or 0.4%) GRBV infections in petiole tissue from asymptomatic vines, a lower percentage than previously reported for PCR or qPCR (Cieniewicz et al. 2017, KC et al. 2022). LAMP is a highly sensitive assay that can outperform PCR and qPCR in terms of virus detection (Romero Romero et al. 2019). However, variable GRBV distribution within the grapevine prior to veraison, in combination with the lower tissue weight requirement for the LAMP assay, can result in decreased sensitivity of the assay (DeShields and KC 2023). Future studies are needed to focus on applications of the LAMP assay for detection of asymptomatic GRBV infections in permanent woody tissues (i.e., cordon, trunk), which are likely to have more consistent virus distribution than unretained, seasonal tissues.

Conclusion

Emerging evidence indicates that removing infected vines is a critical approach to GRBD management (Cieniewicz et al. 2020, KC et al. 2022). Practitioners can improve the effectiveness of this strategy by tracking symptoms over time and selecting an optimal date to map symptoms and mark vines for removal. Following the initial appearance of visual symptoms, practitioners should regularly visit blocks to capture the peak period of symptom expression and search for the late onset of symptoms. Once symptom development has been characterized as early, mid, or late for a particular site, historical records and periodic visits can be used to select an optimal mapping date. Visual assessment protocols should include provisions for GRBD symptoms that are light in intensity, limited in distribution, affected by canopy exposure, or restricted to specific parts of the canopy. The use of diagnostic assays is essential for confirming visual disease diagnosis and increasing the reliability of symptom-based mapping.

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Supplemental Data

The following supplemental materials are available for this article at [ajevonline.org:](ajevonline.org)

Supplemental Table 1 Loop-mediated isothermal amplification (LAMP) assay sample numbers, assay results (POS, NEG), and visual symptom ratings (positive, negative, questionable) for each site and year of the study.

Supplemental Table 2 Results for the overall effect of the survey period on the number of symptomatic vines in 2022, including pairwise comparisons across survey dates (week 1 = 25 Aug, week 2 = 14 Sept, week 3 = 29 Sept, week 4 = 13 Oct, week 5 = 26 Oct). Friedman's test confirmed overall significant effects of sampling date at all sites, because grapevine red blotch disease symptoms continued to develop from late August through October. For Oakville River East (ORE) and Wooden Valley (WV), pairwise comparisons showed significant increases in the number of symptomatic vines between four consecutive survey periods. OW, Oakville West.

Supplemental Table 3 The Oakville West (OW) site had significant differences in symptom incidence between the first week and later weeks (4, 5) of the symptom survey in 2022. Test statistics for the pairwise comparisons compare the number of symptomatic vines across the specific survey periods (week 1 = 25 Aug, week 2 = 14 Sept, week 3 = 29 Sept, week 4 = 13 Oct, week 5 = 26 Oct).

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