

Epidemiology of Grapevine Red Blotch Disease Progression in Southern Oregon Vineyards

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Abstract: Grapevine red blotch disease (GRBD) is an emerging disease caused by grapevine red blotch virus (GRBV). It is widespread in most United States winegrape production regions and differential spread dynamics have been reported between the regions. This study surveyed eight vineyard sites in southern Oregon over four years for the progression of GRBD incidence. The vineyards included five sites that were five years or older and three sites that were three-years-old. The disease incidence in the older blocks ranged from 2.81 to 58.78%, while in the younger blocks it ranged from 0.29 to 1.11%. Some vineyards implemented frequent disease scouting, removing infected vines and replanting. The disease incidence in these blocks remained <5% over the survey period. However, in vineyards with no roguing and replanting, the disease incidence increased nearly 30-fold after three years. We analyzed the spatial distribution of the disease in vineyards surveyed in 2020 and found that the disease distribution is highly aggregated based on Spatial Analysis by Distance Indices (SADIE). In a separate study, we also tested the GRBV infection status of asymptomatic vines next to symptomatic vines to inform decision-making when roguing and replanting. Out of 410 asymptomatic vines surrounding 41 symptomatic vines, only two tested positive for GRBV. Additionally, in 2020 and 2021, we tested the GRBV status of previously identified possible alternative host species: blackberries (*Rubus armeniacus*) and wild/feral grapes (*Vitis riparia*) collected from areas surrounding the four survey sites. GRBV was present in 10 to 70% of wild grape samples in both years and in 10% of the blackberry samples in 2020. However, the virus titer was low in blackberry samples and it was not detected in 2021 samples. These results indicate the potential importance of wild grapes as alternative hosts on GRBD incidence and spread in southern Oregon vineyards, while blackberry is unlikely to be an alternative host with epidemiological significance.

Key words: alternative hosts, grapevine, GRBD, GRBV, virus

Grapevine red blotch disease (GRBD) is an emerging disease of grapevines caused by grapevine red blotch virus (GRBV). It is a single-stranded DNA virus within the family *Geminiviridae* (Krenz et al. 2012). Since its discovery in 2008 in California, the disease has impacted several winegrape-

growing regions in North America (Calvi 2011, Krenz et al. 2014). It has now been reported in three provinces in Canada, 18 states in the United States, Mexico, two European countries (Switzerland and Italy), two Asian countries (India and South Korea), and in Argentina (Fuchs 2020, Bertazzon et al. 2021). The economic losses associated with this disease occur through reduced fruit quality and delayed fruit ripening. The disease alters fruit juice chemistry, reduces total soluble solids up to 4 Brix, and lowers anthocyanin content in berry skin (Calvi 2011, Girardello et al. 2020).

Due to the relatively recent discovery of GRBV, limited information is available on GRBD epidemics and management. The disease is widespread in North American vineyards (Krenz et al. 2014, Sudarshana et al. 2015). Short- and long-distance spread of the disease has been documented (Brown et al. 2012, Al Rwahnih et al. 2013, Krenz et al. 2014, Cieniewicz et al. 2019, Dalton et al. 2019). Spatial patterns of infected vines within a vineyard suggest spread by insect vectors (Dalton et al. 2019). The three-cornered alfalfa hopper (*Spissistilus festinus* Say) can vector red blotch virus (Bahder et al. 2016a, Flasco et al. 2021) and its epidemiological importance in secondary spread of the virus from infected vines within a vineyard ecosystem has been described (Cieniewicz et al. 2018).

GRBV is also a graft-transmissible virus and has been diagnosed in both grafted and self-rooted vines (Al Rwahnih et al. 2013). Preparing planting materials using either infected scions or rootstocks during grafting results in proliferation

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of infected vines. Shipment of infected planting material is responsible for the long-distance dispersal of this disease (Al Rwahnih et al. 2013, Krenz et al. 2014).

Most winegrape cultivars are susceptible to GRBD, with different symptoms expressed on white-fruited and red-fruited cultivars (Sudarshana et al. 2015). Symptoms are more apparent in red-fruited varieties, where the leaf tissue develops interveinal red blotches early in the season, which expand and coalesce across leaf blades as the season progresses. On white-fruited varieties, the symptoms consist of irregular chlorotic areas that become necrotic as the season progresses. The symptoms first appear on older leaves at the base of the canopy and progress toward the top branches later in the season. Severely infected vines defoliate later in the season (Sudarshana et al. 2015).

GRBD research has focused on understanding the virus, rapid and accurate virus detection, identifying and managing vectors, characterizing disease effects on vine physiology, and cultural practices for mitigation (Bahder et al. 2016a, 2016b, Cieniewicz et al. 2018, Martínez-Luscher et al. 2019, Levin and KC 2020, Copp and Levin 2021, Copp et al. 2021). Several extension efforts have concentrated on limiting transport of infected plant material within and between states (Cieniewicz et al. 2017). Along with other cultural and regulatory management practices, frequent scouting of vineyards for the presence of disease symptoms and roguing infected vines are standard practices in vineyards to limit virus spread and disease progression. However, there are several knowledge gaps to these tactics commonly applied by growers. Key questions remain on the status of asymptomatic vines adjacent to symptomatic vines and associated decisions to rogue adjacent vines, when to start roguing a newly planted vineyard, and the epidemiological importance of alternative host species.

In this study, we investigated aspects of GRBV biology and epidemiology that are crucial to developing an integrated disease management program for GRBD. Our objectives were

to understand GRBD prevalence, the rate of progression over time, the status of GRBV infection in nearby asymptomatic vines within vineyard rows, and the GRBV status of possible alternative hosts in proximity to infected vineyards.

Materials and Methods

Survey and GRBD incidence. Six vineyard sites with black-fruited *Vitis vinifera* L. cultivars were surveyed within the Rogue Valley American Viticultural Area (AVA) in southern Oregon in 2016, 2017, 2018, and/or 2020 for GRBD incidence (Table 1). At all sites, the vine rows were established in the same year as planted (Table 1) except for site E. In this site, the vines were originally planted 4.0 m × 1.5 m between and within the rows, respectively, in 2010. In 2015, interplanted rows were added to 78% of the block in the western side, and in 2020, more interplanted rows were added to the remaining 22% of the block in the eastern side. The final spacing was maintained at 2.0 m × 1.5 m between and within the rows, respectively.

At all sites, individual vines were assessed for GRBD symptoms at harvest in all years. The GRBD symptoms included prominent interveinal red blotch patterns on leaves in the vine canopy (Figure 1). The data were recorded as the presence or absence of GRBD symptoms for each vine. Depending on the size of the vineyard, the number of vines surveyed per block ranged from 361 to 12,222 (Table 2). Disease incidence was calculated as the percentage of symptomatic vines per total number of vines surveyed in a given block. For sites where more than one year of disease incidence data were determined, disease progression was calculated as the area under the disease progress curve (AUDPC). The AUDPC was calculated using the formula: $\sum\{[(x_i+x_{i+1})/2][(t_{i+1}-t_i)]\}$, where x_i is the assessment of disease incidence at the i th observation, t_i is the time at the i th observation (Madden et al. 2007a), and time is expressed in years. To confirm the symptom-based GRBD diagnosis, 1 to 12% of the total surveyed vines

Table 1 Southern Oregon vineyards included in the grapevine red blotch disease (GRBD) survey.

Site	City	Soil type	Planted year	Rootstock	Scion	Vine spacing (row × vine) m	GRBD Management	Survey year
A	Jacksonville	Ruch silt loam	2009	Schwarzmann	Pinot noir	2.75 × 1.83	No roguing	2016, 2017, and 2018
B	Eagle Point	Carney clay	2013	3309C	Pinot noir clone 115	2.13 × 1.22	No roguing	2017, 2018, and 2020
C	Talent	Manita and such silt loam	2015	420A	Pinot noir clone 115	2.75 × 1.52	Monitoring and Roguing	2017, 2018, and 2020
D-1	Medford	Carney clay	2009	3309C	Pinot noir clone 115	2.13 × 1.22	No roguing	2017, 2018
D-2	Medford	Carney clay	2017	101-14	Cabernet franc clone 11	2.44 × 1.52	Monitoring and roguing	2020
D-3	Medford	Carney clay	2017	101-14	Petit Verdot	2.44 × 1.52	Monitoring and roguing	2020
E ^a	Medford	Medford silty clay loam	2010	3309C	Pinot noir clone 777	1.98 × 1.52	No roguing	2020
F	Central Point	Central Point sandy loam	2017	110R	Pinot noir clone 113	2.75 × 1.83	Monitoring and roguing	2020

^aThe vines were originally planted 4.0 m × 1.5 m between and within rows, respectively, in 2010. In 2015, interplanted rows were added to 78% of the block on the western side, and in 2020, more interplanted rows were added to the remaining 22% of the block. The new spacing was maintained at 2.0 m × 1.5 m between and within the rows, respectively.

were tested for GRBV using a quantitative polymerase chain reaction (qPCR) assay as described below. The tested vines comprised 0.1 to 1% of the symptomatic vines and 0.4 to 11% of the asymptomatic vines.

Spatial analysis of GRBD across the vineyard sites.

Spatial analysis by distance indices (SADIE) was used to analyze the distribution pattern of symptomatic vines across the vineyard sites. Characterizing the spatial distribution of red blotch disease is important to understand the general epidemiology of the diseases and other risk factors. SADIE is a geospatial technique that uses spatially referenced count data to determine the spatial distribution patterns of plant diseases and other pests (Perry 1995, Turechek and Madden 1999, Madden et al. 2007b, Rijal et al. 2016, Reay-Jones 2017). SADIE measures the overall aggregation by calculating the distance to regularity (D) - the minimum total distance that individual samples need to move to reach the same mean distance. The magnitude of D can be calculated by a randomization test in which permutations of all observed counts from sample points are performed (Perry and Dixon 2002). The test provides an index of aggregation, I_a , and probability, P_a . The index value, $I_a > 1$, $I_a = 1$, or $I_a < 1$, indicates aggregation, random, or uniform distribution patterns, respectively. The probability ($p < 0.025$) determines the statistical significance of the resultant distribution pattern. The spatial aggregation is a product of the “patch” (area representing the presence of the disease), “gap” (area representing the absence of the disease), or both, and is quantitatively indicated by the clustering indices ($>|1.5|$) and their associated probability values ($p < 0.025$) (Perry 1995, Perry and Dixon 2002, SADIShell 2008).

For the spatial analysis, we used the symptom data from three sites, B, C, and E in 2020. Due to low disease incidence ($<1.5\%$), sites D-2, D-3, and F were not included in the analysis. Within site E, the symptom data was divided into three sections based on the year of planting. The data from the original rows planted in 2010 (E-1; Figure 2) and the rows interplanted in 2015 (E-2; Figure 2) were analyzed separately. The data from the rows planted in 2020 (E-3; Figure 2) were excluded from the analysis due to low disease incidence ($<1\%$). The size of the square-grid used for the spatial analysis depended on vine spacing. At sites B and C, the rows and vines were selected to obtain an approximate $6\text{ m} \times 6\text{ m}$ grid, while at site E, the rows and vines were selected to obtain an approximate $8\text{ m} \times 8\text{ m}$ grid pattern. The number of vines (N) selected at these sites ranged from 382 to 791 (Table 3). For each sample vine selected using the square-grid criterion, ‘1’ and ‘0’ were noted for disease presence and absence, respectively. The data were analyzed using SADIShell (Ver. 2) with 153 permutations and 12,345 randomizations.

Sampling for GRBV and infection status of nearby asymptomatic vines. In 2020, 10 symptomatic vines were selected randomly from vineyard sites B and C. Five, three, nine and four symptomatic vines were selected randomly from sites D-2, D-3, E, and F, respectively. For each of the 41 selected symptomatic vines, 10 asymptomatic vines, five from either side and within the same row as the symptomatic vines, were sampled (Figure 2). Altogether, 451 samples were



Figure 1 Symptoms of grapevine red blotch disease (GRBD) on three cultivars included in this study. (A) Pinot noir, (B) Cabernet franc, and (C) Petit Verdot.

Table 2 Progression of grapevine red blotch disease incidence at eight vineyard sites in southern Oregon from 2016 to 2020.

Site	Number of vines surveyed				Disease incidence (%) ^a				AUDPC ^c	Rogued vines (%)	Number of tested vines		Accuracy of the tested vines (%) ^b	
	2016	2017	2018	2020	2016	2017	2018	2020			Symptomatic	Asymptomatic	True positive	True negative
A	9671	9493	9187	-	17.01	31.49	25.39	-	52.69	Vineyard removed	52	62	92.31	100
B	-	4763	5375	5133	-	0.55	0.67	29.48	30.76	-	23	115	95.65	98
C	-	9281	9567	6023	-	3.42	5.49	2.81	12.76	37	20	106	80	100
D-1	-	5522	2253	-	-	30.03	14.65	-	22.34	Vineyard removed	11	9	81.82	100
D-2	-	-	-	600	-	-	-	1.00	-	0.66	6	60	100	100
D-3	-	-	-	1026	-	-	-	0.29	-	1.66	3	30	33.33	100
E	-	-	-	12,222	-	-	-	58.78	-	-	10	100	100	99
F	-	-	-	361	-	-	-	1.11	-	-	4	40	0	100

^aDisease incidence is calculated as ratio of symptomatic vines to the total number of vines surveyed, expressed as percentage.

^bAccuracy is calculated based on the PCR test of the sampled vines identified as symptomatic and asymptomatic. True positive ratio is the ratio of vines that tested positive for grapevine red blotch virus (GRBV) to the total number of vines that were tested and recorded as symptomatic during the survey. Similarly, true negative ratio is the ratio of vines that tested negative for GRBV to the total number of vines that were tested and recorded as asymptomatic during the survey.

^cThe disease progression was measured as the area under the disease progress curve (AUDPC) using the formula: $\sum \{[(x_t + x_{t+1})/2][(t_{i+1} - t_i)]\}$, where x_t is the assessment of disease incidence at the t th observation and t_i is the time at the i th observation (Madden et al. 2007a). AUDPC was not measured at site D-2, D-3, E, and F due to one year of data.

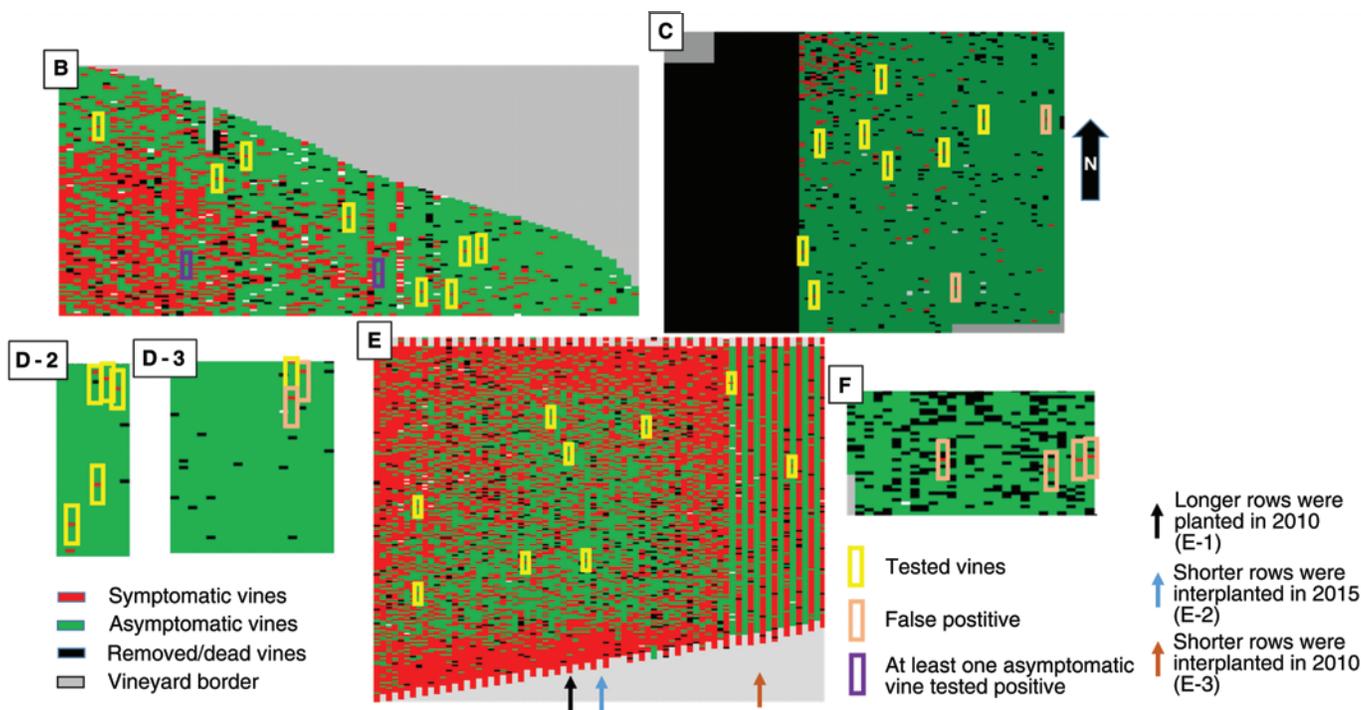


Figure 2 Symptom mapping of grapevine red blotch disease (GRBD) vines in six survey sites in 2020. Details on these sites are presented in Table 1. The red solid cells represent symptomatic vines, green represents asymptomatic vines, black represents removed or dead vines, and gray represents the vineyard border, where no vines were planted. The yellow, orange, and purple blocks represent the test areas where symptomatic vines were selected for grapevine red blotch virus (GRBV) testing of adjacent vines. At each site, five asymptomatic vines from both sides of the symptomatic vines were selected. Eleven vines from each of these blocks were tested for GRBV using qPCR assays. False positives are vines that were collected as symptomatic vines but tested negative for GRBV in qPCR assays. In site C, the block of black area represents the vines that were rogued in 2019 due to GRBD. In site E, the longer rows were planted in 2010 (E-1), shorter rows were interplanted in 2015 and 2020: 78% of the block on the western side was planted in 2015 (E-2) and the remaining 22% of the block on the eastern side was planted in 2020 (E-3).

collected for GRBV testing at harvest, out of which 9% were symptomatic vines and the rest asymptomatic. Each sample consisted of two whole leaves (blades with attached petioles) collected from each bilateral cordon at the base of the canopy. The four petioles were excised from the leaves, combined, and the apical petiole tissues were cut into small pieces (1-mm thick) with sterilized blades to obtain a 100 mg tissue sample for DNA extraction. Total genomic DNA was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) DNA extraction protocol (Richards et al. 1994). The presence of GRBV was detected by using qPCR assays, modified from Krenz et al. (2014). qPCR was performed using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Inc.) with 20 μ L reaction volumes using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Inc.), primers CPfor (5'-AGCGGAAGC ATGATTGAGACATTGACG-3') and CPprev (5'-AACGTATGTCCACTTGCAGAAGCCGC-3') for GRBV detection, and primers 16Sfor (5'-TGCTTAACACATGCAAGTCGGA-3') and 16Srev (5'-AGCCGTTTCCAGCTGTTGTTTC-3') as an internal control, and 1 μ L DNA template. DNA from a grapevine confirmed GRBV-positive and a non-template control were used as positive and negative controls, respectively.

All qPCR reactions were performed with a 32.0 Ct cut-off value, which was determined by subtracting 2.0 cycles from the average Ct value of non-template controls. qPCR results were validated by crosschecking melt curve analysis peaks between samples and the positive control. To further check the validity of samples with Ct values equal to or greater than 30 Ct, multiplex PCR was performed using primers CPfor and CPprev and Repfor (5'-CAAGTCGTTGTAGATTGAGGACGTATTGG-3') and Reprev (5'-AGCCACACCTACACGCCTTGCTCATC-3') (Krenz et al. 2014). PCR products were run through pre-cast 1% agarose gels with 1x GelRed (Biotium). Samples were confirmed GRBV-positive if band

sizes of 257 and 318 bp were observed for the CP and Rep fragments, respectively.

Alternative hosts and GRBV detection. In 2020 and 2021, wild/feral grapes (*Vitis riparia*) and wild blackberries (*Rubus armeniacus*) were collected for GRBV testing as possible alternative hosts. The samples were collected in proximity to the survey sites B, C, E, and F in fall 2020 and 2021, from the same location. The approximate distance between the alternative host sampling site and vineyard survey site was estimated based on Google Map's 'measure distance' tool with Google satellite maps of each vineyard (Table 4). Ten individual vine samples of each species were selected randomly from all available surroundings of the vineyard. The samples included four whole leaves per plant of each species. Total genomic DNA was extracted as with the winegrape samples, where apical petiole tissues were pooled to achieve 100 mg tissue. The presence of GRBV was detected using both qPCR and multiplex PCR assays as described above.

Results

Change in GRBD incidence over time using the symptom-based survey. The total number of vines surveyed for GRBD incidence changed yearly within the same block due to roguing and replanting practices by collaborating growers (Table 2). At site A, the percentage of rogued vines increased from 1.8% in the first year to 3.2% in the second year, culminating in vineyard removal after the third year. At site B, no roguing practices were applied; however, replanting resulted in an increased number of vines by 7% in the third year.

Table 4 Incidence of grapevine red blotch virus (GRBV) in alternative hosts collected near four vineyard sites that were included in disease survey in 2020.

Sites	Alternative host	Incidence (%) ^a		Average distance from the surveyed block (m) ^b
		2020	2021	
B	Blackberry	0	0	8 (8)
	Wild grape	-	-	-
C	Blackberry	10	0	10 (6-21)
	Wild grape	70	60	12 (10-12)
E	Blackberry	10	0	131 (4-222)
	Wild grape	10	10	8 (5-18)
F	Blackberry	10	0	171 (108-220)
	Wild grape	0	0	197 (196-197)

^aIncidence is calculated as ratio of vines that tested positive for GRBV to the total number of vines sampled (n = 10 per species per site), expressed as percentage.

^bAverage distances between the surveyed block and location of alternative hosts. The distances were estimated using Google Map's 'measure distance' tool with Google satellite maps of each vineyard. The numbers in parentheses are the range of distances from survey block and location of alternative hosts.

Table 3 Spatial analysis by distance indices (SADIE) parameters for grapevine red blotch disease distribution across four vineyard sites surveyed in 2020.

Site	N	Disease incidence (%) ^a	I_a^b	P_a	v_j	Pv_j	v_i	Pv_i
B	382	29.3	3.316	0.0002	-3.323	<0.0001	3.365	<0.0001
C	791	2.4	2.782	0.0002	-2.794	<0.0001	2.786	<0.0001
E-1	725	69.9	2.625	0.0002	-2.570	<0.0001	2.676	<0.0001
E-2	555	55.9	2.288	0.0002	-2.237	<0.0001	2.372	<0.0001

^aEstimated disease incidence based on the selected vines for SADIE analysis.

^b I_a , index of aggregation; P_a , p value of I_a ($P_a < 0.025$ indicates the statistically significant aggregation distribution pattern); v_j , mean value of clustering index over the gap units; Pv_j , p value of v_j ($Pv_j < 0.025$ indicates statistically significant gaps); v_i , mean value of clustering index over the patch units; Pv_i , p value of v_i ($Pv_i < 0.025$ indicates statistically significant patches); N, total number of sample vines in individual vineyards used for SADIE.

At site C, nearly 3% of the vines were replanted in the second year and nearly 37% of the vines were rogued in the third year. At site D-1, nearly 40.8% of the vines were rogued in the first year and the vineyard was removed after the second year (Table 2). At sites A and D-1, where the blocks were ultimately removed, the disease incidence reached >30% in 2017 and the AUDPC values at these sites were 52.7 and 22.3 over the three- and two-year periods, respectively (Table 2). Vineyard sites B and C were the only available sites to be surveyed in 2020 that had previous data on disease incidence. At site B, the disease incidence increased from <1% in 2017 and 2018 to nearly 30% in 2020. At site C, the disease incidence was lower than at site B, with 3.42, 5.49, and 2.81% in 2017, 2018, and 2020, respectively. At sites B and C, the AUDPC values were 31 and 13, respectively, over the four years. Among the vineyard sites surveyed in 2020 only, the disease incidence at three newer planting sites (D-2, D-3, and F) ranged from 0.3 to 1.1%. At site E, the rows that were planted in 2010 and 2015, the disease incidence was nearly 59%. The youngest interplanted rows in 2020 had <1% disease incidence (Figure 2E).

The reliability of symptom-based surveys ranged from 33 to 100% depending on the site (Table 2). At six out of eight sites, the ability to identify positive vines (true positive ratio) based on symptoms was greater than at the other sites. At sites A, B, C, D-1, D-2, and E, 80 to 100% of the vines that were recorded as symptomatic also tested positive using PCR-based assays. At sites D-3 and F, where disease incidence was 0.29 and 1.11% respectively, the ability to identify positive vines (true positive ratio) ranged from 0 to 33%. However, the ability to identify negative vines (true negative ratios) was consistently high in all vineyard sites. At sites B and E, 98 and 99% of asymptomatic samples were negative for GRBV, respectively. At the remaining sites (A, C, D-1 to D-3, and F) all asymptomatic samples were negative for GRBV.

Spatial aggregation of GRBD using SADIE. Based on SADIE, all vineyard blocks included in the analysis showed strong aggregation of the red blotch disease, with statistically significant p values ($p < 0.025$). All sites used for spatial analysis had an index of aggregation (I_a) greater than 1, with p values < 0.025 (B: $I_a = 3.316$, $P_a = 0.0002$; C: $I_a = 2.782$, $P_a = 0.0002$; E-1: $I_a = 2.625$, $P_a = 0.0002$; and E-2: $I_a = 2.288$, $P_a = 0.0002$), indicating an aggregated distribution pattern (Table 3). Clustering indices also suggested robust clustering, as all sites had $>|1.5|$ clustering values for both parameters – gap v_j and patch v_i with <0.025 p values (Table 3). Symptom mapping of the analyzed sites also suggested the presence of ‘disease hot spots’ with edge effects at sites B and C (Figure 2). At site E, the estimated disease incidence in rows planted in 2010 was greater than in those planted in 2015 (Table 3). However, in both rows, the disease incidence was greater than 55%. Furthermore, a strong aggregation, indicated by a statistically significant ($p < 0.025$) index of aggregation of the symptomatic vines >1 , was apparent in rows planted in both years.

GRBV status in nearby asymptomatic vines. At 39 out of the 41 test areas, none of the asymptomatic vines near

symptomatic vines tested positive (Figure 2). Out of the 410 asymptomatic vines, only two or 0.5% tested positive for GRBV. One of the two asymptomatic vines that tested positive was located directly adjacent to the symptomatic vine, while the other was four vines to the north, both in the same vineyard site (Figure 2B). The first vine was sampled in the area with a lower percentage of symptomatic vines (Figure 2B, eastern purple block), and the second vine was sampled in an area with a greater percentage of symptomatic vines (Figure 2B, western purple block). Out of 41 symptomatic vines, eight (19%) tested negative for GRBV (Figure 2, orange blocks). These vines were sampled from vineyard sites C, D-3, and F, and were from areas with a lower percentage of symptomatic vines. All adjacent asymptomatic vines within these test areas also tested negative (Figure 2C, 2D-3, and 2F, orange blocks).

GRBV detection in alternative hosts. GRBV was detected in 2020 in both sampled alternative host species, wild grape and blackberry, and from wild grape samples only in 2021 (Table 4). At site B, all the blackberry samples were collected from ~8 m from the surveyed block and GRBV was not detected in any samples. At site C, the blackberry samples were collected at distances ranging from 6 to 21 m from the surveyed block, and GRBV was detected in 10% of the 2020 samples. At this site, wild grape samples were collected from ~10 to 12 m from the surveyed block and 70% and 60% of the samples tested positive for GRBV in 2020 and 2021, respectively. At site E, the blackberry samples were collected from 4 to 222 m from the surveyed block and 10% tested positive for GRBV in 2020. Similarly, wild grape samples were collected from 5 m to 18 m from the surveyed block and 10% of the samples tested positive for GRBV in both years. At site F, the blackberry samples were collected 108 to 220 m from the surveyed block and 10% of the samples tested positive for GRBV in 2020. At this site, wild grape samples were collected 196 to 197 m from the surveyed block and none tested positive for GRBV in either year (Table 4).

Discussion

Our study suggests that GRBD is prevalent in both older and younger vineyards planted in southern Oregon. In general, disease incidence was greater in sites with mature vines (five-years-old or older). At sites where infected vines were monitored frequently for foliar symptoms and rogued, disease incidence was reduced by half. At sites where little to no roguing occurred, disease incidence increased from <1% to nearly 30% in three years. This finding provides a time frame to previously published recommendations to mitigate the economic impact of GRBD management: that losses can be minimized by roguing and replanting infected vines if the disease incidence is <30% (Ricketts et al. 2017). If the disease incidence exceeds 30%, then the entire vineyard should be removed and replanted. Given the disease progression rate reported here, it may take only four years for a grower opting not to rogue and replace infected vines to face a situation where vineyard removal becomes the preferred option. Roguing and replanting as soon as symptoms appear in a vineyard

may be a manageable and profitable strategy to reduce the long-term negative economic impact of GRBD.

As more information on GRBD management becomes available and extension and outreach efforts are in place (Bahder et al. 2016b, Ricketts et al. 2017, Cieniewicz et al. 2018), we observed variations in the practices adopted by growers to manage GRBD. At sites A and D-1, where the disease incidence once reached 30% or more, the entire vineyard block was removed in 2019. At sites C, D-2, and D-3, the symptomatic vines were continuously monitored, rogued, and replanted every year. However, at sites such as E, the vineyard is still under full production even though the disease incidence is as high as 59%. This highlights the unique economic situation that each grower encounters and subsequent decisions on farming practices. Nevertheless, the long-term economic impact of the high disease incidence has yet to be realized, given the disease progression rate, disease spread in newer blocks, and subsequent reduced fruit and wine quality.

When a simple foliar symptom-based diagnosis was compared with a PCR-based diagnosis, we overestimated disease incidence by ~12%. Diagnosing red blotch as characteristic symptoms of GRBD is challenging as the vines produce similar symptoms in response to various biotic and abiotic stresses (Cieniewicz et al. 2017). For example, biotic stress conditions like leafroll-associated viruses, mite damage, and crown gall by *Agrobacterium tumefaciens*; and abiotic conditions such as poor root health, physical injuries, and mineral deficiencies produce red leaves, which can be confused with red blotch disease symptoms (Rayapati et al. 2008, Sudarshana et al. 2015, Singer et al. 2018, Mulder 2019). Alternatively, the lower virus titer in the sampled tissues resulting in reduced sensitivity of the PCR assay could have contributed to overestimation in some vines. The sensitivity of multiplex PCR was reduced when the GRBV total nucleic acid (TNA) was diluted to 5 pg/μL, while the sensitivity of qPCR and Loop-Mediated Isothermal Amplification (LAMP) assays were reduced when the TNA was diluted to 50 and 0.5 fg/μL, respectively (Romero et al. 2019). Furthermore, the variability in virus distribution within individual vines could have contributed to the possibility of selecting tissue samples with low virus titer in the selected symptomatic vines (Setiono et al. 2018). In our survey, we generally overestimated the actual incidence of disease in areas with low disease incidence, particularly in newer planting sites. Nevertheless, the relatively high cost of GRBV testing precludes testing all symptomatic vines in a block and symptom-based assessment is still reliable in 88% of the cases. As such, symptom-based monitoring and preventative strategies to minimize disease spread are still more beneficial than taking no measures.

We observed spatial aggregation of disease incidence in older vineyard sites planted before 2015. At these sites, disease symptoms were strongly aggregated, often at the borders; this is in agreement with previous findings (Cieniewicz et al. 2019, Dalton et al. 2019). As suggested by these studies, the spatial patterns of GRBD incidence resemble

the virus spread by mobile insect vectors. On the other hand, at the newer vineyard sites planted in 2017, GRBV was already detected, but the disease incidence was relatively lower than at the older sites (<1.1%). The symptomatic vines were spread throughout the vineyard at these sites, suggesting the possibility of inoculum originating from the planting material (Cieniewicz et al. 2019). As reported by several other studies, these observations also suggest three possible phenomena: GRBV is introduced into the vineyard from planting materials (Cieniewicz et al. 2018, 2019); vectors are transmitting the virus from infected to healthy vines within the vineyard or from alternative hosts that surround the vineyard (Bahder et al. 2016b, Cieniewicz et al. 2018, Dalton et al. 2019); or some combination of the two. When treehopper girdling was mapped (data not included in this study), we found 0 to 7.48% of treehopper girdling incidence at these sites in 2020. This indicates some level of possible vector activities in these vineyards. These activities were most frequent at site F, with none at sites D-2 and D-3, and <1% at sites B, C, and E. These values, however, did not correlate ($R^2 = 0.04$) with disease incidence and is difficult to justify their epidemiological importance in this study. It is important to consider that the treehopper girdling was mapped only in 2020, the treehopper species causing girdled vines were not identified, and the girdled vines were not tested for GRBV status. The current disease incidence could result from vector activities in previous years or may result in greater disease incidence in the coming years. This hypothesis, however, needs additional validation. The presence of vectors other than the three-cornered alfalfa hopper remains a real possibility and is a topic of considerable research. Other candidate vectors for GRBV such as *Coladonus reductus*, *Osbornellus borealis*, and *Melanorius* sp. have been reported from other GRBD-infected sites in the United States (Cieniewicz et al. 2018). The three-cornered alfalfa hopper and other potential vectors have been collected from the vineyard sites included in this study (Richard Hilton, unpublished data).

When the nearby asymptomatic vines were tested for the possibility of GRBV infection, the vines were GRBV-free in 95.5% of the tested areas. This observation has a critical practical implication in making decisions about roguing vines as a management strategy. While the latency of GRBV is still a subject of ongoing research (Yepes et al. 2018), growers face the question of whether nearby asymptomatic vines should be rogued when they are roguing infected/symptomatic vines. It is typically suggested that the infection status of the vines should be confirmed using PCR-based diagnosis (Sudarshana et al. 2015, Cieniewicz et al. 2018). However, the cost per sample in the United States can range from \$25 to \$30, making this testing procedure inaccessible to some growers. Other cost-effective DNA-based diagnostic tools such as LAMP have been explored (Romero et al. 2019), however, the data on their accuracy in commercial settings are limited. Given the high cost of PCR-based diagnosis and reluctance to rogue vines due to lower confidence in limiting virus spread from potentially-infected nearby

vines, some growers may decide to forego roguing and replanting entirely. Our study suggests that symptom-based management practices are still reliable options, without worrying about nearby asymptomatic vines. Furthermore, roguing and replacing symptomatic vines as soon as they appear in the vineyard could have long-term economic benefits when disease incidence is <30% (Ricketts et al. 2017).

We detected GRBV from both wild blackberry and wild grape samples collected in fall 2020 and from wild grape samples collected in fall 2021. These plant species were selected based on findings that these two species, out of 13 plant species sampled throughout the year in and around California vineyards, tested positive for GRBV (Bahder et al. 2016b) and that none of the cover crop samples collected from GRBV-infected California vineyards tested positive for GRBV (Cieniewicz et al. 2019). In addition, these species are common in riparian habitats in southern Oregon, often in proximity to vineyards. Out of three vineyard sites from where wild grape samples were collected, samples from two consistently tested positive for GRBV in both years. Both quantification cycle threshold (Ct) values in qPCR analysis (Ct ranged from 12.4 to 18.95) and the presence of a strong band of correct base pair size (257 and 318 bp for CP and Rep fragments, respectively) in multiplex PCR confirmed the presence of GRBV in wild grapes. In blackberry samples, we detected GRBV in samples collected in 2020 from three sites with 10% incidence at each site. Similar to wild grape samples, the presence of GRBV was confirmed in these samples by both qPCR and multiplex PCR. However, the virus titer measured as Ct values in qPCR were relatively low in blackberry samples (Ct ranged from 28.35 to 31.97). In addition to these samples, there were five other blackberry samples in 2020 from site E that resulted in Ct values from 29.86 to 31.16, and a faint band of the correct size in multiplex PCR. One sample from the same site in 2021 resulted in Ct values of 31.12, with a faint band of the correct size in multiplex PCR. However, we could not justify the infection status of these samples, and these data were not included in the analysis. Bahder et al. (2016b) reported in their study that the GRBV detection in blackberry samples may be a “form of environmental contamination as a result of vector feeding.” They also confirmed that GRBV did not replicate in these hosts. The lower virus titer in our blackberry samples collected from southern Oregon vineyards could be the result of the same effect of vector feeding in the absence of virus replication. This would explain the high Ct values observed with qPCR, faint bands observed in agarose gels following multiplex PCR, and absence of GRBV in any of the 2021 blackberry samples. The timing of sample collection could have also played a role. In the study by Bahder et al. (2016b), GRBV was only detected in winter and spring samples and was absent in summer and fall samples. All our samples were collected in the fall and the virus was still detected, but at a low titer. Nonetheless, without definite knowledge of vector species and their habitat, the epidemiological importance of these alternative host species remains unknown and should be a subject of future studies, as it may provide useful information for integrated management of GRBD.

Conclusion

Our study suggests that GRBD is present in both older and newer planting sites in southern Oregon and that disease is progressing in the older sites. In those vineyards that implement regular monitoring, roguing, and replanting, the disease incidence has been maintained at 5% or lower. However, in those vineyards that are not implementing any preventative strategies, the disease incidence can increase by 30-fold in three years. Therefore, removing symptomatic vines as soon as they appear in newer planting sites is recommended to limit the spread of GRBD within a vineyard. Our results indicate that only symptomatic vines should be rogued and neighboring asymptomatic vines can be left in place as long as the vineyard is monitored frequently for symptom development. Finally, one should be aware of the surrounding vegetation and potential vector activities if the rate of disease spread remains high despite regular scouting and roguing.

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