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Research Article

Incidence of Grapevine Leafroll Disease: Effects of Grape Mealybug (*Pseudococcus maritimus*) Abundance and Pathogen Supply

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Abstract

Studies of spatiotemporal dynamics are central to efforts to characterize the epidemiology of infectious disease, such as mechanism of pathogen spread and pathogen or vector sources in the landscape, and are critical to the development of effective disease management programs. To that end, we conducted a multi-year study of 20 vineyard blocks in coastal northern California to relate the dynamics of a mealybug vector, *Pseudococcus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae), to incidence of grapevine leafroll disease (GLD). In each vineyard block, a subset of vines were scored visually for relative mealybug abundance, disease was quantified by visual assessment, and virus presence was verified using standard laboratory molecular assays. GLD incidence was analyzed with a classification and regression tree, and with a hierarchical model that also captured variability among blocks and heterogeneity within blocks. Both analyses found strong interannual variability in incidence, with the hierarchical model also capturing substantial between- and within-block heterogeneity, but with significant contributions of vector abundance and pathogen supply (prior disease incidence) to the frequency of newly diseased vines. These results strengthen further the conclusion that mealybug vectors are causally related to pathogen spread in this system and are therefore an important target for management. Moreover, they are consistent with relatively efficient secondary spread of the pathogen, suggesting an important role for the removal of diseased vines as a tool to mitigate further damage.

Key words: disease management, vector pressure, grapevine leafroll-associated virus, roguing

Grapevine leafroll disease (GLD) is one of the most significant viral diseases of grapevines worldwide, described for more than a century (Hoefert and Gifford 1967), occurring in every major grape-growing region, and infecting wine, juice and table grape cultivars, as well as rootstocks (Maree et al. 2013). The pathogens associated with GLD are known collectively as grapevine leafroll-associated viruses (GLRaV) and following a recent taxonomic revision (Martelli et al. 2012) include GLRaV-1, -2, -3, -4, and -7. Of these, GLRaV-3 is the most widely reported, occurring in Europe, Africa, Asia, Oceania, and the Americas (Maree et al. 2013).

Although the pathogen is implicated in graft incompatibility and young vine failure, the disease is not typically lethal, but results in significant economic loss from a combination of factors. Leafroll-diseased vines may experience phloem degradation and decreases of 25–65% in net photosynthesis (Charles et al. 2006), leading to decreased fruit quality and pigmentation (Guidoni et al. 2000), altered amino acid profiles (Lee et al. 2009), delayed maturity and yield reductions (Woodrum et al. 1984, Blaisdell et al. 2016), and (in a 2014 study) economic impacts from $29,902 to $226,405 (United States) per hectare in California (Ricketts et al. 2015). Affected vines may exhibit leaf symptoms including a cultivar-dependent reddening or mild yellowing or chlorotic mottling of the interveinal area, as well as a downward rolling of the margins of the leaf blade (Rowhani and Gugerli 2015). Fruit symptoms, as described above, can also be severe. Increased incidence in coastal vineyards (Golino et al. 2008) elevated the disease to a high-priority issue for California grape growers.

Historically, GLD was considered transmissible only by grafting (Scheu 1935), until Engelbrecht and Kasdorf (1990) demonstrated vine-to-vine transmission by the vine mealybug, *Planococcus*

ficus (Signoret) (Hemiptera: Pseudococcidae). Subsequently, various other mealybug and soft-scale species were shown to transmit GLRaV-3, demonstrating a breadth of vector species and lack of specificity that should cause concern worldwide wherever GLD is found (Almeida et al. 2013, Herrbach et al. 2017). Of the known insect vectors, five are mealybug species present in California vineyards: grape (*Pseudococcus maritimus*), obscure (*Pseudococcus viburni*) (Signoret) (Hemiptera: Pseudococcidae), long-tailed (*Pseudococcus longispinus*) (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), Gill's (*Ferrisia gilli*) Gullan (Hemiptera: Pseudococcidae) (Wistrom et al. 2016), and vine (*Pl. ficus*). The geographic distribution varies by species: *Ps. maritimus* historically had the widest range in the state, although it is rapidly being displaced by the invasive *Pl. ficus* (Daane et al. 2012)

Ps. maritimus, native to North America, is found throughout California's Central Valley and coastal grape regions in Oregon and Washington (Daane et al. 2008b). It is believed to be one of the oldest California vineyard pests (Essig 1914) and has a complex of natural enemies including predators and parasitoids that suppress populations in the absence of tending ants (Cooper et al. 2008; Daane et al. 2008a). Historically, *Ps. maritimus* management programs in northern California vineyards were focussed on reducing economic damage to the fruit and were not based on vector control criteria. This may affect disease spread because transmission of GLRaV-3 under field conditions is efficient (Blaisdell et al. 2016), whereas a larger *Ps. maritimus* population is required to cause direct economic damage from mealybug feeding. In most northern California vineyards, biological control agents keep *Ps. maritimus* at very low density such that it rarely causes direct economic damage to the fruit.

Understanding spatial and temporal dynamics is essential to the development of management strategies for insect-borne diseases, as evidenced by numerous studies of other plant pathosystems (Gottwald et al. 1996, Bassanezi et al. 2005, Park et al. 2006a). These analyses are critical to understanding vector behavior and movement (Park et al. 2006b, Sétamou and Bartels 2015), developing sampling plans (Marcus et al. 1984, Park et al. 2006a), elucidating the influence of surrounding environments (Purcell 1974, Perring et al. 2001), and developing best management practices, such as removal of infected plants and insect population reduction (Tubajika et al. 2004, Sétamou and Bartels 2015).

Studies of spatial distribution patterns in vineyards demonstrated a generally high degree of spatial aggregation of leafroll-diseased vines, consistent with vector-mediated movement of the pathogen (Habili and Nutter 1997, Cabaleiro and Segura 2006, Arnold et al. 2017). These studies included measures of disease incidence, but with few exceptions, did not include structured, consistent sampling procedures to measure vector incidence and distribution. Researchers studying GLRaV-3 epidemics in vineyards in Spain (Cabaleiro and Segura 2006, Cabaleiro et al. 2008) included mealybug monitoring, but only in certain years and select blocks. During recent field trials in New Zealand (Charles et al. 2009, Bell 2014), researchers monitored leafroll disease incidence and mealybug populations in production vineyards, demonstrating the relationship between mealybug populations and disease incidence under New Zealand growing conditions.

Our objective was to develop a mechanistic understanding of the dynamics of GLD epidemics in northern California vineyards by describing the relationship between *Ps. maritimus* populations and GLRaV-3 infection rates. By developing a more comprehensive understanding of the causal relationship between vector populations and rates of disease incidence, we aimed to inform the development and implementation of best management practices for GLD in California vineyards.

Materials and Methods

Field Sites

We selected 20 unique vineyard blocks in the Napa Valley, CA, as study sites. The blocks ranged in location, age, size, cultivar, and rootstock (Table 1). Of these blocks, three were monitored during the 2009 to 2013 growing seasons, 14 were monitored from

Table 1. Description of study blocks in Napa Valley

Cultivar	AVA	Planting date	Rootstock	Block size (hectares)	Years mapped
Cabernet Franc	Oakville	1994	Mix	2.13	2010–2013
Cabernet Franc	Oakville	2000	St. George	2.39	2010–2013
Cabernet Franc	Yountville	1994	3309C	2.11	2010–2013
Cabernet Sauvignon	Oak Knoll	1996	110R	4.26	2010–2013
Cabernet Sauvignon	Oakville	1945	St. George	1.66	2010–2013
Cabernet Sauvignon	Oakville	1986	110R	1.56	2010–2013
Cabernet Sauvignon	Oakville	1986	110R	1.93	2010–2013
Cabernet Sauvignon	Oakville	1986	110R	0.44	2010–2013
Cabernet Sauvignon	Oakville	1992	101–14	1.45	2009–2013
Cabernet Sauvignon	Oakville	1992	101–14	0.51	2009–2013
Cabernet Sauvignon	Oakville	1992	101–14	1.90	2009–2013
Cabernet Sauvignon	Oakville	2000	St. George	1.93	2010–2013
Cabernet Sauvignon	Rutherford	1998	Mix	1.33	2010–2013
Cabernet Sauvignon	Yountville	1988	110R	1.89	2010–2011
Cabernet Sauvignon	Yountville	1988	St. George	1.79	2010–2011
Cabernet Sauvignon	Yountville	1990	110R	3.97	2010–2013
Merlot	Oakville	1986	110R	0.56	2010–2012
Merlot	Oakville	1986	110R	0.55	2010–2013
Merlot	Oakville	2006	1616C & 420A	2.24	2010–2013
Zinfandel	Rutherford	1997	O39-16	1.51	2010–2013

All blocks were planted with *Vitis vinifera*; cultivar and rootstock are indiated in columns 1 and 4, respectively. All blocks are within the Napa Valley appellation and AVA as indicated in column 2.

AVA = American Viticultural Area.

Table 2. For each of the study blocks, we are reporting the total number of vines evaluated for grapevine leafroll disease (GLD) symptoms and *Pseudococcus maritimus* (*Pm*) populations, as well as average disease and mealybug incidence over the course of the study

Cultivar	Planting date	Vines evaluated for GLD (average disease incidence)	Vines evaluated for *Pm* (average incidence)
Cabernet Franc	1994	1,530 (0.07%)	523 (3.00%)
Cabernet Franc	2000	3,280 (0.00%)	1,107 (9.96%)
Cabernet Franc	1994	765 (2.42%)	275 (0.00%)
Cabernet Sauvignon	1996	1,104 (5.89%)	378 (0.61%)
Cabernet Sauvignon	1945	379 (72.53%)	135 (0.49%)
Cabernet Sauvignon	1986	123 (14.63%)	41 (14.63%)
Cabernet Sauvignon	1986	533 (1.14%)	179 (32.96%)
Cabernet Sauvignon	1986	170 (1.79%)	58 (14.39%)
Cabernet Sauvignon	1992	1,469 (2.76%)	500 (4.21%)
Cabernet Sauvignon	1992	462 (23.93%)	157 (4.72%)
Cabernet Sauvignon	1992	2,228 (12.47%)	752 (9.22%)
Cabernet Sauvignon	2000	2,422 (0.02%)	817 (0.74%)
Cabernet Sauvignon	1998	540 (0.37%)	180 (13.30%)
Cabernet Sauvignon	1988	528 (47.03%)	188 (5.32%)
Cabernet Sauvignon	1988	849 (57.63%)	300 (3.16%)
Cabernet Sauvignon	1990	1,253 (3.78%)	470 (1.58%)
Merlot	1986	278 (44.36%)	107 (17.35%)
Merlot	1986	126 (7.79%)	46 (16.08%)
Merlot	2006	1,167 (0.17%)	315 (0.94%)
Zinfandel	1997	639 (0.08%)	215 (8.55%)

Vines were evaluated on an annual basis in late summer or early fall.

2010 to 2013, 1 was monitored from 2010 to 2012, and 2 were monitored only in 2010 and 2011. The latter three vineyard blocks were removed from production due to high leafroll disease pressure ranging from 44 to 57% (Table 2). Within each block, every fifth row was designated as a sampling row. In each sampling row, leafroll disease incidence was evaluated for every vine during all years; mealybug populations were evaluated on every fifth vine in 2009, and every third vine in all other years. This resulted in a range of 123 to 3,280 and 41 to 1,107 vines categorized per site for disease incidence and mealybug populations, respectively (Table 2). Variation among years and blocks resulted from vine removal or replant activities and differences in size and density of planted vines.

Sampling Procedures

Grape Mealybug (Ps. maritimus)

Mealybug populations were categorized using a validated rating system (Geiger and Daane 2001). These categorical ratings facilitated a larger sample size, thereby decreasing variance in the data. This can be especially important when working with a pest such as *Ps. maritimus*, which is unevenly distributed in a clumped pattern. Mealybug populations were categorized for one fruit cluster (grape bunch) on each sampled vine, and morphological inspections confirmed that *Ps. maritimus* was the only species recorded in these plots during the sampling period. Grape clusters in direct contact with woody parts of the vine were preferentially sampled because they are more likely to host mealybug populations (Geiger and Daane 2001). The following rating system was used to categorize the level of infestation of each sampled cluster: a '0' rating was equivalent to a clean cluster with no mealybugs. A '1' rating was assigned to clusters with 1–10 mealybugs (light damage); a '2' rating assigned to those with 11–20 mealybugs, but at least part of the bunch is salvageable, and a '3' rating assigned to clusters with more than 20 mealybugs where none of the bunch is considered salvageable. The cluster assessment using this rating scale was conducted at one timepoint during the growing season, coinciding with the period after veraison but prior to harvest. This specific timing was selected to assess prevalence and distribution of the second generation of mealybugs before they moved to the bark to deposit overwintering eggs. Sampling clusters was more efficient than conducting timed searches for mealybugs under the bark given the large vineyard area (36.11 ha) that was included in the trial.

Grapevine Leafroll Disease

Visual symptoms of leafroll disease were recorded for every vine in the sampled row. Leafroll disease symptoms were rated as present or absent. To evaluate the level of agreement between visual assessment and virus status of the vines, a subsample of rated vines was subjected to laboratory analysis (Table 3), in partnership with a commercial laboratory (Agri-Analysis, LLC, Davis, CA). Samples from each study vine were collected during the dormant seasons of 2010, 2011, 2012, and 2013, and consisted of four to six, 15- to 20-cm sections of basal canes per vine. Phloem extracts were prepared according to the GES protocol of Osman et al. (2012) and Osman and Rowhani (2006). GLRaV-1 and GLRaV-3 were tested with the enzyme-linked immunosorbent assay (ELISA). ELISA reagents for GLRaV-1 and GLRaV-3 were products of Bioreba Ag (Reinach, Switzerland) and Agri-Analysis, respectively. The remaining GLRaV, as well as *Grapevine virus A* and *Grapevine virus B*, were tested with the one-step reverse transcription-polymerase chain reaction (RT–PCR) method (Osman et al. 2012). Once *grapevine red blotch virus* (GRBV) was identified (Krenz et al. 2012), conventional PCR assays using the primers of Al Rwahnih et al. (2013) were included in all subsequent testing. All PCR primers were custom synthesized by IDT DNA Technologies (Coralville, IA). Sampled vines were selected from one of four categories: 1) visually negative for leafroll disease symptoms, 2) visually positive for leafroll disease symptoms, 3) questionable symptoms, and 4) visually positive red blotch disease symptoms (2012 and 2013 only). Vines sampled in categories 1 and 2 were randomly selected from all vines within the block falling in said categories, whereas vines in categories 3 and 4 were tested specifically to determine their status.

Table 3. Laboratory assays were conducted on a subset of vines within each block to confirm the agreement between visual assessments and virus status

Cultivar	Planting date	Visual rating of vines by category (number of vines)			Assay result by category: None, GLRaV-3, other GLRaV, GRBV (number of vines)			Agreement (%) between visual rating and lab results
		Negative	Positive (GLD)	Positive (GRB)	Negative	Positive (GLD)	Positive (GRB)	
Cabernet Franc	1994	12	7	1	12, 0, 0, 0	0, 7, 0, 0	0, 0, 0, 1	100
Cabernet Franc	2000	8	1	4	8, 0, 0, 0	0, 0, 0, 1	0, 0, 0, 4	92
Cabernet Franc	1994	5	3	0	5, 0, 0, 0	0, 3, 0, 0	0, 0, 0, 0	100
Cabernet Sauvignon	1996	16	20	5	16, 0, 0, 0	0, 19, 0, 1	0, 0, 0, 5	98
Cabernet Sauvignon	1945	8	9	0	8, 0, 0, 0	0, 9, 0, 0[a]	0, 0, 0, 0	100
Cabernet Sauvignon	1986	3	6	0	3, 0, 0, 0	1, 5, 0, 0	0, 0, 0, 0	89
Cabernet Sauvignon	1986	7	3	0	7, 0, 0, 0	0, 1, 1, 1	0, 0, 0, 0	80
Cabernet Sauvignon	1986	2	3	0	3, 0, 0, 0	0, 2, 0, 0	0, 0, 0, 0	100
Cabernet Sauvignon	1992	6	6	0	6, 0, 0, 0	0, 6, 0, 0	0, 0, 0, 0	100
Cabernet Sauvignon	1992	6	5	0	3, 1, 0, 2	0, 5, 0, 0[b]	0, 0, 0, 0	73
Cabernet Sauvignon	1992	11	16	3	11, 0, 0, 0	0, 15, 0, 1[c]	0, 1, 0, 2	93
Cabernet Sauvignon	2000	13	1	4	13, 0, 0, 0	0, 1, 0, 0	0, 0, 0, 4	100
Cabernet Sauvignon	1998	9	3	1	8, 0, 1, 0	0, 3, 0, 0	0, 0, 0, 1	92
Cabernet Sauvignon	1988	2	8	0	2, 0, 0, 0	1, 7, 0, 0	0, 0, 0, 0	90
Cabernet Sauvignon	1988	3	6	0	3, 0, 0, 0	0, 6, 0, 0	0, 0, 0, 0	100
Cabernet Sauvignon	1990	13	5	2	13, 0, 0, 0	0, 5, 0, 0	0, 0, 0, 2	100
Merlot	1986	1	4	0	1, 0, 0, 0	2, 2, 0, 0	0, 0, 0, 0	60
Merlot	1986	3	3	0	3, 0, 0, 0	1, 2, 0, 0	0, 0, 0, 0	83
Merlot	2006	22	2	6	22, 0, 0, 0	0, 2, 0, 0	0, 0, 0, 6	100
Zinfandel	1997	12	1	3	11, 0, 0, 1	1, 0, 0, 0	0, 0, 0, 3	94

Visual rating categories included negative (no symptoms of leafroll disease [GLD], positive [symptoms of GLD], and positive [symptoms of red blotch disease {GRB}]). Samples were assayed for all known grapevine leafroll-associated viruses, grapevine red blotch virus (2012 and 2013, only), and *Grapevine virus A, B*.

[a] One vine was co-infected with GLRaV-3 and GRBV, and eight vines marked as questionable were infected with GLRaV-2.

[b] Two vines were co-infected with GRBV.

[c] Seven vines were co-infected with GRBV.

Statistical Analyses

Mealybug and GLD monitoring data were analyzed with two complementary approaches. First, we used a regression tree at the block scale as a relatively flexible and robust way to estimate the effect of prior vector abundance and disease prevalence on the incidence of new disease. Next, we used a more mechanistic hierarchical modeling approach at the row scale that also captured variability among vineyard blocks and heterogeneity in incidence within a block.

Regression trees function by binary recursive partitioning of a dataset into mutually exclusive and exhaustive subsets (Crawley 2007). This involves identification of a threshold value for each of a set of explanatory variables to develop nested partitions that maximize within-partition homogeneity and between-partition heterogeneity of the dependent variable, while avoiding over fitting. At the block scale, we estimated effects of prior GLD (i.e., lagGLD), prior *Ps. maritimus* (grape mealybug; denoted as lagGMB), and year in a regression tree analysis, with the number of new GLD cases per block as the dependent variable. For this analysis, 20 vineyard blocks were used each with between 2 and 5 yr of surveys, translating to 58 block-year observations of effects on new cases of disease. The analysis was carried out using the CART v6.0 software (CART Classification and Regression Trees, Salford Systems, San Diego, CA). The standard relative cost metric, which trades off residual sum of squares of predictions based on the tree with the relative complexity of the tree, was used to select the optimal tree.

Our hierarchical model at the row scale consisted of a generalized linear mixed-effects model (GLMM) with Poisson error and multiple random effects to account appropriately for the repeated measurement of new GLD cases that occurred in each vineyard block (Pinheiro and Bates 2000). The model included fixed effects of year, prior GLD, *Ps. maritimus* abundance, and random effects of vineyard block, block by year, and row nested within blocks, and interaction between row within block and year. Model diagnostics to verify the goodness of fit included normality plots for the predicted random effects and coverage probability for model-based prediction intervals. For this analysis, the addition of within-block row observations translated to 699 total observations to draw upon for analysis, which was carried out with PROC GLIMMIX in SAS v9.0 software.

Results

At the block scale approximately 70% of blocks in a given year (41 of 58) had at least one new case of GLD, with a maximum of 98 new cases (mean ± SD = 11.88 ± 21.85). Over 80% of blocks in a given year (47/58) had *Ps. maritimus* present, with a maximum relative abundance of 157 (mean ± SD = 19.76 ± 31.55). At the row scale, nearly one-third of rows in a given year (227 of 699) had at least one new case of GLD, with a maximum of 31 new cases (mean ± SD = 0.99 ± 2.39). Nearly half of rows in a given year (337/699) had *Ps. maritimus*, with a maximum relative abundance of 25 (mean ± SD = 1.64 ± 2.83).

Visual assessments were in agreement with laboratory assays (Table 3; average: 92%; range: 60–100%). Other than GLRaV-3, only GLRaV-2 was found, and only in 2 of 20 blocks. This is not surprising, given that most blocks were prescreened for inclusion in the trial and at the time of the study, the leafroll disease complex in Napa Valley was dominated by GLRaV-3 (Sharma et al. 2011). Six blocks that were initially included in the study were subsequently removed when no GLRaV-3 was found (those blocks are now known to be infected with GRBV). Nine of the 20 study blocks were infected with GRBV, which had not been identified at the time the study was initiated. However, in most cases, assessors were able to visually distinguish between symptoms of GLRaV-3 and GRBV (Table 3). The general high degree of agreement between visual assessments and assay results are consistent with other studies and suggest that a highly trained visual assessor with molecular tools can identify diseased vines of red-fruited cultivars (Bell et al. 2017).

The optimal regression tree model identified in the analysis included five nonterminal nodes and six terminal partitions (Fig. 1). Three of the five nonterminal nodes hinged on threshold values of prior GLD, with terminal partitions depending on threshold values of prior disease, year, or mealybug abundance. The other two nonterminal nodes hinged on mealybug population or year. Overall, the model structure suggests that disease incidence depends on interactions between prior disease and both vector abundance and year. For example, vineyard block-years with high prior disease (lagGLD > 102) and high vector abundance (lagGMB > 16.5) had more than 4 times as many cases of new disease compared with blocks with high prior disease but lower (lagGMB ≤ 16.5) vector abundance (on average nearly 58 vs 13 cases, respectively). In the 11 block-years with intermediate prior disease (18.5 < lagGLD ≤ 102), incidence of GLD was moderate, averaging approximately 10 new cases, but differed among year groupings (i.e., approximately 5 new cases in 2010 and 2012 vs 13 in 2011 and 2013). Finally, for the 33 block-years with low prior disease (lagGLD ≤ 18.5), incidence of new GLD was low or very low depending on the prevalence of prior disease.

Results for the GLMM at the row scale showed significant effects of year ($F_{3,23}$ = 4.35, P = 0.014), lagGLD ($F_{1,135}$ = 21.58, P < 0.0001), and lagGMB ($F_{1,371}$ = 10.59, P < 0.0012). Overall, both vector abundance (Fig. 2) and prior disease (Fig. 3) were positively associated with disease incidence. Based on the estimated effect of lagGLD (mean ± SE: 0.038 ± 0.008), each additional diseased vine would increase disease incidence by approximately 4%, and based on the estimated effect of vector abundance (0.106 ± 0.032), each additional *Ps. maritimus*-infested vine leads to an approximately 11% increase in disease incidence. However, there was substantial variability in incidence among years, with relatively low incidence in 2010 and 2012 compared with 2011 and 2013 (Figs. 2 and 3). Additional variability is attributable to the random effects of block (estimate ± SE: 1.54 ± 0.71), block by year (0.44 ± 0.20), row nested within block (0.31 ± 0.10), and row nested within block by year (0.34 ± 0.09). Nearly 60% of variance in the estimated effect sizes was attributable to differences among blocks, whose deviations were evenly distributed from strong reductions in incidence to strong increases in

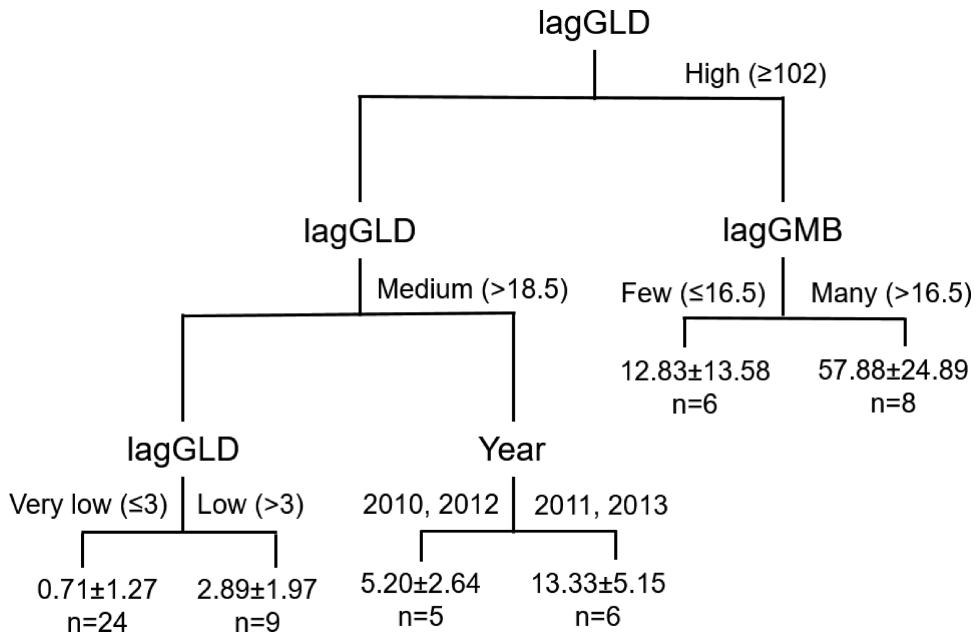

Fig. 1. Classification and regression tree analysis on the effects of prior leafroll disease (lagGLD), mealybug (*Pseudococcus maritimus)* population (lagGMB), and study year on the number of new leafroll disease cases. Variables at nonterminal nodes and the accompanying values to the left and right denote the thresholds that define the arrangement of partitions. Values listed in terminal partitions equate to the mean number of new leafroll disease cases (±SE) and number of block-year observations (*n*, out of 58 total) falling into each partition. Descriptors 'High' to 'Very low' and 'Many' versus 'Few' are intended to characterize broadly relative values for partitions with respect to prior leafroll disease and mealybug abundance, respectively. Partitions further to the left represent block-years for which prior leafroll disease was lower, which corresponded with fewer new diseases cases. For mealybug abundance, lower values (to the left) equated to fewer new cases of disease in some blocks, and the years 2010 and 2012 had fewer cases of new disease than 2011 and 2013 for some blocks.

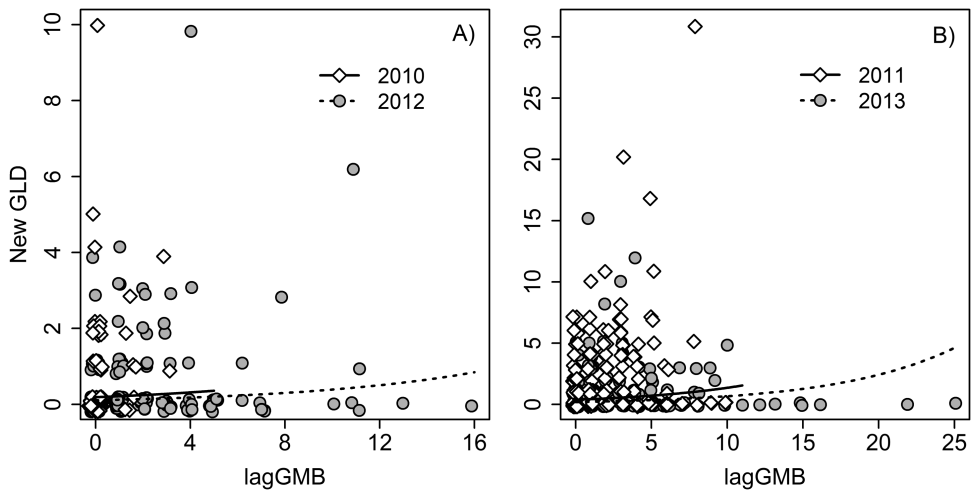

Fig. 2. Effect of mealybug (*Pseudococcus maritimus)* population (lagGMB) on the number of new leafroll disease cases (newGLD), for (A) 2010 and 2012 and (B) 2011 and 2013. Pairs of years were grouped together based on similarities identified in the regression tree analysis (Fig. 1). Lines denote fit of the generalized linear mixed-effects model.

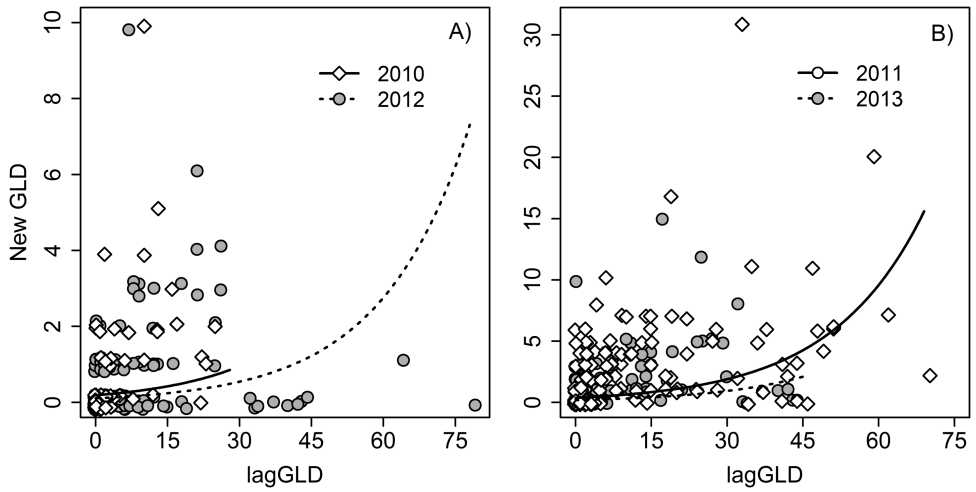

Fig. 3. Effect of prior leafroll disease prevalence (lagGLD) on the number of new leafroll disease cases (newGLD), for (A) 2010 and 2012 and (B) 2011 and 2013. Lines denote fit of the generalized linear mixed-effects model.

disease incidence (Fig. 4). The random effect of row nested within year by block, which reflects heterogeneity within a block, accounted for nearly 12% of the estimated variance, with the vast majority of rows showing little to no deviation but with some hotspots for incidence and a few areas with lower than average incidence (Fig. 5).

Discussion

Temporal and spatial patterns of disease have long been recognized as important elements of plant pathosystems (Vanderplank 1963) because of their potential to inform the development of efficient sampling plans and management strategies (Madden et al. 2007). Common strategies for the management of vector-borne diseases include the use of resistant host cultivars, vector control to reduce pathogen pressure, and removal of infected hosts (i.e., roguing) to reduce pathogen supply (Jeger et al. 2004, Madden et al. 2007). Here, we quantified incidence of an economically important grapevine disease and its vector in 20 vineyard blocks over multiple seasons to clarify the factors driving disease dynamics and identify useful management strategies. Our results indicate that disease incidence is tied primarily to incidence and abundance of pathogen supply in the prior season, secondarily to vector incidence in the prior season, along with substantial interannual, block-to-block, and within-block variability.

Three of five nonterminal nodes in the regression tree hinged on threshold values of prior GLD, suggesting the central epidemiological importance of this variable. This noted effect of pathogen supply suggests that secondary (i.e., vine-to-vine) spread of GLRaV-3 is epidemiologically significant and implies that removal of disease hosts may be a critical management tool (Sisterson and Stenger 2013, Sokolsky et al. 2013), particularly in blocks with low to moderate disease pressure. Analyses suggest an economically sustainable threshold for vine removal for leafroll disease to be 20 to 25% (Bell 2014, Ricketts et al. 2015). Given the importance of vine removal, efforts should focus on developing tools for rapid, accurate, and reliable detection of diseased vines (MacDonald et al. 2016).

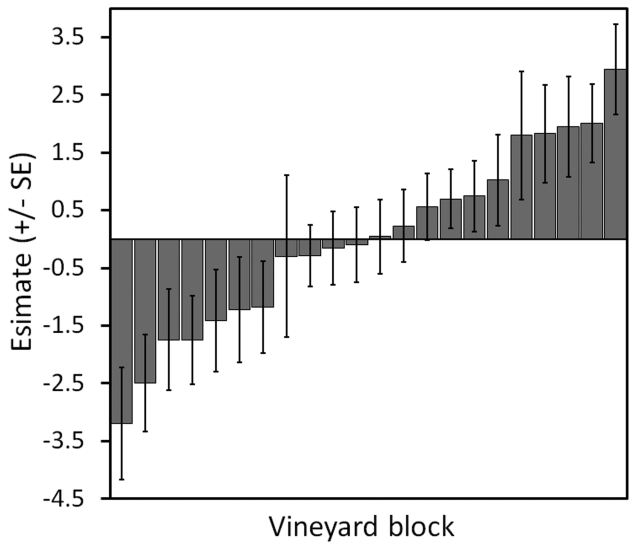

Fig. 4. Range of mean (±SE) deviations in new leafroll disease cases (newGLD) among blocks, which corresponds to the spatial variability in leafroll disease incidence among different vineyard blocks.

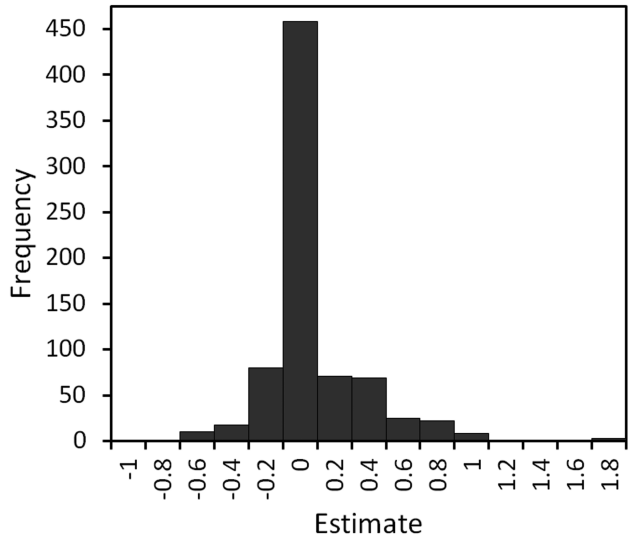

Fig. 5. Frequency histogram of observed deviations among vineyard rows nested within block by year, capturing fine-scale spatiotemporal heterogeneity in leafroll disease incidence.

Since factors in the previous year determine new disease in the current year, and there is a lag between inoculation and symptom development (Blaisdell et al. 2016), disease management programs implemented over multiple years have apparent benefits (Pietersen et al. 2013, Bell 2014). Similarly, the effectiveness of vine removal may in part depend on the disease management practices in surrounding blocks (e.g., primary spread—Purcell 1974). When the surrounding blocks are under the control of the same entity, a consistent management approach can be adopted across the area, whereas when the source is controlled by an outside party, a collaborative, regional approach to disease management that includes multiple partners may be needed to reduce the disease pressure across a larger scale (Sisterson and Stenger 2013).

Our results also suggest that vector pressure within a block may also be important within certain contexts. In vineyards with relatively low disease pressure, mealybug populations within the block contribute little to incidence of new disease. This suggests that relying solely on vector control tactics may not be sufficient to contain the spread of GLRaV-3, as demonstrated by Wallingford et al. (2015). And if vector abundance is low, even relatively efficient vector control measures may be economically impractical (Daugherty et al. 2015). However, in vineyards with greater disease pressure, vector management incorporating the use of insecticides that are compatible with biological control could be used to manage secondary spread.

Vector species may also be a factor. The vector in our study vineyards, *Ps. maritimus*, is generally under excellent biological control in northern California vineyards (Daane et al. 2008b), resulting in populations significantly smaller than what would be expected with other mealybug species, particularly *P. ficus* (Daane et al. 2012, Herrbach et al. 2017). This species has the potential to complete 5–12 generations per year under California conditions (Gutierrez et al. 2008), resulting in increased vector pressure that is likely to require management action (Pietersen et al. 2013). Further studies should explore the development of thresholds for vector populations as a component of disease management. This is complicated by the difficulty of sampling mealybugs, a cryptic pest often found underneath the bark (Daane et al. 2008b), but could be assisted by use of pheromone-baited traps (Walton et al. 2013).

Factors associated with vintage year and vineyard block are important sources of variability in annual rates of leafroll disease spread in vineyards in Napa, CA. Climatic factors such as temperature (average and extremes), relative humidity, and precipitation define a vintage year (Winkler et al. 1974). Vineyard blocks in this study varied by wine grape cultivar, vine age, management practices, and location. Fluctuations in year-to-year rates of disease spread may be influenced by practices adopted in other diseased blocks and/or may result from environmental factors affecting vector populations, with greater rates of spread following years with large mealybug populations (Charles et al. 2009).

In summary, our results suggest that a flexible approach based on disease incidence may be justified with respect to management of GLD. Regardless of the level of disease incidence, removal of diseased vines is a critical practice to limit the number of pathogen sources within the vineyard. In vineyards with low to moderate disease pressure, vine removal practices may be sufficient to control disease spread, whereas in vineyards with moderate to high disease pressure, vector management practices could also be implemented.

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