Environmental variables influencing the incidence of Pierce's disease

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Abstract

Background and Aims: Pierce's disease (PD) of cultivated grape, *Vitis vinifera*, caused by the bacterium *Xylella fastidiosa* (*Xf*) and transmitted by xylem-feeding insects, can lead to vine decline and death. Infection and expression of PD among or within vineyards vary, depending on known environmental variables, such as climate and *Xf* vegetative reservoirs, as well as some which are little studied with respect to PD, such as soil, water and nutrition.

Methods and Results: We collected data on over 30 environmental variables and used canonical correspondence analysis (CCA) and discriminant analysis (DA) to analyse their relationship to PD infection, sharpshooter density and *Xf* reservoir plants. The CCA found high positive correlation between PD and vineyard proximity to citrus orchards, and moderately positive correlation between PD and grape petiole Zn concentration and soil pH. The DA retained eight variables and predicted an increased risk of PD with higher cover of annual grasses, proximity to riparian habitat, increasing soil pH and increasing petiole Zn; PD risk decreased with higher perennial grass cover, higher latitude, increasing vine water stress and increasing soil cation exchange capacity.

Conclusions: We identified several novel variables which contributed to expression of PD and may improve the understanding of the role of environment in the disease triangle.

Significance of the Study: That the models include factors relating to soil and soil/plant relations suggest that these variables and their interactions play a role in constitutive or induced plant defenses to *Xf* and provides a basis for further study into management options for PD.

Keywords*: grape, sharpshooters,* Vitis vinifera, Xylella fastidiosa

Introduction

Pierce's disease (PD) is a disease of cultivated grape (*Vitis vinifera* L.) caused by the xylem dwelling bacterium *Xylella fastidiosa* (*Xf*). Xylem vessel blockage takes place as a result of proliferation of the bacteria and their subsequent masses, of secretion of biofilms, and of tyloses generated as part of vine defense response (Janse and Obradovic 2010), leading to water stress, decreased vigour, loss of productivity and often vine death (Hopkins and Purcell 2002). The bacteria can be transmitted by two groups of insects: the Cercopidae, and members of the Auchernorrhyncha, tribe Proconiini (Hemiptera : Cicadellidae), otherwise known as 'sharpshooters'. In California, sharpshooters that are known vectors of PD are the green [*Draeculacephala minerva* (Ball)], the red-headed [*Xyphon fulgida* (Nottingham)] and the blue-green [*Graphocephala atropunctata* (Signoret)], all of which are native to California, and the glassy-winged [*Homalodisca vitripennis* (Germar)], the origin of which is the south-eastern USA (Purcell and Saunders 1999a), and which is firmly established in southern California.

Pierce's disease has had several major regional outbreaks in California since the beginning of commercial grape production in the 1830s (Purcell and Frazier 1985, Tumbler et al. 2014). Chronic PD incidence occurs in California viticultural regions where conditions and resources are favourable (Hopkins and Purcell 2002). In Sonoma, Napa and Santa Cruz counties, incidence of the disease is generally limited to vines in proximity to riparian corridors (Purcell 1975), whereas in the San Joaquin Valley, vines adjacent to pastures or weedy alfalfa fields have shown the highest incidence of PD (Sisterson et al. 2010). In southern California, the proximity of vineyards to citrus groves led to large vineyard losses in the Temecula Valley of Riverside County in the late 1990s (University of California 2000).

Xylella fastidiosa has over 150 host plants (Janse and Obradovic 2010). Most of these are non-pathogenic, in which the presence of the bacterium leads to little or no disease development or recognisable symptoms (Hopkins and Purcell 2002). In pathogenic hosts, a systemic or localised buildup of the bacteria causes the symptomatic expression of PD and may eventually kill the plant, which is the case for *V. vinifera* (Janse and Obradovic 2010). Most PD infections from *G. atropunctata*, *D. minerva* and *X. fulgida* are a result of springtime feedings from the overwintering generation of adults, which feed on shoot tips (Feil et al. 2003). For these species, most vineyard PD symptoms occur within 100 m of the vineyard edge (Purcell 1974), because the sharpshooters do not move far from any *Xf* reservoir vegetation. In contrast, *H. vitripennis* has an extremely broad host range (Raju et al. 1983) and is quite mobile, flying up to 500 m at a time in search of a suitable host plant. *Graphocephala atropunctata* feeds on a range of landscaping and riparian plants (Purcell 1976), whereas grasses are the host plant preference for *D. minerva* and *X. fulgida* (Purcell and Frazier 1985). The presence of citrus orchards has been associated with a high density of *H. vitripennis* (Park et al. 2006), likely because citrus as well as many other evergreens have relatively high water status in all seasons.

Expression of PD among vineyards, or even among vines within a vineyard, varies considerably, even when the host grape, *Xf* and sharpshooter vectors are present in the same time and place, indicating that environmental variables influence expression of the disease. Climate plays a role, in that winter cold limits the northern extent of *Xf* and its sharpshooter vectors in the south-eastern USA (Hopkins and Purcell 2002). Meyer and Kirkpatrick (2008) found that in vitro cultures of *Xf* did not survive more than 24 h at −10°C. Johnson et al.

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(2006) found that the glassy-winged sharpshooter (GWSS) does not feed below 10°C and does not survive if held at this temperature for >15 days. Nutrition may also be a factor in PD incidence, in part because the form and concentration of nitrogen influence sharpshooter development (Brodbeck et al. 1999), and also because adult sharpshooters are sensitive to plant amino acid concentration (Bi et al. 2005). In addition, *Xf* is affected by the concentration of micronutrients, including calcium (Cruz et al. 2012), and Zn, copper, iron and boron (Darjean et al. 2000). Plant water stress is an important variable to sharpshooters because they have to feed against the tension of the xylem fluid. Anderson et al. (1992) found an exponential decrease in *H. vitripennis* feeding rate as a function of increasingly negative xylem tension. In contrast, some work has found associated intensity of scorch symptoms from *Xf* infection with increasing water stress (Hopkins 1985). McElrone et al. (2001) found that water stress in Virginia creeper (*Parthenocissus quinquefolia*, Vitaceae) increased the severity of *Xf* infection.

Our observations were that there are many viticultural areas in California that appeared to have all of the elements of the 'disease triangle'—a susceptible host (*V. vinifera*), the causal agent (*Xf*) and its vectors (sharpshooters) and suitable environmental conditions—but exhibited little PD expression. We therefore undertook field studies to understand better how the incidence of PD is impacted by these variables, and rank their relative influence. We collected data from eight commercial vineyards, located in northern and southern California, during the 2002 and 2003 growing seasons. Our assumption is that the environmental conditions monitored during this period were a reasonable representation of those during the life span of the vineyard.

Materials and methods

Vineyard field sites

Descriptions of the vineyard field sites are summarised in Table 1. Beringer was located in the Knight's Valley (Sonoma County, northern California) and was bisected by Redwood Creek, much of which has been disturbed by human activity, and consisted of a mixture of native riparian plants and naturalised vegetation. Cain was located at the upper altitudes of Spring Mountain (Napa Valley, northern California), which was not near a riparian corridor, but was surrounded by undisturbed oak woodland. There were two sites managed by the Callaway vineyards in the Temecula Valley (Riverside County, southern California). Callaway-citrus was bordered on the north by a large citrus orchard, and Callaway-non citrus was located >3 km from the nearest citrus orchard. The DeBerard vineyards in Rancho Cucamonga (San Bernardino County, southern California) were divided into DeBerard-irrigated, which was under drip irrigation, and DeBerard-non irrigated, which relied on water stored in the soil from winter rainfall, otherwise known as 'dry farming'. Both sites were surrounded by urban landscape. The Guadagni Brothers vineyard was located in the Dry Creek Valley (Sonoma County, northern California), and bordered Dry Creek, a riparian corridor which was fairly undisturbed. Finally, the Hofer vineyard in Ontario (San Bernardino County, southern California) was surrounded by urban landscape.

Nutrient analysis

We sampled soil from each of the field sites, taking five separate 500 cm^3 core samples, pooled from a depth of 0.5, 0.67 and 1 m. We estimated soil pH, electrical conductivity, exchangeable cations (calcium, magnesium, potassium and sodium), sulfate-sulfur, plant-available micronutrient metal cations (iron, manganese, copper, Zn, nickel and cobalt) and plant-available phosphorus, using methods described in Sparks et al. (1996). We took petiole samples at flowering time of each study year according to the protocol of Christensen et al. (1978); 60–80 petioles per site were analysed by a commercial laboratory (Dellavalle Laboratories, Fresno, CA, USA) for nitrate-nitrogen, phosphorus, potassium, Zn, manganese, sodium, boron, calcium, magnesium, iron and copper, using the methods of Gavlak et al. (2005).

Soil and vine water status

We monitored soil moisture continuously from May–October of each year using modified gypsum blocks (electrical resistance blocks) (Watermark, Irrometer, Riverside, CA, USA). We placed the sensors at a depth of 30 cm, 60 cm and 1 m within the vine

Table 1. Vineyard sites and associated variable codes used in the discriminant analysis and canonical correspondence analysis, and site descriptions with respect to surrounding vegetation, grape cultivar and soil texture.

Vineyard site (variable code)	Location	Surrounding vegetation	Grape cultivar	Soil texture
Guadagni Brothers (guad)	Dry Creek Valley, Sonoma County, Northern California (latitude 38.7°N)	Riparian	Merlot	Sandy clay
Cain Vineyard and Winery (cain)	Spring Mountain, Napa County, Northern California (latitude 38.5°N)	Oak woodland	Merlot	Sandy clay loam
Beringer (beri)	Knight's Valley, Sonoma County, Northern California (latitude 38.6°N)	Disturbed riparian	Cabernet Sauvignon	Sandy clay loam
Callaway Vineyard and Winery-citrus (callcitr)	Temecula Valley, Riverside County, Southern California (latitude 33.5°N)	Bordering citrus orchard	Merlot	Sandy clay loam
Callaway Vineyard and Winery-non citrus (callnocit)	Temecula Valley, Riverside County, Southern California (latitude 33.6°N)	No citrus border	Sauvignon Blanc	Sandy loam
Hofer Vineyard (hofer)	Ontario, San Bernardino County, Southern California (latitude 34.0°N)	Urban landscape	Grenache	Sand
DeBerard Vineyard-irrigated (debirr)	Rancho Cucamonga, San Bernardino County, Southern California (latitude 34.1°N)	Urban landscape	Zinfandel	Loamy sand
DeBerard Vineyard-non irrigated (debnoirr)	Rancho Cucamonga, San Bernardino County, Southern California (latitude 34.1°N)	Urban landscape	Zinfandel	Loamy sand

row and at least 1 m from an existing drip emitter and connected to a data logger (M.K. Hansen, Wenatchee, WA, USA).

We estimated vine water status with a pressure bomb (PMS, Corvallis, OR, USA), taking samples from ten randomly selected vines at each vineyard every 2 weeks between 1100 and 1400. Selecting a leaf in full sun, we placed a small plastic bag around it, then cut the petiole with a razor blade and immediately placed the leaf into the chamber. The units corresponding to negative xylem tension are in megaPascals (−MPa), but for purposes of the DA and CCA analyses, we entered the data as positive units (the variable H_2Ostress indicated increasing vine water stress).

Monitoring grape and non-grape vegetation for Xylella fastidiosa

We estimated incidence of *Xf* in two ways: (i) through visual observation of PD symptoms of individual grapevines in mid-season to late-season (July–September), scoring each vine as infected or not infected; and (ii) by polymerase chain reaction (PCR), by which all non-grape vegetation was tested for the presence of *Xf*, and a subsample of ten visually symptomatic grapevines was tested at each study site from September–November 2003. The primers were RST31/RST33, which do not distinguish among *Xf* strains (Minsavage et al. 1994). We took samples for PCR in May–June and again in August–September in each study year, from adjacent citrus groves, natural riparian and woodland habitats and from weedy vegetation within the vineyard. The PCR protocol of Minsavage et al. (1994) was applied to DNA isolated from plant tissue with the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), and the protocol of Voytas (2001) was used for agarose gel electrophoresis.

Sharpshooter monitoring

The density of the sharpshooter population was monitored with yellow sticky cards (178 x 101 mm, Seabright, Emeryville, CA, USA), placing them on the end posts of each sample vineyard, and in or near the closest alternative vegetation (riparian zones or urban landscaping). The cards were placed at equal distant intervals (approximately 15 m) and changed every 2 weeks; the sharpshooter counts were recorded per location.

Climate

Climatic data were gathered with the California Irrigation Management Information System, National Climatic Data Center and the University of California Integrated Pest Management TouchTone and PestCast databases. The data collected were: (i) average seasonal high temperature (1 April–31 October for 2002 and 2003); (ii) average summer high temperature (1 June–31 August for 2002 and 2003); (iii) average seasonal low temperature (1 April–31 October for 2002 and 2003); (iv) average winter low temperature (1 December–28 February for 2001/02 and 2002/03); (v) rainfall total (1 October–30 April for 2001/02 and 2002/03); and (vi) cumulative degree days, 10°C base temperature (1 April–31 October for 2002 and 2003).

Statistical analysis

Plant and sharpshooter species and abiotic factors that might be associated with the incidence of PD at a given vineyard site over the 2-year study period were assessed with CCA (CANOCO, Ter Braak and Šmilauer 2002) and DA ([PROC DISCRIM and PROC STEPDISC (SAS for Windows Release 9.4, SAS Institute, Cary, NC, USA)] (Der and Everitt 2002). The plant species data were a compilation of up to 20 1 x 1 m quadrats placed randomly at each of the sites, and the proportion of cover by plant species was estimated visually. For exploratory purposes CCA was utilised to reveal underlying community composition that could provide important, easy to measure indicators for PD incidence and associated environmental variables. By comparison, DA was intended to quantify the effects attributable to species and environmental variables that made a site more or less likely to become infected with PD.

The environmental variables used for analysis are described in Table 2. To account for multicollinearity issues, a correlation matrix was generated (PROC CORR, SAS Institute) and when two environmental variables were significantly correlated, one was eliminated based on the reliability of the measurement. Site variables were categorical, and therefore depicted on the bi-plot as centroids. All data were log ten (x + 1) transformed.

The best discriminant function was developed with stepwise DA (PROC STEPDISC, SAS Institute), to distinguish PD incidence among vineyard sites (Der and Everitt 2002). A positive threshold PD incidence at each site was arbitrarily set to greater than 5.5%, and the level of significance for retaining variables at each step was set to $P < 0.15$. A discriminant function was generated (PROC DISCRIM, SAS Institute), from which a z score, z(i), was computed for each site, i, by

$$z(i) = -z_{critical} + \sum_{j=1}^{n} a_j x_j \quad (1)$$

where a_j is the discriminant coefficient for variable j, and x_j is the observed value for variable j. The critical z value, $z_{critical}$ was computed as the mean of the average z score for all sites where PD was absent, and the average z score for all sites where PD incidence was at least as high as the 5.5% threshold. A compounded z score less than 0 would be indicative of an increased potential for grapes to be infected with PD. The exact F tests used to assess the overall model of variables (sharpshooter species and plant functional groups) selected in the stepwise DA were Wilks' Lambda, Pillai's Trace, Hotelling–Lawley Trace, and Roy's Greatest Root. The main assumption with this approach is that vineyard sites without PD incidence had potential exposure to infection by *X. fastidosa*, but the biotic and abiotic conditions were not suitable for PD expression. The alternative is that sites not expressing PD could become symptomatic, given enough exposure time.

A CCA biplot depicting sites, species (sharpshooters or plant functional groups) scores and environmental variables was generated for species abundance relation to PD incidence (grapePD) and how the other environmental variables related to each other and grapePD. A Monte Carlo permutation test was run within CANOCO to construct a model containing only the environmental variables that explained a significant portion ($P < 0.05$) of species variation. The CCA and the corresponding biplot are exploratory, an attempt to tease apart a complex, multidimensional system, one which could be explained by each independent variable as a dimension. The point of the analysis is to reveal the pertinent interrelations that assist in explaining why species occur where they occur, and which variables are encouraging or discouraging them, as a well as which variables do not have any measurable effect.

The plant species were combined into functional groups because many species were unique to a vineyard site, which resulted in a high number of zero values for the other sites, and using unique species in the analysis would have created a highly nested situation statistically. Plant functional groups were origin (California native or non-native), life cycle (annual

Table 2. Environmental variable categories, categorical or continuous variable descriptions and associated variable codes used in discriminant analysis and canonical correspondence analysis. Only variables that were found to have significant contribution to the analyses are described.

Environmental variable	Variable code	Description
Urban landscape	urban	Estimate of vineyard proximity to urban development combined with the degree of landscaping vegetation in the surrounding neighborhoods: 5, within 1 km and a high degree of urban development; 0, greater than 5 km and a low degree of urban development.
Citrus orchards	citrus	Estimate of vineyard proximity to citrus orchards: 5, within 1 km; 0, greater than 5 km.
Riparian vegetation	riparian	Estimate of vineyard proximity to riparian vegetation: 5, within 1 km; 0, greater than 5 km.
Maximum summer temperature	tmaxsum	Average maximum temperature (°C) for summer days (defined as 1 June–30 August).
Rainfall	rain	Seasonal rainfall (mm) (defined as 1 October prior to each growing season to 30 April of the current growing season).
Latitude	latit	Latitude based on Geographical Information Systems coordinates at center of each vineyard site.
Pierce's disease	grapePD	PD frequency in grapevines (% of symptomatic vines).
Xf in non-grape vegetation	non-grape*Xf*	Frequency of *Xylela fastidiosa* in non-grape vegetation (% weeds or surrounding plants infected).
Soil pH	soilpH	Soil pH
Soil CEC	CEC	Soil cation exchange capacity.
Grape water stress	H_2Ostress	Degree of grapevine water stress, based on leaf water potential.
Grape petiole zinc (Zn)	petZn	Grapevine petiole Zn concentration.

or perennial) and classification (grass or broadleaf). *Cyperus* spp., though not true grasses, were categorised as such for simplicity of the functional group.

The CCA biplot arrows (eigenvectors) point in the direction of maximum change for a given variable across the biplot diagram, and the length of the arrows is proportional to the rate of change. Environmental variables that are longer are more correlated with the CCA ordination axis and thus explain species distribution across that axis. All correlations represented were extracted from the correlation matrix weighted by sample totals (Ter Braak and Šmilauer 2002). To interpret the CCA biplot for each of the species (sharpshooters and plant functional groups), the biplot score for the species (open triangles) is projected perpendicularly (this is the ranking) to the eigenvector of the environmental variable or its extension from the axis origin (0, 0). The order of each of these species projections on an eigenvector corresponds approximately to the ranking by abundance of the species along a gradient of the environmental variable, with the highest ranking closest to the arrowhead of the eigenvector.

Results and discussion

The incidence of visually symptomatic vines for PD is presented in Table 3. The study sites with the highest incidence were Callaway-citrus (37.1%), Callaway-non citrus (26.2%), Guadagni (23.7%) and DeBerard-irrigated (25.8%), and those with the lowest incidence were Cain and Beringer (0.02%), DeBerard-non irrigated (0.2%) and Hofer (5.5%). Visual incidence was confirmed using PCR on a subset of the 2003 samples, and all tested positive for presence of *Xf*.

The significant effects of CCA did not depend on year, which was not selected in the Monte Carlo permutation. The first two axes of the CCA biplot of species–environmental variables explained 79.8% of the variance in the species data and 81.8% of the variance in the weighted averages and class totals of the species with respect to the environmental variables over the 2002 and 2003 assessment years (Figure 1). The latitude variable was consistently correlated with the pattern of community variation based on its eigenvector length on the first two CCA axes ($r_{latitude\ :\ CCA1} = 0.887$ and $r_{latitude\ :\ CCA2} = 0.119$) and the Monte Carlo permutation test ($P = 0.001$). Because the first two CCA axes account for the most variation in species cover, the pattern reflects a mostly southern to northern California trend, with most of the higher incidence PD sites at the southern latitudes. The riparian and rainfall variables were highly correlated with latitude ($r_{latitude\ :\ riparian} = 0.824$ and $r_{latitude\ :\ rain} = 0.935$), and the riparian and latitude variables were inversely correlated with the urban variable ($r_{riparian\ :\ urban} = -0.932$ and $r_{latitude\ :\ urban} = -0.782$) (Figure 1). This reflects the facts that most of the southern California sites were nearer to human populated areas, while those in northern California were nearer to natural vegetated areas along waterways, and that the annual rainfall total was higher in northern than that in southern California.

Table 3. Visually symptomatic incidence of Pierce's disease at the eight vineyard study sites. Vine counts and the number of symptomatic vines are totaled from autumn sampling (September–November) in 2002 and 2003.

Vineyard	Total vines	Symptomatic vines	Incidence (%)
Guadagni	500	118	23.7
Cain	500	1	0.02
Beringer	500	1	0.02
Callaway-citrus	1179	437	37.1
Callaway-non citrus	1448	379	26.2
Hofer	6056	333	5.5
DeBerard-irrigated	4021	1037	25.8
DeBerard-non irrigated	3192	6	0.2

The CCA analysis did not find that PD incidence (Table 3) and sharpshooter counts (Table 4) were significantly correlated (Figure 1, grapePD with shrpshtr, glassywn, redhead or bluegrn), which is surprising given that sharpshooters are the primary vector group for transmission of *Xf* to grape. The likely

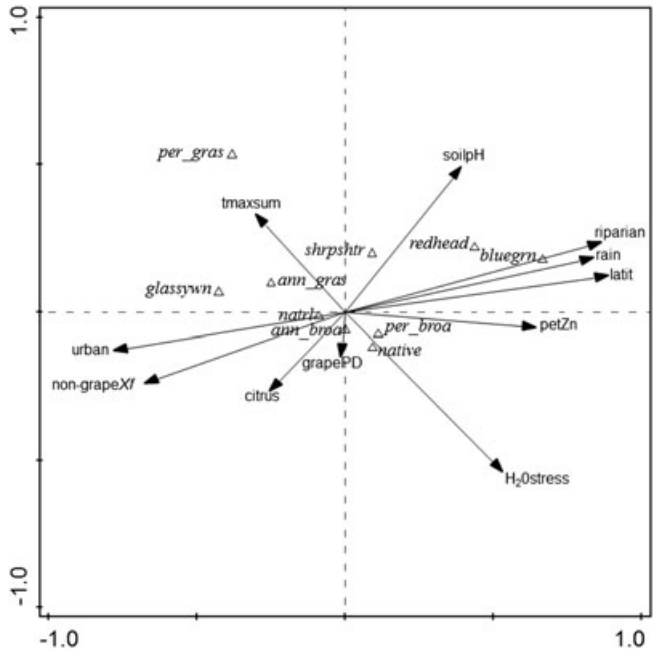

Figure 1. Canonical correspondence analysis biplot of species (Δ), compiled as functional plant groups and sharpshooters and environmental variables [(▲) attached to arrows (the environmental eigenvectors)]. The environmental variables were selected based on Monte Carlo permutation tests (conditional effects threshold for selection $P < 0.05$).

confounding factor here was the area-wide insecticide treatment for *H. vitripennis* in the Temecula Valley, which began in 2001 and lowered the GWSS population at the Callaway vineyards far beyond what would be expected from these high PD incidence sites. The citrus variable, however, showed a high positive correlation with PD (Figure 1, $r_{grapePD\ :\ citrus} = 0.719$), reflecting a relationship which has been recognised for many years (Perring et al. 2001) and is the basis for the area-wide insecticide treatment programs against GWSS in the Temecula Valley and parts of the San Joaquin Valley where citrus orchards and vineyards are in close proximity (Sisterson et al. 2008). The CCA showed PD as moderately positively correlated with petiole Zn concentration (petZn) (Figure 1, $r_{grapePD\ :\ petZn} = 0.484$) and soil pH (Figure 1, $r_{grapePD\ :\ soilpH} = 0.303$). To our knowledge, a field correlation between PD incidence and these two variables has not been previously reported. Attempts to inhibit reproduction of *Xf* in vitro using Zn (Darjean et al. 2000) and iron (Toney and Koh 2006) have been attempted in laboratory studies but were inconclusive.

The non-grape plants that tested positive for *Xf* and were included in the CCA are shown in Table 5. The frequency of non-grape vegetation with positive tests for *Xf* (nongrape*Xf*) and grape PD are essentially uncorrelated (Figure 1, $r_{non\text{-}grapeXf\ :\ grapePD} = -0.044$), indicating that merely because *Xf* is present in surrounding vegetation does not necessarily mean that it will be expressed in the adjacent vineyard, either because the strain is not pathogenic to grape, or that there are other factors in the pathogen triangle (e.g. host, disease and environment) and disease transmission process (e.g. vector) that affect pathogenicity.

GrapePD is a major explanatory variable in the third dimension of the CCA analysis ($r_{grapePD\ :\ CCA3} = -0.686$) and some of the same patterns are retained, such as a strong correlation with citrus ($r_{citrus\ :\ CCA3} = -0.561$) but less so with petiole Zn ($r_{petZn\ :\ CCA3} = -0.244$) and not with soil pH ($r_{soilpH\ :\ CCA3} = 0.065$). All other variables had a low correlation with grapePD, not exceeding 0.10.

Data from weather variables that contributed to the CCA are presented in Table 6. The average maximum temperature for summer days (tmaxsum) and grape leaf water stress (H_2Ostress) were inversely correlated ($r_{tmaxsum\ :\ H2Ostress} = -0.451$), and the perennial grasses functional group (per_gras) correlates closest with the maximum summer temperature variable (tmaxsum), followed by the GWSS variable (glassywn) and the annual grass functional group (ann_gras) (Figure 1). No cold temperature variables (data not shown) correlated with grapePD, indicating that winters are not sufficiently severe at our field sites to impact *Xf*.

The stepwise DA found eight variables that could be used to predict the potential for grapevines to be symptomatic of PD with a significant ($P = 0.021$) multivariate exact F test (Table 7). Two of these were soil variables (CEC and soil pH), two were grapevine variables (vine water stress and petiole Zn), two were plant functional groups (annual grasses and perennial grasses) and two were location variables (latitude and riparian). Variables with a negative coefficient increase the predicted risk of PD, in contrast to those with positive coefficients (Table 7). Therefore, in this model, increased annual grass

Table 4. Sharpshooter species (scientific and common names) and associated codes for the canonical correspondence analysis biplot, counted on yellow sticky traps placed at the periphery of the vineyard sites.

Sharpshooter species	Common name	Code for CCA biplot	Total count from sticky cards, 2002/03							
			Guadagni	Cain	Beringer	Callaway-citrus	Callaway-non citrus	Hofer	DeBerard-irrigated	DeBerard-non irrigated
Homalodisca vitripennis	Glassy-winged sharpshooter	glassywn	0	0	0	3	0	62	16	14
Xyphon fulgida	Read-headed sharpshooter	redhead	100	0	0	0	4	0	0	0
Graphocephala atropunctata	Blue-green sharpshooter	bluegrn	138	0	46	0	0	0	0	0
	Total sharpshooters	shrpshtr	238	0	46	3	4	62	16	14

CCA, canonical correspondence analysis.

Table 5. Plant species at the field sites which tested positive for *Xylella fastidiosa*, including scientific and common names, functional groups (origin, life cycle and classification) and associated codes for the canonical correspondence analysis (CCA) biplot. Functional groups enabled unique species to be part of the discriminant analysis and CCA. *Cyperus* spp., although not true grasses, were classified as such for simplicity of the functional group.

Plant species		Functional group (codes for CCA biplot)		
Scientific name	**Common name**	**Origin with respect to California (native, natrl)**	**Life cycle (ann, per)**	**Classification (gras, broa)**
Conyza canadensis	Horseweed	Native	Annual	Broadleaf
Erodium spp.	Filaree	Naturalised	Annual	Broadleaf
Brassica spp.	Mustard	Naturalised	Annual	Broadleaf
Eremocarpus setigerus	Turkey mullein	Native	Annual	Broadleaf
Heterotheca grandiflora	Telegraph weed	Native	Annual	Broadleaf
Salsola tragus	Russian thistle	Naturalised	Annual	Broadleaf
Sonchus spp.	Sowthistle	Naturalised	Annual	Broadleaf
Chenopodium murale	Common Lamb's quarters	Naturalised	Annual	Broadleaf
Amaranthus spp	Pigweed	Naturalised	Annual	Broadleaf
Portulaca oleracea	Common Purslane	Naturalised	Annual	Broadleaf
Hordeum murinum ssp. *Leporinum*	Foxtail barley	Naturalised	Annual	Grass
Bromus spp.	Brome grass	Naturalised	Annual	Grass
Echinochloa grus-galli	Barnyard grass	Naturalised	Annual	Grass
Rubus ursinus	California Blackberry	Native	Perennial	Broadleaf
Vitis californica	California Wild grape	Native	Perennial	Broadleaf
Salix spp.	Willow	Native	Perennial	Broadleaf
Eriogonum spp.	Buckwheat	Native	Perennial	Broadleaf
Atriplex spp.	Saltbush	Native	Perennial	Broadleaf
Eucalyptus spp.	Eucalyptus	Naturalised	Perennial	Broadleaf
Arctostaphylus spp.	Manzanita	Native	Perennial	Broadleaf
Quercus agrifolia	Oak	Native	Perennial	Broadleaf
Cyperus spp.	Nutsedge	Naturalised	Perennial	Grass
Cynodon dactylon	Bermudagrass	Naturalised	Perennial	Grass

The number of samples tested for each species varied from 1 to 16, and the samples testing positive varied from 75 to 100%.

Table 6. Variables which contributed to the canonical correspondence analysis or discriminant analysis, averaged from 2002/03, from the eight vineyard study sites.

Vineyard	Soil CEC (meq/L)	Soil pH	Petiole Zn (mg/kg)	Vine leaf water potential (MPa)†	Average summer high temperature (1 June–31 August) (°C)	Rainfall total (1 October–30 April) (mm)
Guadagni	21.4	7.7	88.5	−1.33	32.8	1101.5
Cain	11.2	6.6	—	−1.53	30.1	981.9
Beringer	13.9	6.4	33.5	−1.38	32.1	1217.1
Callaway-citrus	14.9	6.8	66.5	−1.39	30.9	263.9
Callaway-non citrus	13.4	6.8	—	−1.22	30.8	187.1
Hofer	17.3	7.2	36	−1.05	34.4	271.5
DeBerard-irrigated	4.1	5.7	42	−1.19	34.4	271.5
DeBerard-nonirrigated	3.4	5.2	48.5	−1.34	34.4	271.5

†More negative vine leaf water potential values (MPa) indicate higher water tension. CEC, cation exchange capacity.

cover heightens the predicted risk of PD, as does proximity to riparian habitat, increasing soil pH and increasing petiole Zn. The variables which reduce the predicted risk of PD are perennial grasses [although the predictive power of perennial grasses may not be significant, given the high *P*-value ($P = 0.12$, Table 7)], higher latitude, higher vine water stress and higher soil cation exchange capacity. The misclassification rate using the model, that is classifying one of our sites as PD infected when it indeed was not, by the re-substitution and cross-validation methods was 0.0 and 18%, respectively (Der and Everitt 2002). The CEC and soil pH, annual and perennial grass and vine water stress were the most significant predictors of

Table 7. Discriminant analysis results using variables selected from forward selection in PROC STEPDISC. Variable descriptions can be found in Table 2.

Variable and code(x_j)	Coefficient (a_j)	*P*-value
Annual grasses (ann_gras)	−0.8753	0.038
Perennial grasses (per_gras)	8.81211	0.120
Latitude (latit)	5.65632	0.110
Riparian (riparian)	−3.2933	0.110
Vine water stress (H_2O stress)	3.85447	0.037
Soil pH (pH)	−11.85806	0.046
Cation exchange capacity (CEC)	0.54514	0.003
Petiole zinc concentration (petZn)	−0.0222	0.051

The coefficients can be used to compute a *z* score for a new unknown location to predict its potential to have grapes infected with Pierce's Disease (PD). Using Equation 1 where the $z_{critical} = -180.00$, the values observed for each variable (j) measured at the location, are inputted. A compounded *z* score less than 0 would be indicative of an increased potential for grapes to be infected with PD. Any variable with a negative coefficient is indicative of an increased potential for infection as the value of that variable increases.

grape PD incidence based on the order and retention of variable selection during the stepwise DA. For Equation 1, the critical *z* value was found to be −180.00 (Table 7).

Of the two soil characteristics selected in the DA, CEC was highest at Guadagni at 21.4 meq/L, and lowest at the DeBerard-irrigated and DeBerard-non irrigated sites, with a CEC of 4.1 and 3.4 meq/L, respectively (Table 6). It is generally accepted that the optimal range in soil pH for grape cultivation is 5.5–8.0 (Lanyon et al. 2004); one of our sites was slightly basic (Guadagni at pH 7.7), one was slightly acidic (DeBerard-irrigated at pH 5.7) and one was out of range and acidic (DeBerard-non irrigated at pH 5.2) (Table 6). Petiole Zn was at adequate concentration, >26 mg/kg (Christensen et al. 1978) at all sites, the lowest of which were Beringer and Hofer, averaging 33.5 and 36 mg/kg, respectively, with Guadagni representing the highest average, 88.5 mg/kg, of all the study sites (Table 6). Using a midday leaf water potential threshold of −1.4 MPa to indicate high water stress of grapevines (Prichard et al. 2004), the Cain vineyard, with the most negative average seasonal water potential of the study sites (−1.53 MPa), was also the only one in the high vine water stress category (Table 6). Calloway-citrus, Beringer, DeBerard-non irrigated and Guadagni, however, were close behind at −1.39, −1.38, −1.34, and −1.33 MPa, respectively. The Hofer site had the least negative average water potential (lowest degree of water stress) at a seasonal average of −1.05 MPa.

It is the DA that allows us to take a broader view of the variables and the interactions among them that contribute to PD. Within our model, two of the variables have a fairly simple explanation, those being latitude and riparian. The positive association of the latitude variable with PD incidence is reflective of our study sites, because three of the high PD-incidence vineyards were in southern California (Callaway-citrus, Callaway-non citrus and DeBerard-irrigated), whereas only one was in northern California (Guadagni) (Table 1). Vineyard proximity to riparian areas and its relationship to higher PD risk is a relationship that is well known (Purcell and Saunders 1999b), because the riparian zone is a preferred habitat of *G. atropunctata*, which takes advantage of the relatively high water status of plants there throughout the year (Purcell 1975). The riparian zone is also prime habitat for many host plant reservoirs of *Xf* (Baumgartner and Warren 2005).

Why the six other variables were included in the model is less certain. It is not clear why the annual grass variable would increase the risk of PD and the perennial grass variable would reduce the risk, given that all grasses tested in this study were positive for *Xf*. In addition, the CCA found no correlation between grape PD and the incidence of *Xf* in alternative vegetation. An explanation might be in vector efficiency; it is possible that sharpshooter transmission of *Xf* from the annual grasses in our data set (*Hordeum murinum*, *Bromus* spp. and *Echinochloa grus-galli*) is more efficient than from the perennial 'grasses' (*Cynodon dactylon*, *Cyperus* spp.) (Table 5). This may be partly due to the phenology of these plants, in that *H. murinum* and *Bromus* spp. are winter annuals and biologically active in the spring, when there is a higher frequency of *Xf* establishment in grapevines via sharpshooter transmission. In contrast, *C. dactylon* remains dormant until spring, and likely does not contribute much to springtime grapevine infections. In a study on the *Xf* transmission efficiency by sharpshooters, Hill and Purcell (1997) found no transmission of *Xf* from *C. dactylon* by *G. atropunctata* or *D. minerva*. The sharpshooter species in these studies that prefers to feed on grasses is *X. fulgida*, which has not been studied as to its preference for the annual or perennial grasses. The lowered risk of PD because of higher vine water stress may not be attributable to its effect on *Xf* alone, as some studies have shown that water stress exacerbates the negative impacts of *Xf* on grapevines (Choat et al. 2009). Choi et al. (2013), however, found 138 *Xf*-induced genes that were transcribed when *V. vinifera* vines were subjected to *Xf* infection and water stress, indicating that a plant defense response to *Xf* is triggered and accentuated by drought. The influence of water stress might also be explained by the effect on sharpshooters, again because these insects prefer plants with high water status. As was found in the CCA, increasing petiole Zn concentration and increasing soil pH, are DA variables which resulted in higher predicted risk for PD. It is possible that within the acceptable range of Zn concentration for optimal vine growth, a concentration at the low end (again, in combination with the other variables in the model), may be less favourable for *Xf* establishment or growth. Similarly, Toney and Koh (2006) found that Fe can be a limiting element in allowing *Xf* to form the extracellular matrix within which it exists. Although a comparable role for Zn has not been established, it is possible that Zn could be a limiting factor for *Xf*. Why the soil pH plays a significant role in the model is unclear, although the comparison between the DeBerard sites is noteworthy. Among the eight variables in the model, these two sites differed only in water stress, petiole Zn and soil pH. Although both DeBerard sites had acidic soils, the slightly lower soil pH at the DeBerard-non irrigated vineyard (Table 6), in conjunction with overall lower leaf water potential (Table 6) and notwithstanding the slightly higher petiole Zn (Table 6), was enough to decrease PD incidence substantially (Table 3). These findings highlight the complexity of the interactions among these environmental variables and provide a basis for further study into management options for PD.

The finding from our model that variables relating to soil (CEC and pH) and soil/plant relations (water stress and Zn concentration) influence PD incidence is of interest and suggests that these variables may play a role in constitutive or induced plant defenses to *Xf*. Many studies have shown that abiotic factors, such as drought, salinity and plant/soil microbe interactions, can affect the production of plant defense chemicals such as phenolic substances or defense proteins (Santino et al. 2013). Sun et al. (2011) found that grape genotypes varying in susceptibility to *Xf* differed in the composition and structure of polysaccharides composing the primary cell walls of xylem vessels at the intervessel pit junction, which are potentially degraded by cell wall digestive enzymes from *Xf*. Basha et al.

(2010) identified several plant defense proteins that were present in PD-tolerant grapevines (*V. rotundifolia* and *V. vinifera* hybrids) but absent in *V. vinifera*. Maddox et al. (2010) found that some phenolic substances, which are found at relatively high concentration in *V. vinifera*, had anti-*Xf* properties in vitro. Field studies, however, on the interaction of environmental variables on xylem primary cell wall structure, phenolic substances or defense proteins, with respect to PD, have not been conducted on grape. The possibility that the genes for grape constitutive or induced defenses might be up-regulated or down-regulated with respect to the interaction of soil pH, CEC, plant water stress and petiole Zn concentration, is intriguing, and could be the basis for further study.

Conclusions

In addition to variables, such as latitude (significant in this study because most of our high PD-incidence study sites were in southern California) and vineyard proximity to riparian corridors and citrus orchards (known to influence PD because of habitat for sharpshooters and *Xf* reservoir plants), we identify several novel variables which contributed to the expression of PD in this study and may allow us to more fully understand the role of environment in the disease triangle. These include soil pH and grapevine petiole Zn, which, from the CCA, showed a moderately positive correlation with PD incidence at our study sites. These two variables were also selected in the DA model, which can be used to predict the potential for grapevines to be infected with PD. The model showed increased predicted risk of PD with proximity to riparian habitat and higher cover of annual grasses, as well as increased soil pH and petiole Zn. Risk of PD is reduced with higher latitude, higher cover of perennial grasses, increased vine water stress and increased soil CEC. These findings provide a basis for further study in understanding the contribution of these variables to PD incidence, as well as their interactions, and the role that they may play in grapevine defense responses to *Xf*. We consider that these results and interpretations will provide insight to other investigators who might take a more reductionist approach into specific mechanisms involved in expression of PD.

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