Vector Preference and Inoculation Efficiency as Components of Resistance to Pierce's Disease in European Grape Cultivars

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I thank Allan Finlay and John Sorensen for assistance, and cooperating growers for providing field plots. Peter Rauch provided help with computer graphics. This work was supported in part by the Napa Valley Viticultural Research Fund, the California Table Grape Commission, and Minnehoma Land and Farming Company.

Accepted for publication 12 September 1980.

ABSTRACT

Purcell, A. H. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. Phytopathology 71:429-435.

Feeding preference and inoculation efficiency of the leafhopper, *Graphocephala atropunctata* (the blue-green sharpshooter) were evaluated as factors affecting the rate of natural spread of Pierce's disease (PD) in European grape (*Vitis vinifera*) cultivars in California. Significant feeding preferences for certain cultivars were observed in field counts of sharpshooters and in cage trials, but preference was not correlated with resistance. In young potted grapevines no major differences were noted in the resistance of cultivars to infection following exposure to feeding by infectious leafhoppers. Older foliage of cultivars Sylvaner, Cabernet Sauvignon, Chenin blanc, Thompson Seedless, Petit sirah, and Ruby Cabernet was more resistant to infection than was young foliage, but this was not true for cultivar Pinot noir. Older foliage of Flora, Chardonnay, and Mission also was more resistant to infection than was younger foliage in greenhouse tests. Exposure of mature vines growing in the field to leafhopper vectors produced infections that spread much less rapidly from the point of inoculation in some cultivars than in others. In some cultivars fewer infections persisted through the following dormant season than in others. Inoculations by leafhoppers in April through June produced more persistent infections in all cultivars than did inoculations made in July or early August. A theoretical model of the probability of infection using conservative estimates of vector density, infectivity, and transmission efficiency predicted much higher infection levels than those commonly observed under the assumed conditions. Adjusting predicted disease incidence for the persistence of infections through the dormant season as a function of inoculation date produced more realistic rates of disease spread and differences in PD incidence among European grape cultivars.

Additional key words: almond leaf scorch, alfalfa dwarf, rickettsialike organism, epidemiology, mathematical model, resistance.

Pierce's disease (PD) is a serious disorder of grapevines (*Vitis vinifera* L.) caused by bacteria (1) transmitted by xylem-feeding leafhoppers and spittlebugs (3,11). In California, PD is most severe near leafhopper vector source areas such as weedy alfalfa fields, pastures, or stream banks (3,11). In the southeastern United States, PD is a major factor precluding the successful culture of *V. vinifera* (5).

Although all *V. vinifera* cultivars are susceptible to PD, relative differences in resistance or tolerance have been noted (3,7,8). In California, cultivars Pinot noir, Chardonnay, and Flora have been observed to have a higher incidence of PD and to develop more severe symptoms than cultivars White Riesling, Chenin blanc, or Petit sirah (8). Tracy (17) evaluated vector preference among grape cultivars and concluded that, although the principal vector species in coastal California, the blue-green sharpshooter (*Graphocephala atropunctata* (Signoret) (= *Hordnia circellata* [19]), definitely preferred feeding upon some cultivars over others, such preferential feeding was not a significant factor in resistance.

In this investigation I also studied differences in feeding preference of the blue-green sharpshooter along with foliage age, and susceptibility to infection by vector inoculation as possible causes of differences in PD incidence that are observed among grape cultivars and attempted to directly test the hypothesis (9) that the date of inoculation would significantly influence successful infection. A theoretical model was constructed to assess the relative contributions to PD spread of vector density, cultivar susceptibility to infection by vector feeding, and date of inoculation.

MATERIALS AND METHODS

Vector preference. Preference was evaluated in field samples and in observations of caged insects. In the field, comparisons were made between grape cultivars in adjacent plantings of similar age along transects perpendicular to a vector source area such as a wooded stream bank or a weedy vineyard margin. Adults and nymphs of *G. atropunctata* on 20 randomly selected canes or on all the canes of a single vine (9) were carefully counted along the distal 40 cm of each cane tip, which is the preferred feeding site on grape (9). Eight to 15 samples were taken on each sampling date from each cultivar plot and repeated for several years in late spring in the same locations in the Napa Valley. Each autumn, the incidence of PD in these plots was mapped as described previously (8).

In cage experiments to evaluate varietal feeding preferences, 35 to 80 field-collected adult *G. atropunctata* were released into a cubical cage (.75 m^3) containing four potted vines, each of a different cultivar. During subsequent inspections, each plant was carefully lifted and the number of leafhoppers upon it was recorded. Dead leafhoppers were removed, and the number of leafhoppers not on plants or missing was noted. After each census, each vine was shaken to dislodge the insects and the position of the plant in the cage was rotated. Two identical cages with cultivars arranged in a mirror image in relation to a central lamp were examined several times daily for several days. Newly-collected insects from natural vegetation were used when cultivar comparisons were changed. Because plant vigor and succulence strongly influence leafhopper preference (9), I selected plants of equal age and vigor.

Plant inoculations by leafhoppers. *Greenhouse tests.* Dormant two-bud cuttings of Barbera, Cabernet Sauvignon, Chardonnay, Chenin blanc, Flora, Mission, Pinot noir, Ruby Cabernet, Sylvaner, Thompson Seedless, and White Riesling were obtained from registered, disease-free vines of the Foundation Plant Material Service at the University of California, Davis. Cuttings were stored inverted in damp vermiculite in a cold room (4 C) until needed and then were rooted and transplanted to 10-cm square pots. Blue-green sharpshooters were collected from native populations from various host plants at Berkeley and placed for two or more days on greenhouse-maintained vines that had advanced symptoms of PD. Concurrent experiments (1,14)

0031-949X/81/04042907/$03.00/0

indicated that most (but not all) individuals initially were free of the PD agent. After acquisition feeding, the insects were caged singly on potted grapevines and transferred to new plants. In some transmission tests, infective *G. atropunctata* were caged in 2.5-cm-diameter leaf cages fastened to leaves with a metal clip or rubber bands. In each test, additional plants (at least 1 in 10) were caged without insects to serve as uninoculated controls and to detect accidental spread of PD. No check plants developed PD symptoms during this study. All inoculated test plants and controls were held at least 6 mo in a greenhouse (23–37 C, heated to 27 C) and diagnosed for PD. The PD bacterium was isolated from selected vines with PD symptoms (1) but not from symptomless ones.

Field tests. Vines in commercial vineyards were inoculated with infective leafhoppers in small cages at several times during spring and summer. Vines previously free of PD symptoms of the cultivars Chenin blanc, Flora, White Riesling, Pinot noir, Chardonnay, Cabernet Sauvignon, and Petit sirah were inoculated in 1973–1975 in the Napa Valley. Portions of these vineyards in which inoculations were made were mapped for PD each autumn from 1973 until 1977. Barbera and Ruby Cabernet were inoculated in a Fresno County vineyard in 1975 and each experimental plot was mapped annually, 1974–1978, for PD.

For the field inoculation tests, adult *G. atropunctata* were fed for 2 or more days in the greenhouse on PD source plants and then transported to the field either in plastic bags in an ice chest or on caged grapevines. Two leafhoppers were aspirated through an entry hole into the 2.5-cm plastic cages. The bottom of the cage was fastened flush against the lower side of a primary leaf that was at least 3 cm wide near the end of a cane. A 5-cm-square × 1-cm-thick foam pad stapled to a wooden plant label was secured to the cage with several rubber bands. Each cage location was tagged with a plastic label. In some tests leafhoppers remained caged for 1 wk; in other tests they were removed after 24 hr. Surviving insects were returned to the insectary at Berkeley and checked for infectivity on rooted cuttings. The development of PD symptoms at each inoculation site was monitored for at least 2 yr.

RESULTS

Vector preference. Counts of blue-green sharpshooters on cultivars located with similar proximity to adjacent vector source areas showed consistent differences among cultivars in the numbers of sharpshooters per sample unit (Table 1). The order of cultivar feeding preference in caged preference tests was the same for paired comparisons of Chardonnay-White Riesling, Sylvaner-Cabernet Sauvignon, Chenin blanc-Pinot noir, or Chenin blanc-Flora (Table 1). Mapping of adjacent blocks (300 vines each) of Sylvaner from 1975 to 1977 gave overall incidence of vines with PD symptoms (8) of 8, 3, and 11%, respectively. The incidence of PD in Cabernet Sauvignon was 30, 35, and 48% for the same years.

Greenhouse transmission tests. Ninety percent of 11 cultivars exposed for 4 days to infective blue-green sharpshooters developed PD, with small but significant differences ($\chi^2 = 21.53$, df = 10, $P < 0.05$) among individual cultivars (Table 2). Results in Table 2 do not reflect differences in test plant age, which in some experiments were considerable—up to 2 mo. Symptoms of PD have proven to be a reliable though not infallible indication of infection of grapevines with the causal bacterium, if plants are observed long enough (1,2).

In further greenhouse tests, care was taken to minimize differences in test plant age, and the inoculation access period was reduced to 1 day. Young plants (less than 6 wk from first leaf) were more sensitive to infection than were older plants (more than 3 mo from first leaf), and in cultivars other than Barbera, Pinot noir, and White Riesling, the differences were highly significant ($P < 0.01$) (Table 3).

Leaf age seemed to influence susceptibility to infection in plants over 6 mo old. Single infective *G. atropunctata* were caged for 1 day on older basal leaves or on young apical leaves that had been induced by pruning within the previous month. Of 52 plants in which a young leaf was inoculated, 83% developed PD symptoms, whereas 55% of 62 plants became infected after exposure of an older, basal leaf to an infectious leafhopper vector (Table 4).

Field transmission tests. Experiments to test leafhopper transmission of the PD bacterium to mature grapevines in commercial vineyards produced results that differed from greenhouse tests in two respects. First, foliar symptoms of leaf discoloration and "scorching" appeared several weeks later in field inoculations compared to those that appeared on potted vines kept in the greenhouse. Symptoms appeared in greenhouse plants 7–12 wk after inoculation, whereas in the field, PD symptoms appeared only after 10–12 wk or longer. Second, inoculations made during the spring and early summer resulted in a greater number of chronic infections that persisted from one growing season to the next. Many late-season inoculations caused infections that did not persist through the following year.

The youngest leaves at the growing shoot tip of 3-yr-old vines of Barbera and Ruby Cabernet in a Fresno County vineyard were inoculated on five different dates in 1975 (Table 5). The earliest symptoms of PD appeared in August of the same year. Symptoms in Barbera vines from April, May, and June inoculations were pronounced and widespread throughout each vine by October, 1975. Five of six Barbera vines inoculated in April had PD symptoms that fall; four of those vines died or were removed during

TABLE 1. Average numbers of blue-green sharpshooters per plant in cage choice experiments and in paired comparisons of field populations on grapevines (*Vitis vinifera*, except cultivar St. George) in Napa County, CA[a]

Cultivars	Average field counts[a] (adults and nymphs)				Average cage counts (no.)
	1973	1974	1975	1976	
Chardonnay vs:	20.1 A[b]	16.0 A	...	...	12.4 A
White Riesling	9.3 B[b]	5.8 B			7.7 B
Sylvaner	...	...	...	...	13.8 A
St. George (*V. rupestris*)	...	...	...	...	9.9 B
Sylvaner vs:	8.3 A	6.9 A	12.4 A	4.5 A	21.5 A
Cabernet Sauvignon	1.9 B	0.5 B	3.4 B	2.1 A	12.2 C
Pinot noir	...	...	...	...	16.1 B
St. George	...	...	...	...	10.4 C
Chenin blanc vs:	3.1 A	0.2 A	0.0 A	...	16.9 AB
Pinot noir	10.2 B	...	...	...	19.4 A
Flora	1.7 A	0.0 A	0.0 A	...	12.8 B
St. George	...	...	...	...	18.3 A

[a] In caged choice experiments 35–80 insects initially were placed into each of two cages and examined four times daily for 4 days. Field counts were made in April or June and consisted of 8 to 15 whole-vine counts or of samples of 20 cane tips.

[b] Twenty-cane counts are underlined. Averages followed by the same letter in each column were not significantly different ($P < 0.05$) according to Duncan's multiple range test for cage tests and paired Student's *t*-tests for field counts.

winter pruning in 1976 or 1977 (Table 5). In contrast, Ruby Cabernet vines of the same age in an adjacent planting developed only a few discolored leaves with slight marginal necrosis at the inoculated leaf and on a few leaves distal to the inoculation point. Five of 24 Ruby Cabernet vines inoculated in April–June developed infections that persisted through successive years. Only one of these five Ruby Cabernet vines had PD symptoms in 1976; two of them developed the first PD symptoms 2 yr after inoculation; and the other two vines took 3 yr to develop symptoms. None of the Barbera vines exhibited this pronounced incubation period; however, 11 of the 29 Barbera vines with PD symptoms the same year of inoculation had no PD symptoms in following years. Six of 25 Barbera vines that were inoculated on 9 July or 1 August remained chronically infected. None of an equal number of Ruby Cabernet vines became chronically infected on the same inoculation dates. There was very little natural spread of PD (probably caused by leafhoppers that accidentally escaped from leaf cages) in both of these adjacent plots; nine of 837 (1.1%) uninoculated Barbera vines and 28 of 952 (2.9%) uninoculated Ruby Cabernet vines in the experimental plot developed PD.

Leafhopper inoculations made in Napa Valley vineyards in 1973–1975 (Table 6) produced results similar to those obtained in Fresno County. Again, late-season inoculations produced few chronic infections. The spread of foliar symptoms—leaf discoloration and scorching followed by leaf abscission from the distal end of the petiole—was rapid and extensive in the cultivars Flora, Chardonnay, and Pinot noir. In contrast, 6 mo after inoculation, foliar symptoms of PD often were limited to two or three leaves in Chenin blanc, White Riesling, Petit sirah, and Cabernet Sauvignon. Fewer inoculations made in the Napa Valley produced PD symptoms compared to the Fresno inoculations; however, the same varieties were not compared in both locations.

TABLE 2. Infections by Pierce's disease bacterium in cultivars of *Vitis vinifera* resulting from a 4-day inoculation access by infective blue-green sharpshooters

Cultivars	Transmissions/attempts (ratio and percent)	
Ruby Cabernet	25/25	(100)
Pinot noir	55/56	(98)
Chardonnay	32/33	(97)
Barbera	23/24	(96)
Mission	67/71	(94)
Petit sirah	60/69	(87)
Sylvaner	18/21	(86)
Cabernet Sauvignon	17/20	(85)
Flora	38/46	(83)
White Riesling	32/39	(82)
Chenin blanc	51/62	(82)
Total	418/466	(90)[a]

[a] Chi-squared significant among cultivars ($\chi^2 = 21.53$; df = 10; $0.05 > P > 0.01$).

TABLE 3. Effect of grapevine plant age on the number of Pierce's disease infections resulting from 1-day inoculation access by infective blue-green sharpshooters

Cultivar	Transmissions/attempts (ratio and percent)		
	Young plants[a]	Old plants[b]	Significance[c]
Pinot noir	57/59 (97)	37/39 (95)	N.S.
Cabernet Sauvignon	29/30 (97)	14/26 (27)	**
Mission	81/90 (90)	17/30 (57)	**
Chenin blanc	71/79 (90)	4/11 (37)	**
Chardonnay	64/72 (89)	19/30 (63)	**
Ruby Cabernet	26/30 (87)	20/30 (67)	**
Barbera	35/41 (85)	25/36 (69)	N.S.
Thompson Seedless	34/41 (83)	10/22 (45)	**
Flora	65/80 (81)	24/42 (57)	**
Petit sirah	39/48 (81)	...	...
Sylvaner	48/63 (76)	7/26 (27)	**
White Riesling	16/23 (70)	10/17 (59)	N.S.
Totals	563/656 (86)	187/309 (61)	**

[a] Less than 6 wk from leaf bud stage.
[b] More than 3 mo after leaf bud stage.
[c] Paired asterisks indicate $P < 0.01$ using χ^2 for comparison of "old" and "young" plants of the same cultivar. N.S. = nonsignificant.

DISCUSSION

Each of the factors evaluated or noted in this study—vector preference, susceptibility to infection via leafhopper feeding, the rapidity of tissue invasion by the causal bacterium as evidenced by the extent of foliar symptoms, and the persistence of infections from one season to the next—may contribute to cultivar susceptibility or resistance to PD under field conditions.

The well-established association between vineyards having a high incidence of PD and nearby vector source areas (9,11,12,18) supports the notion that the incidence of PD is a function of vector abundance. A previous study of the within-vineyard distribution of the blue-green sharpshooter in the Napa Valley (9) suggested that early season populations of this leafhopper were most important in the spread of PD and that most late season inoculations might not establish chronic infections. The present investigation was undertaken in part to test these predictions and provides direct evidence that early season inoculations are more likely to lead to chronic infections than are midseason to late-season inoculations.

The influence of time of inoculation on the persistence of PD infections was even more pronounced for the cultivars Ruby Cabernet and Chenin blanc than for cultivars such as Barbera and Flora. The results of field inoculations are in closer agreement with the data from greenhouse transmission tests in leaf cages, if the effects of plant age on susceptibility to infection are considered and

TABLE 4. Infections caused by Pierce's disease bacterium following exposure of old or young grapevine leaves to 1 day of feeding by single infective blue-green sharpshooters[a]

Cultivar	Ratio (no. transmissions/no. test plants)	
	Young leaf	Old basal leaf
Cabernet Sauvignon	5/6	3/6
Chardonnay	5/5	6/8
Chenin blanc	5/6	2/5
Flora	6/7	5/11
Mission	7/8	5/7
Pinot noir	8/10	9/10
Sylvaner	3/5	0/8
White Riesling	4/5	4/7
Total	43/52 (83%)	34/62 (55%)

[a] All plants were over 6 mo old. Plants of the same cultivar were the same age.

TABLE 5. Development of Pierce's disease symptoms in two grape cultivars following inoculation feeding by infective blue-green sharpshooters[a] on different dates during the growing season of 1975 in Fresno County, CA

Date inoculated	Cultivar	Vines (no.)	Vines with PD symptoms in autumn (no.)			
			1975	1976	1977	1978
April 17[a]	Barbera	6	5	4(1D)[b]	4(2R)[c]	(6R)
	Ruby Cabernet	6	2	1	1	1
May 22	Barbera	12	6	4	4	(4R)
	Ruby Cabernet	12	5	0	1	2
June 12	Barbera	11	7	4	(4R)	(4R)
	Ruby Cabernet	11	8	0	1	2
July 9	Barbera	12	5	3	2(1R)	(3R)
	Ruby Cabernet	12	4	0	0	0
Aug 1	Barbera	13	6	2(1R)	(2R)	(2R)
	Ruby Cabernet	13	5	0	0	0

[a] Leafhoppers were exposed to test plants for 1 wk in April inoculations. In other trials, exposure was 24 hr. Two leafhoppers per cage were confined in a cage on a single leaf on each vine.
[b] D = dead.
[c] R = removed.

the data on late-season (after June) field inoculations are excluded. In any case, the field resistance to PD among the *V. vinifera* cultivars considered in this study is entirely relative. All cultivars evaluated could be infected chronically during the early growing season.

What is the contribution of vector preference to the natural spread of PD? Both field and cage observations of relative abundance of the blue-green sharpshooter indicated that there were significant differences among cultivars in leafhopper feeding preference. These results are in general agreement with previous studies (17) which also included comparisons of Pinot noir, White Riesling, Chardonnay, Chenin blanc, and Cabernet Sauvignon. Vector preference was not consistently correlated with field resistance. Field observations of differences in the incidence of PD (8,11) indicated a higher percentage of vines of Pinot noir, Chardonnay, and Flora were infected compared to Chenin blanc, White Riesling, or Petit sirah located in similar proximity to vector source areas in the Napa Valley. The increased sensitivity to field spread of PD matched the order of vector preference for the paired comparisons of Pinot noir vs Chenin blanc and Chardonnay vs White Riesling. However, in comparisons of Chenin blanc vs Flora and Sylvaner vs Cabernet Sauvignon, the apparently more resistant cultivar was more highly preferred.

Vector preferences should influence the average number of vectors per plant. In order to assess quantitatively this impact of vector preference, we can estimate the effect of vector numbers upon the likelihood of infection. The general binomial probability model for the likelihood of transmission (p) or no transmission ($q = 1 - p$) by n vectors per plant during a given inoculation access period (IAP) is

$$P_n = 1 - q^n. \quad (1)$$

If the probability of transmission by a single vector is equal to the product (iE) of the contingent probability i that the insect is infective and the probability E that if infective it will transmit successfully during a given time interval $\Delta t = t - t_0$, then $q = 1 - iE$. If we define $t_0 = 0$, then $\Delta t = t$. The parameter i is simply the fraction of potential vectors that are infective. Similarly E can be interpreted as vector transmission efficiency per unit time (t). If random samples of a natural population of a vector species are tested for their ability to transmit to test plants per unit time, the fraction of transmitting vectors is an estimate of the product iE. Thus the probability of infection by n vectors in t days is

$$P_{nt} = 1 - (1 - iE)^{nt} \quad (2)$$

The probability model just described predicts levels of infection that are far larger than commonly observed. If conservative estimates of the average numbers of blue-green sharpshooters per vine for a given distance from a vector source during April–May and estimates of the likelihood of transmission from field-collected sharpshooters (9) are used to calculate the daily probability of infection, the predicted number of infections is much higher than observed, as the following example illustrates. The Poisson probability distribution of X:

$$p(X) = \lambda^x e^{-\lambda} / X! \quad (3)$$

in which $X = 0,1,2,\ldots n$ and λ = the average X, estimates the per vine probability of no infection ($X = 0$) where infections are randomly

TABLE 6. Development of Pierce's disease symptoms in grape cultivars following inoculation feeding by infective blue-green sharpshooters[a] on different dates in 1973–1975, Napa County, CA

Cultivar Date inoculated	No. of vines	Inoculation per vine[b]	Vines with PD symptoms (no.) 1974	1975	1976	1977
Cabernet Sauvignon						
23 September 1973	6	3	0	0	1	0
29 April and 1 May 1974	17	2	3	3	2	2
3 July and 24 July 1974	4	2	2	0	0	0
20 May 1975	5	2	...	2	1	1
3 July 1975	5	2	...	1	0	0
Vines with PD symptoms in test plot[c] (%)	360	...	8.0%	9.3%	11.7%	10.1%
White Riesling						
20 May 1975	7	2	2	0	0	...
29 May 1975	6	1	1	1	1	...
3 July 1975	6	1	0	0	0	...
Vines with PD symptoms in test plot (%)	200	...	5.5%	4.0%	4.1%	...
Chenin blanc						
11 June 1975	7	2	...	2	1	...
17 July and 23 July 1975	12	2	...	3	0	...
Vines with PD symptoms in test plot (%)	400	...	8.5%	7.8%	6.8%	
Flora						
17 April 1974	8	2	5	5	(all[d])	...
23 July 1975	8	2	...	2	2	...
6 August 1974	6	2	0	2	2	...
Vines with PD symptoms in test plot (%)	252	...	5.4%	15.8%	16.1%	...
Chardonnay						
20 May 1975	4	2	...	4	5	4
23 July 1975	5	2	...	2	3	3
Vines with PD symptoms in test plot (%)	322	...	5.2%	5.8%	6.7%	8.1%

[a] Two blue-green sharpshooters per leaf cage on most distal leaves on cane.
[b] Number of cages per vine; each cage on a separate cane.
[c] Vines including the ones inoculated in each plot were mapped yearly. The percentage of vines with PD symptoms was calculated excluding missing and dead vines.
[d] All removed.

distributed. For $X = 0$, this expression (eq. 3) can be simplified as $p(0) = e^{-\lambda}$. The probability of infection (P_I) therefore is $1 - e^{-I}$ where I is the average number of infections per unit time produced by n insects, of which a fraction (i) are infective and of those a fraction (E) actually transmit ($I = iE$). E may vary among vector species, being close to zero for very poor vectors, and close to 1.0 for very efficient vectors. Using estimates of three adult *G. atropunctata* per vine and 10% of the population transmitting once daily (9) to generate an average of 3/10 infections per vine, a random distribution of infections will produce a probability of 0.25 ($= 1 - e^{-.3}$) that a vine will have one or more infections per day. If it is further assumed that plant-to-plant vector movements occur at least daily, that population densities are constant, and that susceptibility to infection is constant, then the likelihood of infection is

$$P_{nt} = 1 - e^{-niEt} \quad (4)$$

After 30 days, $P_{nt} = 0.9999$. This level of infection is far above what is commonly observed in susceptible cultivars exposed to high vector densities in three seasons, much less 30 days. If we reduce the hypothetical average number of sharpshooters to 1.0 per vine and 5% of this population transmits once daily, the 30-day cumulative probability of at least one infection is 0.78, which is still a much higher figure than normal even for areas of heavy spread with much higher independent estimates of vector density (n) and infectivity (iE). Fig. 1 illustrates the expected probability of infection for values of the product nt of 1, 2, 5, 10, or 30 insect-days as a function of the percentage of the vector population that transmits (iE). Again, random daily movements of vectors from plant to plant are assumed. Actual plant-to-plant movements of the blue-green sharpshooter vectors may be higher, but this is a conservative estimate based on changes in per vine vector density in space and time (9). The model also assumes no change over time in adult vector inoculativity, which seems reasonable (14,16). The choice of daily time steps for this model minimizes the effects of any circadian rhythms in transmission efficiency. The probability of transmission by *G. atropunctata* increases only slightly with IAPs longer than 12 hr (14). If actual vector movements are more frequent, then the average number of inoculations per plant per day would be greater because transmission efficiency increases rapidly with increased IAP for up to 6 hr (14), after which efficiency asymptotically approaches 100%. The net probability of infection P_{nt} is plotted in Fig. 1 for both a random (Poisson) distribution of vectors among vines at the same distance from a vector source area and for an even (binomial) distribution. The actual distribution of vectors is most likely to be random or aggregated. Only extreme aggregation of vectors would reduce P_{nt} substantially below that expected for a random distribution, and this difference due to aggregation would decrease with increased nt, as Fig. 1 illustrates for the differences between the binomial and Poisson distributions.

Because the model just described projects unrealistically high levels of infection even with a conservative choice of parameter values, the *observed* natural spread of PD apparently must be relatively insensitive to vector density in terms of the general relationship just described. This conclusion also is supported by the fact that in the Central Valley of California, where the principal vectors are grass-feeding sharpshooters which are extremely rare on grape (18), the rate of spread of PD can be just as rapid with no measurable per vine density of vectors as rates of spread in the Napa Valley, where the principal vector often is common on grape. To confound this comparison further, the Central Valley vectors are much less efficient (lower E) and generally fewer are infective (lower i) than the blue-green sharpshooter in transmitting the PD bacterium to grape (11,14,16). The effect of transmission efficiency (E) is therefore of interest in considering different vectors.

Percent transmission varied little among cultivars in tests using 1-day or 4-day inoculation access periods and test plants no older than 1 mo (Tables 2 and 3). However, these differences in cultivar susceptibility to infection in young foliage cannot explain observed differences in field spread of PD. Sylvaner and White Riesling were possible exceptions; significantly fewer percentages of both cultivars were infected compared to other cultivars when subjected to a 1-day IAP (Table 3). We can express the decreased susceptibility to daily infection by single vectors in Sylvaner, for example, as approximately 70% that of Cabernet Sauvignon (Table 3). Thus, with an average of three infections daily to Cabernet Sauvignon, the daily probability of any single plant of this cultivar being infected is 0.97. For Sylvaner, 76% of the test plants (Table 3) would have at least one infection from the random distribution of one and four-tenths infections per plant. We have assumed so far that n insects transmit once daily. We can define n′ as the number of *calculated* infections or "infective punctures" (15) from n insects per day. If we postulate that

$$n' = Kn \quad (5)$$

in which K is an expression of cultivar resistance to infection, differences in n′ can be used to express differences in cultivar resistance to infection. Cultivars more resistant to infection by vector feeding should have a lower n′ per vector. In our example $n = 1$ and $n' = 3.0$ for Cabernet Sauvignon and for Sylvaner with $n = 1$, $n' = 1.4$ on young foliage. The calculated values of K are equal to n′ for each cultivar if $n = 1$ for each plant, as was the case for the results shown in Tables 2–4. From the foregoing example, the net probability of transmission after 30 days with $n = 0.1$, and $iE = 0.1$ is 0.59 for Cabernet Sauvignon and 0.34 for Sylvaner. However, if the period of infection is extended to 90 days, or more realistic levels of n and iE (9) are substituted, the probability of infection approaches 1.0 for both cultivars.

Is the model described so far of any use? It describes very high levels of acute infections with conservative estimates of vector densities. It assumes that all infections persist indefinitely. Previous work (8,9) suggested and the present study confirms that this is not the case. Field inoculation tests (Tables 5 and 6) clearly indicate a trend of decreasing overwinter survival of most late-season infections, that is, the likelihood of establishing chronic infections decreases as t increases.

The data from the field inoculations of the cultivars Barbera and Ruby Cabernet (Table 6) can be fitted by linear regression to estimate empirically the probability of infections persisting through the dormant season as a function (v) of date of inoculation

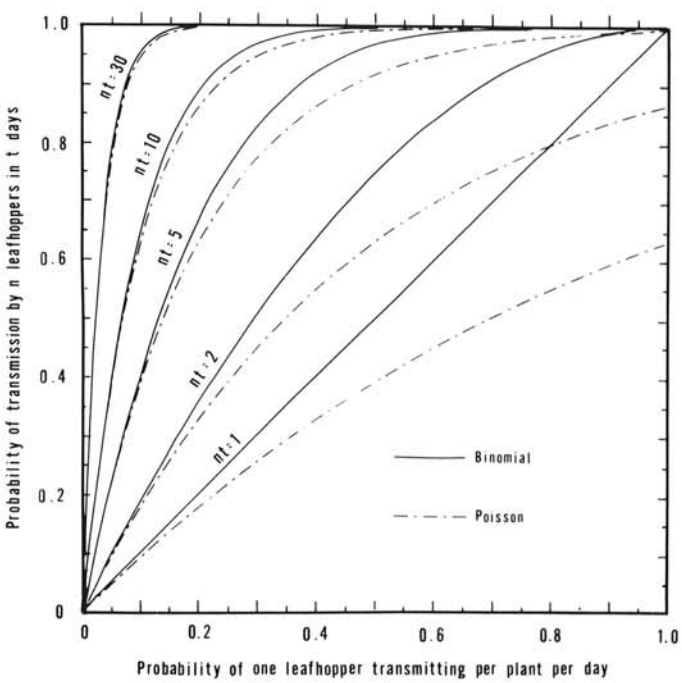

Fig. 1. Probability of infection (P_{nt}) by n vectors after t days for different probabilities of single vector transmission (iE). For equal numbers of vectors per plant (binomial distribution, solid line), $P_{nt} = 1 - (1-iE)^{nt}$. For vectors randomly distributed among plants (Poisson distribution, broken line), $P_{nt} = 1 - e^{-niEt}$.

(t). The equation for a line of best least squares fit for the probability that an infected vine will not recover (y) as a function of t days from the start of the growing season (approximately March 21) is:

$$y = b - at \quad (6)$$

in which b = y when t = 0 and a is the slope of the regression line. For Barbera: $y = 0.9857 - 0.0062t$ ($r^2 = 0.92$, $P < 0.02$). For Ruby Cabernet: $y = 0.2033 - 0.0016t$ ($r^2 = 0.91$, $P < 0.02$). In this example we have assumed hypothetically that all inoculated vines were infected. The value of y, of course, cannot be less than zero or more than unity, so the utility of this simple approach is limited.

For each time step of 1 day, the probability that a plant will be free of PD the following year can be attributed to either (i) the chance avoidance of infection ($= e^{-n'E\Delta t}$) or (ii) the liklihood that infected plants will recover during the following winter, which is equal to 1 − y, the complement of Eq. 6. The probability that a vine will not be chronically infected (P_H) is therefore

$$P_H = (1 - P_{nt}) + P_{nt}(1 - y)$$

$$P_H = e^{-n'E\Delta t} + (1 - e^{-n'E\Delta t})(1 - b + at) \quad (7)$$

for which Δt = 1 day.

Eq. 7 is somewhat unwieldy, having so many independent variables. However, estimates of n and i (or iE) can be made by sampling vector populations, and E, K (= n/n'), b, and a can be estimated experimentally. The product of P_Hs for each successive day is the net probability of no chronic infection for the total time period considered. For example, for the cultivar Barbera, if iE = 0.1, and n' = 1, then after 1 day (eq. 7) $P_H = 0.91$; after 10 days $P_{H(10)} = 0.39$; and after 30 days, $P_{H(30)} = 0.07$. This means that after 30 days, the net probability of chronic infection under these conditions would be $1 - P_{H(30)} = 0.93$. For Ruby Cabernet with the same values of iE and n', after 1 day, $P_H = 0.98$; after 10 days, $P_{H(10)} = 0.83$; and after 30 days, $P_{H(30)} = 0.60$. In these hypothetical examples, n is constant over time. However, vector density could be expressed as a time-dependent variable based on sampling estimates.

The data from field inoculations (Tables 5 and 6) support the contention (9) that chronic infections are most likely to be established early in the growing season and, conversely, not likely to develop later in the season. If adjustments for recovery (eq. 6) of most late-season infections are of the magnitude observed in the trials summarized by Tables 5 and 6, it is clear that a high percentage of chronic infection will result only under the heaviest inoculation pressure early in the growing season in cultivars with resistance to chronic infection such as demonstrated by Ruby Cabernet, Cabernet Sauvignon, Chenin blanc, and White Riesling. The prolonged period during which "field susceptible" cultivars can be chronically infected may explain why the spread of PD in the very susceptible cultivars Pinot noir and Chardonnay was not reduced by early season vector control, whereas some control was noted in the more resistant cultivar Sauvignon blanc (12).

The probability of infections persisting from year to year is empirically described by linear regression (eq. 6). This is at best a crude first approximation. The actual theoretical relationship may be quite different, for example, hyperbolic. The underlying processes determining the trend described here are not known. Conceivably, the extensive pruning of grapevines during the dormant season eliminates more late-season infections in less susceptible cultivars because of cultivar differences in the rate of systemic movement of the PD bacterium. It is also possible that winter survival of PD infections in small or young stems is poor. A low rate of overwinter survival in field inoculations of the PD bacterium into almond twigs was noted even though most inoculations were made in March and a high percentage of current season infections were verified (2). Cold treatments and overwinter exposure to various climates is therapeutic for PD in potted vines (10,13). Climate as well as cultivar may determine the values of a and b for eq. 6. Resistance of the sort discussed here would be impractical if climate reduced the values of b or decreased the values of a substantially for most cultivars. In fact, this may be the case for southern Florida, where European grapes are uniformly very susceptible to PD (5).

If the model is correct in principle, the effects of vector control—that is, in reducing n—can be examined. If the values for i, E, and t are high, n must be reduced to a very small number for P_{nt}, the probability of a current season infection, to be small. For example, if we set iE = 0.1, reducing n from 10 to 1 (90% vector control) reduces the probability of infection (P_{nt}) from nearly 100% to about 65% after 10 days, but to only 96% after 30 days (Fig. 1). Very small numbers of efficient vectors and not much higher numbers of less efficient vectors can quickly saturate the number of uninfected suscepts in a host plant population planted in a low density. In this context, it is understandable how PD spread can be substantial even in vineyards with very low vector populations. Adjusting n for cultivar susceptibility to infection by vector feeding (n' = Kn) similarly fails to significantly reduce the probability of infection.

From a traditional entomological perspective (6), resistance to an insect vector—especially resistance due to vector preference—may promote the increased spread of some virus diseases (4), such as those caused by nonpersistent aphidborne viruses. Both vector cultivar preference and vector control directly affect vector density. Vector preference has been suggested as an explanation for observable field resistance for a number of vectorborne viruses, based upon correlations of preferences with rates of disease spread (4). The results of this study indicate that observed differences among grape cultivars to the natural spread of PD in California are due mostly to their overwinter recovery rates from acute infections and probably only to a minor extent to differences in vector preference or susceptibility to acute infection. In the later phases of localized epidemic spread, the overwinter recovery rate undoubtedly is the most important factor in determining the number of "uninfected" plants. In the early stages of spread where infection is rare, P_{nt} (eq. 4) is the critical factor governing final disease incidence.

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