Reduction in Yield and Vigor of Grapevine Caused by Crown Gall Disease

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ABSTRACT

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The crown gall disease caused by *Agrobacterium tumefaciens* affected yield and vigor of the grapevine (*Vitis vinifera*) cultivar Zinfandel. A comparison of berry yield, weight of prunings, and trunk diameters with disease ratings over a 4-yr period revealed that vines with galls covering >50% of the circumference of the crown region at the beginning of the assessment period were less vigorous and yielded fewer berries than vines with no galls. Reductions in berry yield and prunings ranged from 20 to 40% and 10 to 40%, respectively, over a 4-yr period, and trunk diameters (20 cm below forking) averaged 9% less than those of healthy controls. No significant reductions in yield or vine vigor were detected with mildly diseased plants. The incidence and severity of the crown gall disease changed over the period of the test. New galls formed on some previously healthy plants, and galls on diseased plants sometimes rotted and sloughed off without being replaced by new galls. Trunk diameters of plants subjected to continuous heavy disease pressure were significantly less than those of continuously healthy plants. Plant performance in some categories of mildly diseased plants, however, was significantly greater than that of continuously healthy controls. The stripping of berries by starlings was another factor affecting the correlation of disease and yield. Loss of berries was negatively correlated with overall plant vigor and canopy density, with birds preferring to feed on less vigorous vines. We concluded that the crown gall disease has both long- and short-term effects on plant performance as mediated by the environment.

The effect of the crown gall disease caused by *Agrobacterium tumefaciens* on yield and vigor of numerous plant species has been debated since the bacterium was isolated from grape in Italy in 1894 (4). The first written record of the disease was by Fabre and Dunal in France in 1853 (5). Opinions vary greatly on the importance of the disease because many factors affect disease severity, including climate, host resistance, time of infection, and the number, size, and location of galls. Assessment of disease loss is further complicated in that diseased plants may be more susceptible to invasions by other organisms (14) such as *Pseudomonas syringae* pv. *syringae*, *Armillaria mellea*, and insect borers in the case of *Prunus* spp.; the latter two organisms invade directly through the galls. These and other secondary invaders can cause more serious damage than the crown gall disease. Gloyer (7) suggested that horticultural factors, such as branch and root pruning and shape and distribution of the root system, may have more influence on the top growth of an individual plant than the mere presence or absence of crown gall infections. The difficulty of controlling and assessing the importance of these variables in field plot experiments has largely prevented quantitative evaluations of the impact of the crown gall disease on plant development. Moore and Tingey (10) provided quantitative evidence of the effect of the disease in greenhouse studies using radish as a host.

Despite the absence of published quantitative data on field losses to crown gall, most growers and researchers consider it to be a serious disease and feel that current regulations to curtail the production and distribution of infected stock are necessary. Those familiar with the disease have long observed that symptoms of infected plants vary from no visible effects to stunting or death. Some of the better field evidence of the importance of crown gall and the related disease hairy root, caused by *Agrobacterium rhizogenes*, in affecting plant growth is found in reports by Riker et al (11,12) and Gloyer (7). Hedgecock (8) and Garcia and Rigney (6) provided good observational data for the seriousness of crown gall on grapes in 1910 and 1913, respectively.

An ideal opportunity to quantitatively assess the importance of crown gall on yield and vigor of grapes arose in Shandon, CA. Several new plantings of the cultivar Zinfandel were found to have a high incidence of infection in fields that had previously been planted only to grains. The circumstantial evidence clearly indicated that the mist-propagated plants had been infected in the greenhouse. An inspection of plants in surplus containers revealed that they were diseased at the crown area below the soil surface. An 8-yr study was therefore initiated to determine the effect of crown gall on plant development, beginning with the inception of plant growth in the

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field. The climate of the region is relatively arid, representative of an environment generally considered to prevent the crown gall disease from becoming a significant problem. This report presents data from the study.

MATERIALS AND METHODS

Established plants were examined for galls after the crown regions were exposed with a hydraulic water system (13) to remove the sandy loam soil from the infected region. Presence of galls on plants was evaluated in three plantings located near Shandon, CA. Field A was about 10 km from the other two fields, and fields B and C were within 0.2 km from each other. These fields had been planted to grapes in successive years. Hereafter, temporal references for these fields will be made relative to the transplant date (year 1) for each field. In the summer of the second year of field B, the crowns of 33 plants were exposed and evaluated for disease incidence and severity. A similar evaluation was made on a block of eight rows by 30 plants in field A, year 4. Because the sizes of the galls did not appear to be related to the proportion of the crown affected, disease severity of individual plants was rated both for size of gall(s) and fraction of the crown circumference that was affected. Galls were rated as small (<3 cm in diameter), medium (3–5 cm), or large (>5 cm). The fraction of the crown affected was visually estimated to the nearest 10%. At the same time, cane diameter (trunk) was measured with calipers 5 cm above the soil line and 20 cm below the branching point (about 100 cm above the soil line); the average value of the two measurements was used to calculate group means.

A plot was set up in field C in the spring of year 3 to evaluate the effect of crown gall on plant vigor and yields over a period of years. The crowns of all plants in a seven-row block, average of 30 plants per row, were exposed, scored for disease severity, and assigned to one of four ratings (0, 1–25, 26–50, and >50%) on the basis of visual approximation of the proportion of the circumference of the trunk region covered with galls. One-half of the plants (98 plants) were tagged, and soil was replaced around the crowns. Plants with the four ratings were randomly spaced throughout the plot.

Beginning in year 3 of the planting, weight of berries and prunings of individual plants were collected in October of each year and in January of the following year, respectively, in field C. The prunings (spur-pruned using the cordon system) were the result of shaping the vines, as is customary in vineyards. Pruning weights were not taken in year 5 because workers inadvertently entered the plot and pruned the plants without supervision. In year 5, when stripping of berries by starlings was a major problem, the plants were also rated for degree of fruit stripping at harvest time. Each plant was rated and assigned to one of five categories, ranging from 0 (none to mild fruit stripping) to 4 (severe to complete stripping). These numerical values were also used to calculate an average rating of the fruit stripping associated with the various categories of galled plants.

In the summer of the last year of the study, the crowns of all plants were reexposed in both field A (year 8) and field C (year 6) to determine the

Table 1. Relationships among crown gall size, disease severity, and trunk diameter for year 4 in field A

Crown gall size			Disease severity		
Rating	No.	Trunk diameter (mm)	DSI[x] (%)	No.	Trunk diameter (mm)
None	70	25.2 a[y]	0	70	25.2
Small	69	25.8 a	1–10	27	24.4
Medium	20	26.2 a	11–20	17	26.7
Large	25	24.6 a	21–30	32	27.5
			31–40	13	25.2
			41–50	20	25.8
			>50	6	20.6

[x] DSI = disease severity index as measured by percentage of galled circumference of crowns.
[y] Figures followed by the same letter are not significantly different ($P = 0.05$). Statistical analysis of trunk diameters vs. DSI is given in Figure 1.

Table 2. Relationship between initial and final crown gall disease severity indexes and yield and vine vigor

	Plant performance[w]			
	Final DSI			
Initial DSI[x]	0	1–25%	26–50%	>50%
Field C				
	Berries (kg/vine)			
0	14.4 (22)	19.0 (8)	21.7 (3)	21.7 (1)
1–25%	12.2 (10)	21.3 (7)	20.3 (7)	8.1 (1)
26–50%	14.4 (4)	16.7 (5)	18.7 (4)	8.7 (1)
>50%	13.6 (6)	12.5 (3)	14.7 (14)	9.0 (2)
	$Y = 15.1 - 7.36\ ID + 41.7\ FD - 71.5(FD)^2$			$(R = 0.40^{**})^y$
	Prunings (kg/vine)			
0	1.37	1.50	1.44	1.79
1–25%	1.29	1.62	1.65	0.68
26–50%	1.40	1.19	1.34	0.99
>50%	1.13	1.05	1.41	1.06
	$Y = 1.37 - 0.409\ ID + 1.70\ FD - 2.9(FD)^2$			$(R = 0.30^{**})^y$
	Trunk diameters (mm)			
0	33.3	36.6	39.5	39.0
1–25%	32.2	35.0	35.8	29.0
26–50%	33.9	33.4	37.1	35.0
>50%	30.8	31.0	32.9	31.0
	$Y = 33.6 - 5.70\ ID + 19.6\ FD - 27.5(FD)^2$			$(R = 0.46^{**})^y$
Field A				
	Trunk diameters (mm)[z]			
0	45.9 (18)	45.4 (14)	43.6 (9)	45.5 (5)
1–25%	48.3 (5)	43.6 (10)	45.4 (9)	46.0 (11)
26–50%	··· (0)	46.9 (3)	41.9 (4)	41.4 (7)
>50%	··· (0)	··· (0)	50.0 (1)	44.5 (1)

[w] Values in parentheses refer to number of plants in each group. Numbers read across reflect data from initial disease rating; numbers read down reflect data from final disease rating.
[x] DSI = disease severity index as measured by percentage of galled circumference of crowns. Initial DSI was obtained in year 3 for field C and in year 4 for field A. Final DSI was obtained in year 6 for field C and in year 8 for field A.
[y] ID and FD are, respectively, initial and final disease severity in units of fraction of circumference galled (fraction rather than percentage of circumference galled was used to avoid very small numbers for the regression coefficients in the equation). R = multiple coefficient of correlation. Significance of regression equation, as evaluated by both R and F tests, is * = $P = 0.05$, ** = $P = 0.01$. In regression analysis, addition terms were included in regression equation only if their contribution to explanation of variability was significant at $P = 0.05$ as determined by the F test.
[z] All regressions were nonsignificant.

condition of the galls or if the disease ratings were the same as those at the beginning of the experiments. At the same time, the maximum and minimum diameters of the trunk 20 cm below the point of branching were measured with calipers and averaged as another assessment of vine vigor. The study was terminated in year 7 because the vineyard (field C) was sold to new owners.

Because disease severity ratings changed over the test period, plant performance was evaluated on the basis of continuing disease pressure in fields A and C in an attempt to reflect changes that occurred in the intervening years. This was analyzed using multivariate (initial and final disease severity ratings of each plant) regression. In the regression analysis of plant performance vs. disease severity, only terms that made a significant ($P = 0.05$) contribution to explaining observed variability, as determined by the F test, were included in the regression equation. In these analyses, the midpoint values (0, 12.5, 37.5, and 62.5%) for the disease severity range for each group were used. The last value was used because it more closely approximated the midpoint value for that group; over 90% of the members fell within the 57–75% galling of circumference range. For cases where the regression equation was not significant ($P = 0.05$), the data and the simplest regression were included in the figures for comparative purposes.

RESULTS

The frequency of galled plants in fields A, B, and C was 62, 76, and 61%, respectively. Disease severity (fraction of the circumference of the crown affected) differed between the fields. In field A, the proportion of plants falling into the 0, 1–25, 26–50, and >50% disease ratings were 38, 41, 18, and 3%, respectively, whereas the values for field B were 24, 33, 18, and 24% and those for field C were 39, 25, 15, and 21%.

The disease affected the vigor of the newly planted vines as measured by trunk diameter (Table 1, Fig. 1). In field A, the trunk diameters of plants with a disease severity rating of >50% (galling over 50% of the circumference) were 20% smaller than those of healthy controls. However, the trunks of plants with mild disease severity (11–30% galling of the circumference) had larger diameters than those of the controls, and the overall relationship was best described by a quadratic expression (Fig. 1, field A, year 4). In field B, plants with mild disease severity also had larger trunk diameters than the controls. Although the addition of a quadratic term to the regression equation was not significant at $P = 0.05$, it came close, being significant at $P = 0.07$ ($Y = 10.6 + 4.18(ID) - 12.2(ID)^2$, $R = 0.60$). In contrast to the above, no significant differences were observed when gall sizes only were related to trunk diameters (Table 1).

Berry yield and weight of prunings showed a negative linear relationship with disease ratings of plants over a 4-yr period in field C (Figs. 2 and 3). For the most severely affected plants, yield reduction ranged from 20 to 40% over the test period. Reduction in vine vigor as measured by pruning weight ranged from 10 to 40%. The reductions in pruning weight and berry yield in year 6 were not statistically significant. However, there was a significant reduction (7%) in vine vigor as evaluated by trunk diameters (Fig. 1). In year 5, stripping of berries by starlings was severe (14% of test plants were completely stripped) and significantly affected yield. The degree of berry stripping of individual plants was highly correlated with overall plant vigor and canopy density; plants with sparse foliage were the most affected. The average yields per vine and percentage of vines in each of the five categories of bird damage

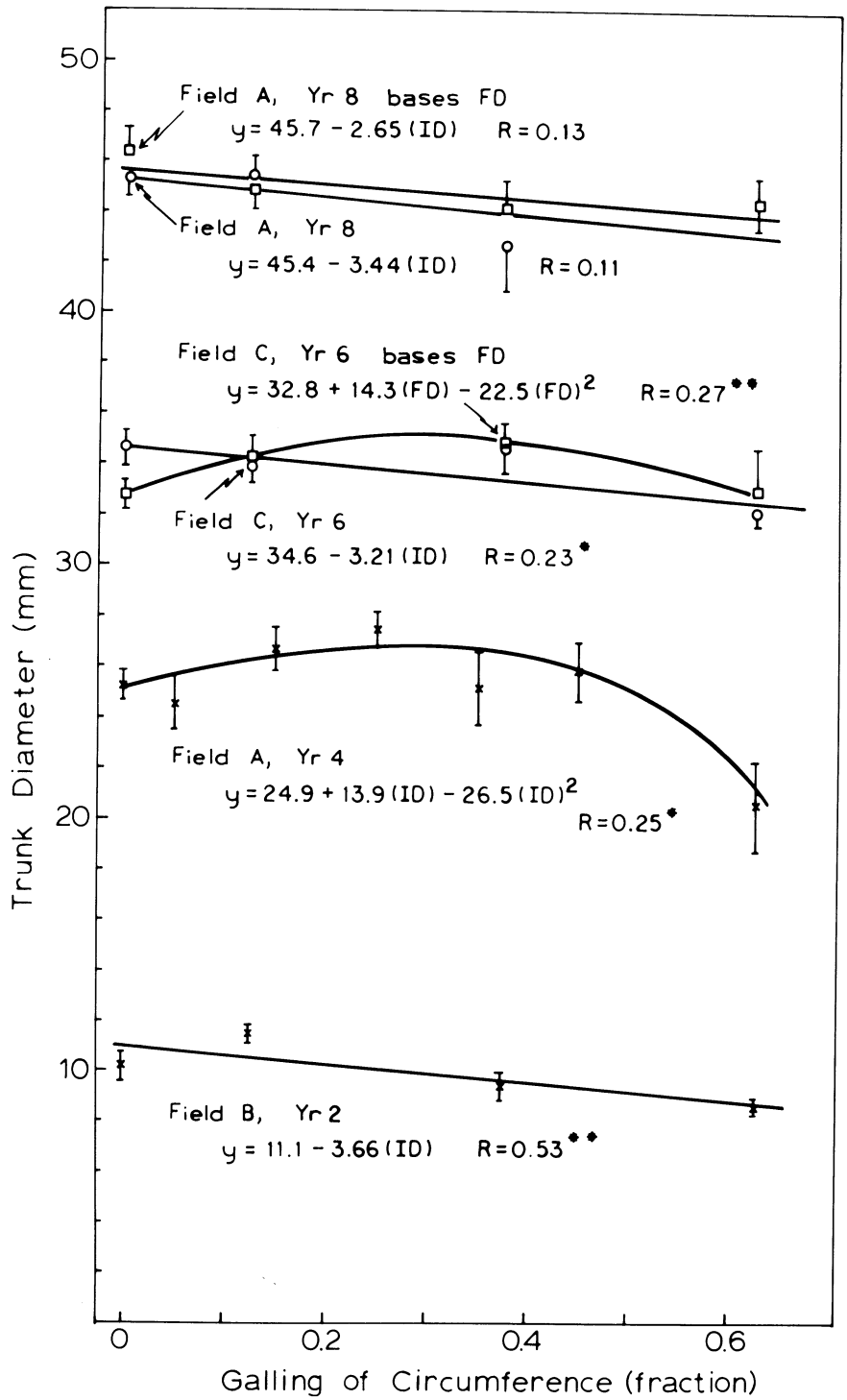

Fig. 1. Relationships between crown gall disease severity and trunk diameters. Meaning of terms in the regression equations and additional details on regression analysis are given in Table 2. Unless otherwise specified, plots are a function of the initial disease severity rating. Bars indicate standard error.

(0 = none to mild, 4 = severe to complete stripping) were 6.94 kg and 25%, 4.04 kg and 32%, 2.63 kg and 24%, 1.13 kg and 5%, and 0.01 kg and 14%, respectively; all were significantly different from each other at $P = 0.001$. The average values of bird damage (0 through 4, as above) for vines in each of the four disease severity categories specified in the figures (from no disease to severe) were 1.18, 1.44, 1.50, and 2.12, respectively. Birds were present in other years, but in fewer numbers, so stripping of berries was not obvious in the test plot and no data were collected.

Substantial changes in disease severity occurred between the years when initial and final disease ratings were made in fields A and C (Table 2). In field C, disease increased in 21% of the plants and decreased in 43% over a 3-yr period. In field A, however, the respective percentages were 56 and 10 over a 4-yr period.

The final disease severity rating provided another means for detecting the effects of disease on plant performance. In contrast to the linear relationships observed for the plant performance variables with respect to the initial disease severity rating, curvilinear relationships were observed with respect to the final disease severity rating (Figs. 1–3). Although the regression equations for pruning weights and berry yields were not statistically significant for field C in year 6, they were significant when evaluated on the basis of year 3 disease ratings when evaluations were based on year 6 disease ratings. In all cases, the coefficient of correlation for the regression equations was higher for equations based on the final disease severity rating than for those based on the initial disease severity rating (Figs. 1–3). To be strictly comparable, the slightly higher R values resulting from inclusion of a quadratic term to linear equations should be used. However, such inclusions had only a minor effect on R and did not materially affect the above cited comparison.

A consideration of the combined initial and final disease severity ratings with respect to plant performance (multivariate analysis) yielded better correlations (Table 2) than when these were treated separately (Figs. 1–3). The relationship was not a simple linear one but had the form of a curved surface. Plants subjected to continuous severe or recent severe disease pressure underperformed plants of all other categories. Although there were only a few individuals in these categories, the data and the regression analysis suggest reductions of around 40, 25, and 10%, respectively, for berry yields, prunings, and trunk diameters associated with the most severe group (Table 2).

Plants with continuous mild or recent mild disease pressure (disease severity index of 0–25% in year 3 but 1–50% in year 6) performed significantly better (berry yield, prunings, and trunk diameter) than those free from galls in years 3 and 6 (Table 2). Berry yields, prunings, and trunk diameters for the mildly diseased categories were 40, 15, and 10% greater, respectively, than the average values of healthy plants. For field A, a trend toward a reduction in trunk diameter of plants subjected to continuous heavy disease pressure was again evident (9% reductions) as compared with healthy plants (Table 2). However, the regression equation ($Y = 46.3 - 1.78\,ID - 9.63\,FD + 11.71\,(FD)^2$, $R = 0.18$) was not statistically significant ($P = 0.05$).

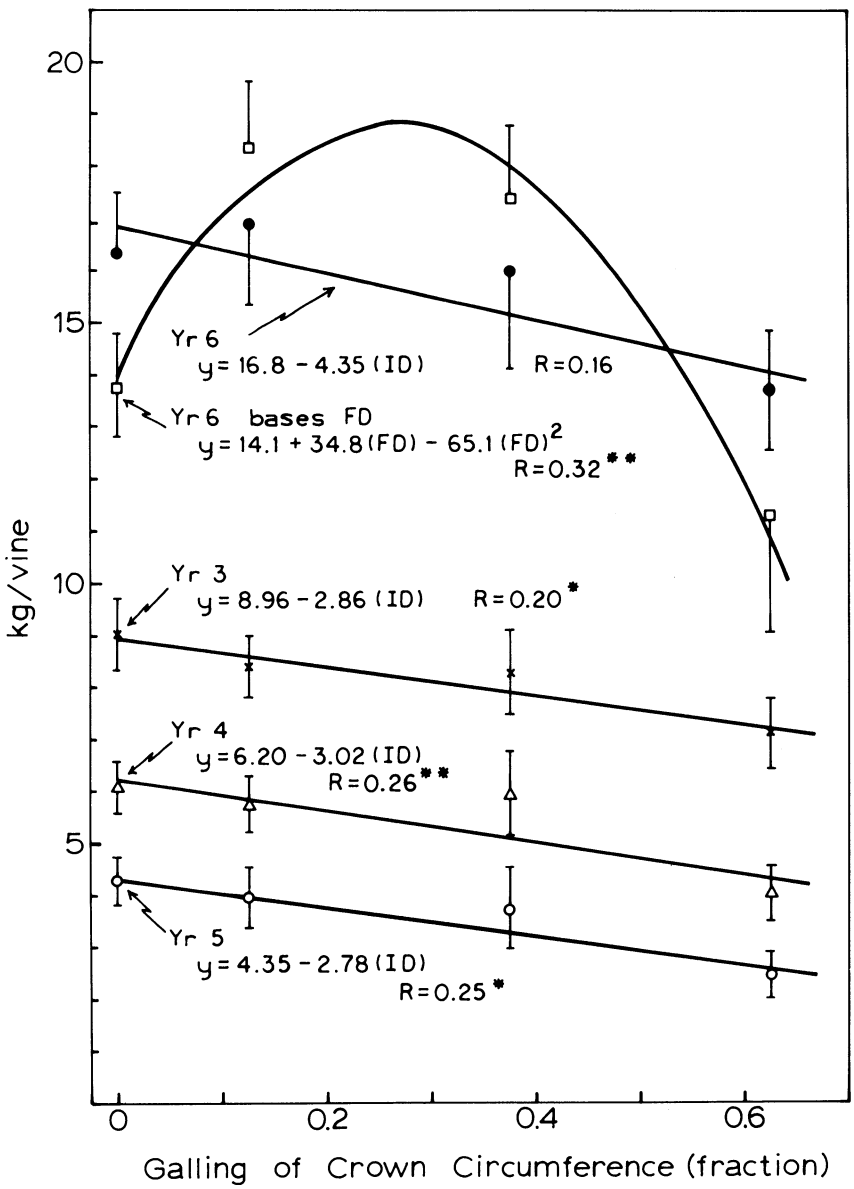

Fig. 2. Relationships between crown gall disease severity and berry yields for field C. Meaning of terms in the regression equations and additional details on regression analysis are given in Table 2. Unless otherwise specified, plots are a function of the initial disease severity rating. Bars indicate standard error.

DISCUSSION

The results of this study quantitatively show the importance of crown gall in reducing the vigor and yield of grapevines. The data are in line with the early observations of Garcia and Rigney (6), who observed that there were no noticeable effects on slightly diseased vines but that vigor, growth, and productiveness were reduced in badly affected plants. When plant performance was assessed on the basis of disease ratings made in the spring of year 3, berry yield and pruning weights were reduced in all 4 yr for severely diseased plants, although the data were not statistically significant in year 6. However, the trunk diameters of severely diseased plants in year 6 were significantly smaller than those of the healthy controls, indicating a substantial reduction in overall vigor.

Although the regression equations were statistically significant ($P = 0.05$), the overall correlations were not high in most cases. Of the various factors that contribute to reducing correlations, two were of particular importance. The disease severity rating of each plant was a subjective evaluation. Thus, this indepen-

dent variable had its own variance, which could be considerable. In addition, the assigning of plants to disease severity intervals also introduced considerable variance, since plants with ratings at the low or high end of the interval were assigned the median value during regression analysis. Although this approach makes presenting the data easier (less cluttered graphs and tables), the disadvantage is that it increases the variability observed in regression analysis.

An analysis of the effects of diseases on plant performance would be incomplete without attempting to account for changes in disease patterns that occur over the years. Significantly higher correlations between plant performances and disease ratings were observed when initial and final severity ratings were considered jointly (Table 2) vs. separately (Figs. 1–3). This supports the view that consideration of disease pressure over time is important in assessing plant performance. The establishment of the continuous disease pressure categories, however, has faults in that it was not feasible to determine when new galls were formed or when old galls died and were sloughed off. For example, some of the galls observed in the year 6 disease index could have developed during the season rather than in the intervening years. On the basis of continuous disease pressure, those few vines that were severely diseased at the beginning and end of the test period also produced substantially fewer berries in year 6 than did the continuously healthy plants that had no galls at either observation.

The finding that plant performance was increased when subject to continuous mild disease pressure was not a complete surprise in that table grape growers routinely girdle plants to improve yields. It also is of interest to note that *Agrobacterium* and tumor tissues produce both auxin and kinetin hormones (2). There were some inconsistencies in the stimulation phenomenon, notably among the trunk diameters in field A for year 8 (Table 2) and the berry yields in field C for year 3 (Table 2). This was not unexpected in that we believe stimulation is associated with recent or continuous mild disease pressure and we could not closely follow the disease dynamics.

It would have been desirable to have continued the study over a long time period to determine some of the more subtle effects of the crown gall disease on plant health, such as predisposition to other pests. It also would have been helpful because of the yearly variation encountered. Environmental disturbances can account for some of the variation. Starlings and other birds that feed on grapes are a problem, as they have selective feeding patterns, generally preferring vines with reduced canopy. The large yield reduction observed for plants in the high disease severity index category in year 5 was ascribed to the fruit-stripping activity of birds. The net effect was that the berry stripping by the birds compounded and magnified the apparent low yields of the less vigorous plants.

The determination of the effect of disease on yield and vine vigor as presented in this study can be deceptive because of the dynamic nature of the disease and because only one location, time period, and situation were involved. For example, grapes in California are grown in relatively arid environments, and aerial galls are not common. This same planting in a more humid environment or in an environment where winter freezes are frequent could become a disaster. Freezes are dangerous when there is a source of bacteria present, as many new portals of entry are generated. A rare freeze in year 6 demonstrated this point, as vines in low-lying areas were injured and consequently many aerial galls developed. Fortunately, such galls usually dry up and disappear in arid climates. Thus, when assessing losses caused by the crown gall disease, it is important to consider how various environmental and cultural factors affect disease severity as well as the long-term effects of the disease. Once present in orchards and vineyards, the inoculum poses a potential threat for the life of the planting. Case in point is the debacle that occurred when the new owners of the vineyard decided to graft the Zinfandel vines over to white grapes after the termination of the study. The graft unions became heavily diseased, and the grafts did not take. The inoculum either came from galls or was already in the vines because of its systemic nature (3,9,15).

The effects of the crown gall disease on plant health may be quite subtle and escape detection for years. The disease could well hasten the eventual decline of a vineyard, even in areas where aerial galls are not common. For example, Farm Advisors in California have observed with *Prunus* species that diseased plantings are usually removed 5–10 yr before crown-gall-free plantings. This is why in a 1963 study of crop losses and disease-control costs in California

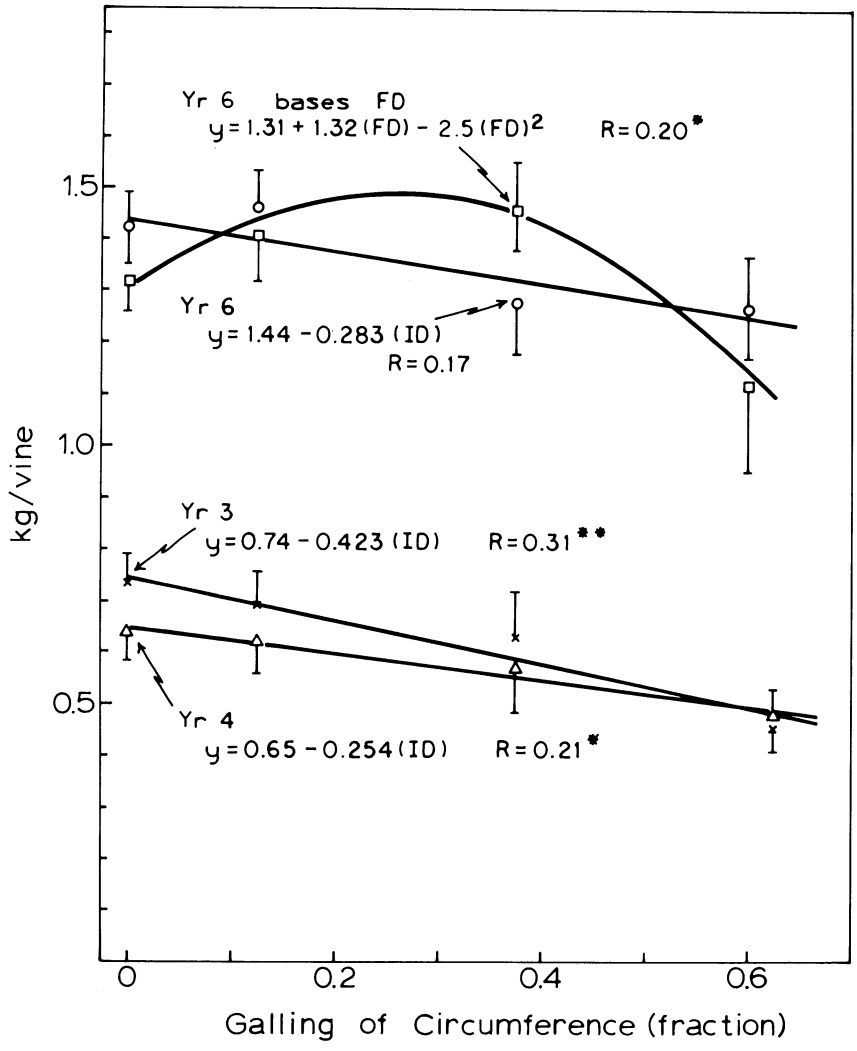

Fig. 3. Relationships between crown gall disease severity and cane prunings for field C. Meaning of terms in the regression equations and additional details on regression analysis are given in Table 2. Unless otherwise specified, plots are a function of the initial disease severity rating. Bars indicate standard error.

(1), the crown gall disease was rated as one of the three most important pathogens on eight different crops.

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