Analysis of Progress and Spatial Pattern of Corky Bark in Grapes

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ABSTRACT

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The corky bark disease of grapevine is believed to be caused by a viral agent. The present study provides information on the distribution of the infection in a commercial vineyard of the seedless cultivar Thompson. An analysis of temporal progress of infection from April 1983 to April 1990 shows a gradual increase in the cumulative number of infected vines with a logistic rate parameter of 0.598 per year. The spatial pattern of infection was clustered in the first three monitoring dates. However, as the incidence of infection increased, the pattern became random. Biological explanations for the phenomena are discussed.

The collection of data on spatial distribution, the determination of spatial patterns, and their interpretation have helped in understanding the process by which a pathogen is disseminated in fields (1,6, 16,23). Little is known about the spatial arrangement of grape (*Vitis* spp.) viruses, and for the corky bark disease knowledge is practically nonexistent (22). The corky bark disease of grapevine was first described by Hewitt (9) in California and named "rough bark." Graft transmission performed in California in 1959 was the basis for its designation as a viral disorder (4). The disease has since been reported from many grapevine-growing countries in South America, Europe, and Japan. In Israel, it was first identified in 1982 in a Thompson Seedless vineyard.

Cultivars and rootstocks differ in their susceptibility to the disease. Some are symptomless carriers or exhibit only mild symptoms such as poor growth, while others suffer rapid decline. This causes difficulties in determining the economic effects of the disease. The disease is graft-transmissible and transmitted by budwood. Mechanical transmission trials were negative. However, vector transmission was proven by us in laboratory tests (21). The etiology of the disease is unknown, but there have been a few reports of viral particles found in infected tissues and associated with the disease (2,7,14,15).

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In view of the recent information about the insect transmission of the corky bark disease agent and several other grape viruses (8,19,20), there was an interest in determining if the spatial distribution of corky bark phenomena in the field corresponds to a disease that is vector-borne. Therefore, this study undertook monitoring of infection in the plot followed by an analysis of its spatial pattern. Approaches used for analyzing spatial pattern are given in reference 5 (see also Madden and Hughes [12] for a review).

MATERIALS AND METHODS

A commercial vineyard of Thompson Seedless (=Sultanina) grafted on rootstock 1613 was chosen for the survey. The plot is located in the Lakhish region in southeastern Israel. Climatic conditions in this area are suitable for this cultivar, and the Lakhish Sultanina grapes are famous for their high yield and quality. The vineyard is planted on lime soil in a hilly region and is drip-irrigated. The vineyard chosen is surrounded by others in which the same variety is grown on various rootstocks.

The surveyed vineyard was planted in 1981 at a 3 × 1 m spacing with the rows oriented from east to west. Within 2 years of planting, reduction in growth vigor was noted and abnormalities were observed in some plants, accompanied by leaf symptoms. At the first monitoring date, there were 45 infected plants, based on symptoms and indexing. In the following year, trunk swelling and corky bark were observed. On peeling the bark lengthwise, cracks, pitting, and grooving were found. The vineyard was surveyed twice yearly for symptoms on the trunk, canes, and leaves, and diseased plants were labeled. The grove included 16 rows, with 50 vines in each row. Assessment of infection was conducted in April 1983, 1984, 1985, 1986, 1987, 1989, and 1990, and in December 1983. Samples from diseased plants were tested in the laboratory by graft indexing on a series of indicator plants, including Rupestris du Lot, LN-33, Mission, Kobber TBB, and Baco 22A. These were grown in containers in a screenhouse and observed for symptoms for 2 to 3 years.

Data analysis. A logistic model was fitted to disease incidence data (y) as a function of time (t). This model is given by the equation (eq. 1): $y = k/[1 + (k/y_0 - 1)\exp\{-rt\}]$ in which y_0 is the initial amount of infection, r is an infection rate parameter ($r > 0$; units of yr^{-1}), and k is the maximum disease incidence ($0 < k \leq 1$). Parameters r and k were estimated by an ordinary least-squares procedure using a nonlinear regression program available on the SAS computer package.

The grove was divided into M quadrats of $n = 800/M$ vines each, and the number of infected vines showing symptoms out of the maximum of n in each quadrat was counted. A beta-binomial distribution (BBD) was fitted to disease incidence data at each assessment time for each of the grove divisions, with $n = 5, 10, 20, 40$. This distribution, introduced by Hughes and Madden (11) for describing aggre-

Fig. 1. Corky bark symptoms on Sultanina X41B at the graft union.

gated disease incidence data, depends on two parameters, p and θ, representing the expected value of the variable probability of infection estimated as mean disease incidence, and an index of an aggregation, respectively. The special case $\theta = 0$ corresponds to binomial distribution (BD). Thus, a random spatial pattern of infected plants is indicated when the value of θ is close to or equal to 0. A program written in FORTRAN by Madden and Hughes (10) was used for obtaining maximum likelihood estimates of p and θ and calculating chi-square and goodness-of-fit tests to BBD and BD for each assessment time. When the number of degrees of freedom (df) of a chi-square statistic was ≤0, the significance probability of the test was set to zero. The program also provides a normal score called $C(\alpha)$ statistic for testing the null hypothesis of randomness ($\theta = 0$) in favor of the alternative that BBD is appropriate ($\theta > 0$).

An alternative method for determining the spatial pattern of infected vines is based on the distance of an infected vine from its nearest infected neighbor. This approach was used by Marcus et al. (13) to analyze the spatial distribution of citrus tristeza virus disease (see Campbell and Madden [5, pp. 324-325] for a discussion). Maps of the distribution of infected vines were drawn for each assessment time. The distance from each infected vine to its nearest infected neighbor was measured. The following statistic for randomness, proposed by Clark and Evans (6), was used: $CE = [\bar{d} - E(\bar{d})]/[\text{var}(\bar{d})]^{1/2}$, where $\bar{d}$ is the average nearest neighbor distance, and $E(\bar{d})$ and $\text{var}(\bar{d})$ denote the expected mean and the variance of the distances. Approximations for $E(\bar{d})$ and $\text{var}(\bar{d})$ calculated for a number N of infected vines within a grove of area A and a perimeter L, as given by Ripley (18), were $E(\bar{d}) = 0.5(A/N)^{1/2} + 0.514L/N + 0.412L/N^{3/2}$ and $\text{var}(\bar{d}) = 0.0703A/N^2 + 0.037(A/N^5)^{1/2}$.

The null hypothesis of randomness was rejected in favor of nonrandomness or clustering if $CE < Z_P$, where Z_P is the lower P-th percentile of the standard normal distribution.

RESULTS

The typical symptoms of corky bark as seen in the vineyard are depicted in Figures 1 and 2. These symptoms resemble those described by Beukman and Goheen (4). Grafted LN-33 indicator plants exhibited typical CB symptoms: swelling and cracking of the canes, grooving of the stem, and in some cases leaf distortion and chlorotic spots.

The total numbers of infected vines in the grove at the different assessment times from 1983 to 1990 were 45, 53, 65, 119, 125, 137, 174, and 193. The number of newly infected vines increased initially, reached a maximum when disease incidence was approximately 0.15, and then decreased. The estimated parameters of the logistic curve given in eq. 1 were: r = 0.598/yr (asymptotic standard error [SE] = 0.075/yr) and k = 0.244 (SE = 0.017), respectively. The coefficient of determination, R^2, defined by 1 – [(error sum of squares)/(uncorrected total sum of squares)], was 0.994. Figure 3 presents the observed and predicted values of infection incidence. Predicted values were obtained by fitting the logistic model given in eq. 1.

The distribution of infection for the years 1983, 1985, and 1990 is presented in Figure 4. The grove was divided into M quadrats, so that the corresponding quadrat sizes were n = 5 (five vines per row), 10 (10 vines per row), 20 (two rows and 10 vines per row), and 40 (four rows and five vines per row). For each of the four grove divisions and at each of the eight disease assessments made in the grove, the number of infected vines out of the maximum of n in each quadrat was recorded and the BBD fitting program was carried out. The results are presented in Tables 1–3. Table 1 gives the maximum likelihood estimates of p and θ and goodness-of-fit statistics to BD and BBD when the grove was divided into M = 40 quadrats of n = 20 vines each. Table 2 presents the corresponding observed and expected (binomial and beta-binomial) frequencies for corky bark data in spring 1983 and 1990 for n = 20. Table 3 gives the maximum likelihood estimates of θ and significance probability of chi-square goodness-of-fit tests to BD and BBD for quadrat sizes n = 5, 10, and 40 vines. The symbols * and ** indicate nonrandomness at the 0.05 and 0.01 significance levels, respectively; the symbol – indicates that the number of df of the chi-square test was ≤0.

The results are summarized as follows: dividing the grove map into quadrats of different sizes had, as expected, no effect on the mean disease incidence (p), which increased over time, and very little effect on its standard error. For example, in April 1983, p = 0.056, SE = 0.009 for n = 5, and SE = 0.011 for n = 40; in April 1990, p = 0.241, SE = 0.016 for n = 5, and SE = 0.019 for n = 40. Tables 1 and 3 show that at each assessment time, θ and its standard error decreased with n. For example, in April 1984, when p = 0.081, θ decreased from 0.137 (SE = 0.056) when n = 5 to 0.048 (SE = 0.025) when n = 40. For each of the four quadrat sizes, θ increased to a peak at around p = 0.081 and then decreased. At the earliest assessment time, when p = 0.056, the chi-square goodness-

Fig. 2. Pitting and grooving caused by corky bark shown after bark peeling.

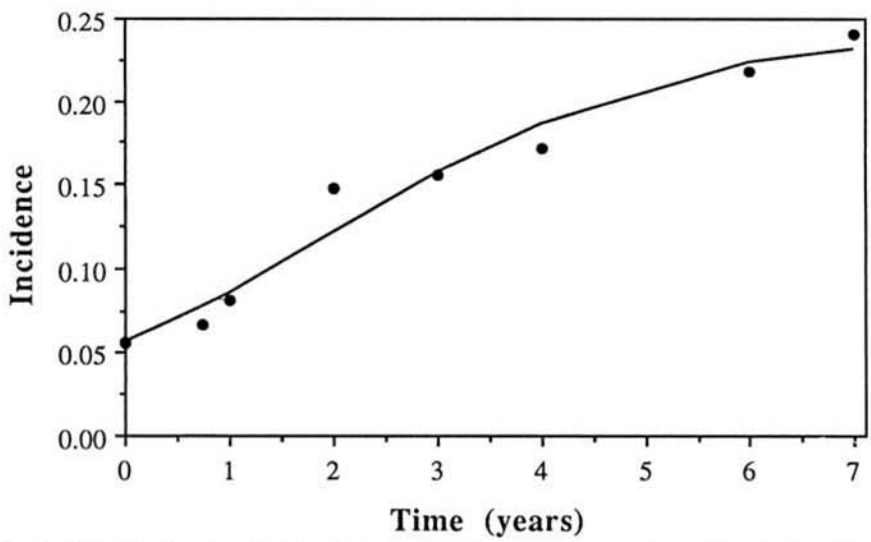

Fig. 3. Observed (•) and predicted (—) disease incidence for corky bark data. Time refers to years after April 1983.

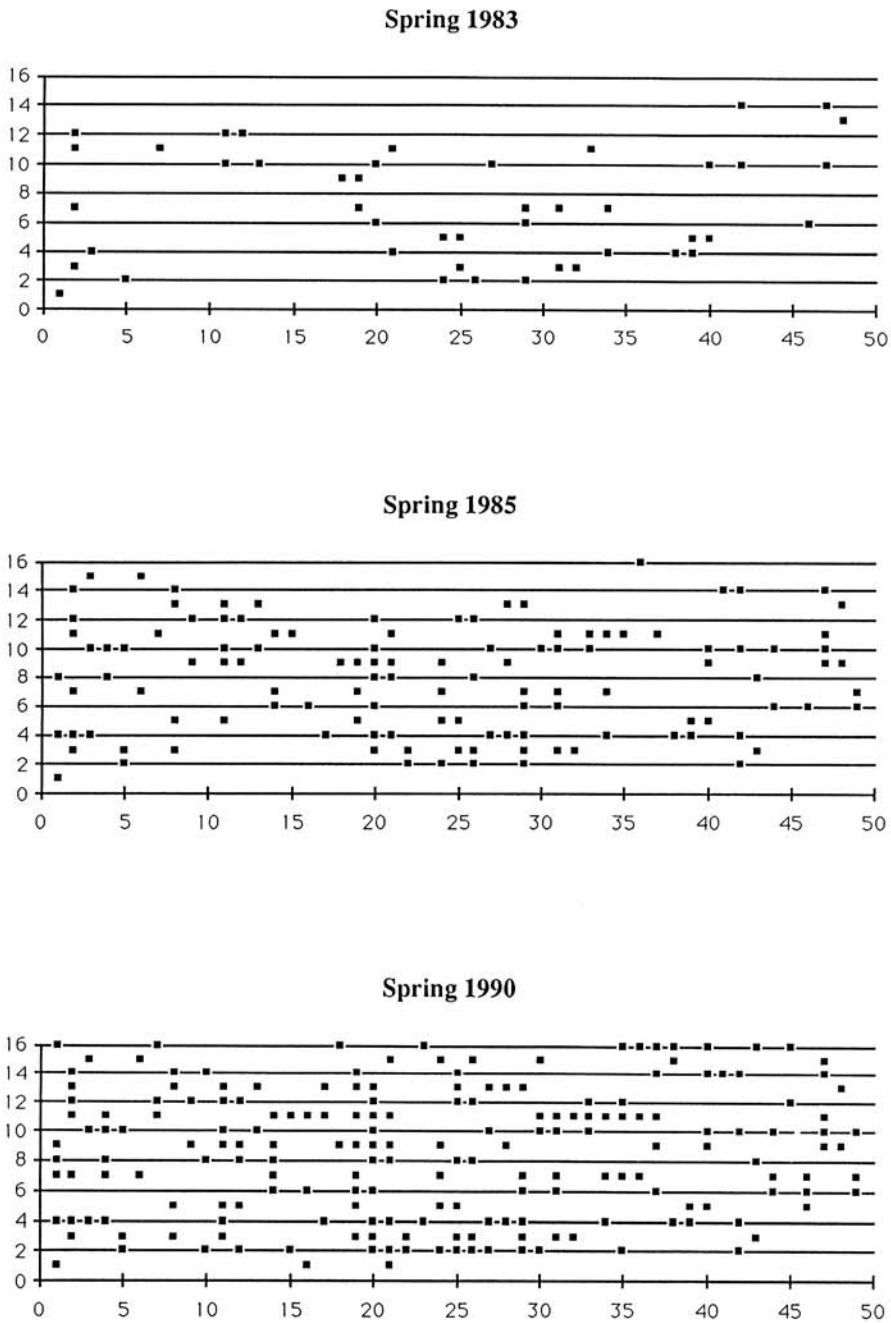

Fig. 4. Distribution of corky bark–affected grapevines cultivar Thompson Seedless (Lakhish, Israel) at three assessment dates.

of-fit tests to BD were significant ($P < 0.05$) for $n = 10$ and 20, and not significant for $n = 40$; when $0.066 \leq p \leq 0.081$, the tests to BD were significant ($P < 0.01$) for $n = 5$, 10, and 20. When $p \leq 0.081$, the chi-square goodness-of-fit tests indicated (when df ≥ 1) that a BBD provided an appropriate fit to the observed frequencies data and a significant improvement over a BD. When $p \leq 0.081$ and the number of df of the chi-square tests was ≤ 0 ($n = 5$ or 40), the standard-normal score of the $C(\alpha)$ test indicated that the hypothesis of randomness (BD) was rejected in favor of clustering (BBD). When the incidence of infection reached or passed the level of 0.148, a random pattern of infected vines was noted. This was true except in the case of $p = 0.148$ (in April 1985), where infected vines still exhibited a clustered pattern ($P < 0.05$) for grove division with $n = 10$.

The *CE* statistic was calculated for different assessment times. For this grove, $A = 2{,}550$ m^2 and $L = 202$ m. The average nearest neighbor distances in April of the years 1983, 1984, and 1985 were: $\bar{d} = 4.74$, 4.05, and 3.44 m, respectively. The corresponding calculated mean, variance, and *CE* values were $E(\bar{d}) = 6.58$, 5.04, and 3.33 m, $[\text{var}(\bar{d})]^{1/2} = 0.30$, 0.21, and 0.11 m, and $CE = -6.13$, -4.95, and 0.91. Thus, the use of the *CE* test indicated that the hypothesis of randomness of corky bark–infected vines was rejected ($P < 0.01$) for the first three assessments when infection incidence was ≤ 0.081; whereas for other assessments, when $p \geq 0.148$ it was not rejected.

DISCUSSION

Two methods were used to analyze the spatial pattern of corky bark in grapes at eight assessment times from 1983 to 1990. One approach was based on fitting discrete distributions to count data consisting of the number of infected vines per quadrat; the second method was based on distance measurements. It was shown that a BBD, which required estimating two parameters (p and θ), provided an acceptable fit to the

Table 1. Beta-binomial parameter estimates and goodness-of-fit tests for corky bark in a vineyard in the Lakhish region of Israel (when each quadrat consists of 20 plants)

Date	Parameter estimates[a]				Goodness-of-fit[b]					
					Binomial			Beta-binomial		
(mo, yr)	p	SE	θ	SE	χ^2	df	P	χ^2	df	P
04, 1983	0.056	0.010	0.044	0.028	4.714	1	0.030	0.549	1	0.459
12, 1983	0.066	0.012	0.069	0.029	9.078	2	0.010	0.143	1	0.705
04, 1984	0.081	0.014	0.070	0.031	8.084	2	0.017	0.561	1	0.454
04, 1985	0.148	0.016	0.041	0.020	3.279	3	0.349	0.663	2	0.717
04, 1986	0.156	0.016	0.038	0.020	4.664	3	0.197	1.728	2	0.418
04, 1987	0.171	0.017	0.035	0.020	4.254	2	0.117	3.601	2	0.163
04, 1989	0.218	0.016	0.009	0.014	0.343	3	0.965	0.540	3	0.914
04, 1990	0.241	0.018	0.021	0.016	1.045	3	0.790	1.661	3	0.645

[a] p = Mean disease incidence; θ = estimated aggregation index; SE = standard error.
[b] χ^2 = Chi-square goodness-of-fit statistic; df = degrees of freedom, determined by pooling frequency classes so that expected frequencies were >5; P = significance level.

observed frequencies data when infected vines exhibited an aggregated (i.e., clustered) pattern.

The spatial pattern of infected plants varied with time. For each of the four grove divisions, the estimated aggregation parameter of the BBD increased over time to a peak of $p = 0.081$ and then decreased. This finding is similar to that obtained by Hughes and Madden (10), who fitted BBD to other virus diseases. The variation of θ with p was related by Hughes and Madden (10) to a power law describing a relationship between p and the variance. Depending on the assessment time, either randomness or clustering could be found (10). The significant P values of the goodness-of-fit tests to the BD and BBD depended on the number of quadrats of the grove division.

Analyzing the data by the average nearest neighbor method also showed that at the first three assessments, when $p \leq 0.081$, infected vines exhibited a clustered pattern ($P < 0.01$). However, when $p \geq 0.148$, randomness was indicated.

An increase in virus infection over space and time could be attributed either to biological spread or to slow development of symptoms in infected budwood, which could be discerned only after a prolonged period. We tend to accept the first explanation, as one would expect a reduction rather than an increase in the number of newly infected plants during each time period if the budwood had become infected before planting. The pattern of infection in the plot does not suggest that it derives from infected propagation material. By default, it may suggest vectorial transmission. The reduction in the infection rate over time may be caused by adverse climatic conditions or by an agricultural practice that affects vector activity and thereby the rate and shape of virus spread.

Spatial distribution of infection may be affected by the form of virus transmission by the vector. Thresh (23) brought up examples of spatial patterns of persistent and nonpersistent viruses. A simulation of forms of spread as a consequence of virus–vector dependence was proposed by Berger and Ferris (3). The mode of transmission of the corky bark disease by the mealybug vector is not yet characterized. However, since closteroviruses were shown to be transmitted semipersistently by their aphid vector (17), we believe that if spread was vectorial, it could be semipersistent, too. In view of the new findings on mealybug transmission of certain viruslike disease agents (20,21) in grape, we are now testing their potential transmission of corky bark.

Table 2. Observed and expected (binomial and beta-binomial) frequencies for corky bark data

	April 1983			April 1990		
Diseased vines per quadrat	Observed frequency	Expected binomial frequency	Expected beta-binomial frequency	Observed frequency	Expected binomial frequency	Expected beta-binomial frequency
0	18	12.62	17.35	0	0.16	0.42
1	9	14.99	10.93	4	1.02	1.76
2	7	8.45	5.98	2	3.07	3.86
3	4	3.01	3.06	4	5.86	5.88
4	0	0.76	1.48	8	7.92	6.95
5	2	0.14	0.69	9	8.06	6.73
6	0	0.02	0.30	5	6.41	5.53
7	0	0.00	0.13	3	4.07	3.94
8	0	0.00	0.05	0	2.10	2.46
9	0	0.00	0.02	5	0.89	1.35
10	0	0.00	0.01	0	0.31	0.66
11	0	0.00	0.00	0	0.09	0.29
≥12	0	0.00	0.00	0	0.02	0.15

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Table 3. Estimated values of θ (and standard error [SE]) and goodness-of-fit tests for beta-binomial (BBD) and binomial (BD) distributions for corky bark in a vineyard for different quadrat divisions

	Quadrat size											
	5				10				40			
Mo, yr	θ	SE	BBD[a]	BD	θ	SE	BBD	BD	θ	SE	BBD	BD
04, 1983	0.060	0.042	–	–	0.044	0.026	–	*	0.032	0.016	–	ns
12, 1983	0.131	0.058	–	**	0.089	0.039	ns	**	0.048	0.021	–	*
04, 1984	0.137	0.056	–	**	0.091	0.038	ns	**	0.048	0.025	–	–
04, 1985	0.047	0.033	ns	ns	0.040	0.023	ns	*	0.024	0.016	–	ns
04, 1086	0.041	0.032	ns	ns	0.041	0.024	ns	ns	0.029	0.019	–	ns
04, 1987	0.054	0.034	ns	ns	0.046	0.026	ns	ns	0.032	0.020	–	ns
04, 1989	0.029	0.029	ns	ns	0.019	0.020	ns	ns	0.007	0.010	–	ns
04, 1990	0.042	0.031	ns	ns	0.039	0.024	ns	ns	0.015	0.014	–	ns

[a] – = Number of df of chi-square test was ≤0; * = nonrandomness at the 0.05 significance level; ** = nonrandomness at the 0.01 significance level; and ns = not significant.

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