Evidence for Random Local Spread of Aphid-borne Mild Yellow-edge Virus in Strawberries

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ABSTRACT

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The spread of aphid-borne strawberry mild yellow-edge virus was studied along short rows in a field in western Oregon during a 37-mo period. The number of infected plants adjoining other infected plants (doublets) was compared with the total number of infected plants per plot. The methods of van der Plank and Freeman were used to predict doublet occurrence, but we revised their methods for computing variance. The pattern of doublet occurrence approximated the pattern predicted from a random distribution and is consistent with the assumption that mild yellow-edge virus infections occurred at random within plots.

Additional key words: *Chaetosiphon fragaefolii*, *Fragaria* × *ananassa*.

Mild yellow-edge virus (MYEV) is a major virus pathogen affecting strawberry. The literature on this virus was reviewed by Mellor and Frazier (9). It is aphid-borne, and in the Pacific Northwest region of the United States, where MYEV is common, the strawberry aphid, *Chaetosiphon fragaefolii*, occurs widely. The rate, season, and general pattern of spread of MYEV and the seasonal population trends of *C. fragaefolii* have been studied (2,4,8,11). Techniques for studying the patterns of movement of pathogens from plant to plant were considered by Bald (1), Cochran (3), Freeman (5), Iyer (6), Kranz (7), Pielou (10), Swed and Eisenhart (12), Todd (13) and van der Plank (14,15).

The detailed pattern of spread of MYEV among adjoining plants in fields has not been investigated. This article describes the pattern of local spread of MYEV in plots with short rows (usually five plants) in a strawberry planting in western Oregon. If virus spread resulted from movements of viruliferous aphids between adjoining plants along the row, maps of the resulting infections should show a clumping pattern of contagious distribution instead of a pattern of random spread.

MATERIALS AND METHODS

Records were kept of virus infection of individual strawberry plants (*Fragaria* × *ananassa* Duch. 'Hood') grown in the hill system over a 37-mo period (1971-1974) in an experimental planting at the North Willamette Experiment Station in the Willamette Valley in Oregon. Before planting, the plants were determined to be free from known viruses by sample indexing. Throughout the period strawberry plantings of various ages, sprayed and unsprayed with insecticides, were near the experimental planting. Management and virus testing procedures in the planting have been described (8).

Virus movement was studied in 22 plots each consisting originally of five adjoining plants spaced 38 cm apart in a single row and two three-plant and two two-plant plots that developed at the outset of the test because of dead plants. Test rows were located in a larger planting that received similar treatment. Only virus spread within each plot was considered, and cross-row virus movement (107-cm row width) was ignored. *C. fragaefolii* was collected in the experimental planting during the course of the study and was presumed to be a major vector involved in transmission of MYEV. In 1974 the average population was seven *C. fragaefolii* apterae per 50 Hood leaves in the planting from June through September. Similar weekly counts for alate *Chaetosiphon* sp. in Corvallis, OR, were 10/50 from May through July 1973, and 2/50 in July 1974.

To detect movement of viruses from plant to plant, we modified methods proposed by van der Plank (14) and Freeman (5). Runner plants were allowed to form on the test plants each July and then were brought into the greenhouse for detection of MYEV. Otherwise test plants were kept free from runners. These runner plants were individually indexed for MYEV content by leaflet grafting to *Fragaria vesca* L. var *semperflorens* (Duch.) Ser. 'Alpine.' When data for individual plants in a plot were missing, the plot was reduced in size or split, as appropriate.

RESULTS

Statistical considerations. To detect spread of diseased plants in a sequence of diseased and healthy plants, van der Plank (14) suggested comparing the observed number of doublets (two adjacent diseased plants) with the expected number of doublets computed under the null hypothesis of a random distribution of diseased plants. To apply van der Plank's method to our situation, the idea of vacancies introduced by Freeman (5) was used.

Suppose that there are r rows of plants in a homogeneous area with the i-th row consisting of n_i plants. The total number of plants, N, is then $n_1 + \ldots + n_r$. Further suppose that M of the N plants are diseased. Let T denote the sum of the doublet counts in each of the r rows. If the r rows are placed together to form a single row with a

vacancy (a missing plant) between each row, then Freeman's basic formulae (5) can be used to compute the mean μ_T and the variance σ_T^2 of T. In our notation, these formulae are:

$$\mu_T = A\gamma_1$$
$$\sigma_T^2 = A\gamma_1 + 2B\gamma_2 + [A(A-1) - 2B]\,\gamma_3 - \mu_T^2$$

where $\gamma_1 = M(M-1)/[N(N-1)]$
$\gamma_2 = \gamma_1(M-2)/(N-2)$
$\gamma_3 = \gamma_2(M-3)/(N-3)$
A = number of adjacent pairs
B = number of dependent adjacent pairs.

Freeman gave formulae for A and B for a rectangular array of plants and claimed that a single row is a special case. His adjustments for vacancies are not correct for the single-row situation, but the correct formulae are easy to obtain. In particular, since row i has $n_i - 1$ adjacent pairs, $A = \Sigma_i(n_i - 1) = N - r$. To compute B we first mention that any two adjacent pairs are dependent provided that a common plant is involved in both pairs. Therefore, each row with $n_i \geq 2$ has $n_i - 2$ dependent adjacent pairs and each row with $n_i = 1$ has no dependent adjacent pairs. Thus, $B = \Sigma_i(n_i - 2) + f = N - 2r + f$ where f is the number of rows consisting of a single plant.

Biological data. Plants were classified each year as being MYEV-infected or healthy, and the positions of infected and healthy plants were recorded (Table 1). If two infected plants adjoined each other in a plot, they were classified as a doublet, as described by van der Plank (15).

As a preliminary test for clumping, we evaluated the homogeneity of binomial variances among plots as described by Cochran (3). This statistical test did not provide evidence for clumping.

Table 2 shows the observed number of doublets (T) in the plots (r) each year and the maximum possible number of infected plants (M) of N total plants that could be involved in doublets in plots each year (that is, the total number of plants in the plots that were determined to be virus-infected each year, regardless of the presence or absence of adjoining infected plants). In later years some single-plant plots occurred as a result of missing data and appropriate corrections were made when single-plant plots (f) occurred. Random clumping increases as M approaches N. Therefore, it is only when the degree of clumping significantly exceeds the clumping predicted by the random model that the observed disease pattern can be attributed to spread between adjoining plants. Table 2 also shows the expected number of doublets (μ_T) and associated standard error (σ_T) for all plots for each year, calculated under the null hypothesis of a random distribution of diseased plants.

In Table 2 we compare T and μ_T via the standardized variate $Z =$

TABLE 1. Occurrence of mild yellow-edge virus symptoms in selected plants of Hood strawberry in plots at Aurora, OR, from 1971 through 1974

		Year[a] symptoms appeared by plant and plot no.:							
Plant no.	Plot no.	1	2	3	4	5	6	7	8
1		73 OK⁻	73	74	73	73 OK⁻	OK	OK	74
2		72	72	73 OK⁻	73	73	74	72 OK⁻	74
3		72	73 OK⁻	OK	72	72	74	74	72
4		71	72 OK⁻	74	72	72 OK⁻	OK	72 OK⁻	73
5		73	73	72	73	73	72 OK⁻	74	73
	Plot no.	9	10	11	12	13	14	15	16
1		72	72	73	73	OK	73	73	72
2		73	73	OK	OK	72 OK⁻	OK	74	73
3		73*	73	72	72	72	OK	72	73
4		73	74	74	72	73	X	73	74
5		OK	OK	73 OK⁻	71	73	73 72	72	73
	Plot no.	17	18	19	20	21	22	23	24
1		72	73 OK⁻	73*	72	73 OK⁻	73 OK⁻	72 OK⁻	72 OK⁻
2		72	73	72	72	OK	72	73	72
3		X	72 OK⁻	71	74	73 OK⁻	74	72	73
4		72	73	73	73 OK⁻	72	72 OK⁻	73	73 OK⁻
5		72 72 OK⁻	73*	74	72	74	72	74	72

[a]The numbers 71, 72, 73, or 74 indicate the year the plant was infected with MYEV; 73* = plant infected in 1973 but was not indexed in 1972. OK = plant indexed free from MYEV 1971-1974. Year OK⁻ = plant indexed free from MYEV through the year shown but was not indexed thereafter. X = skip of one plant in the row.

TABLE 2. Comparison of the number of pairs of mild yellow-edge virus-infected Hood strawberry plants observed to be next to each other (doublets) in the same plot, with the number of doublets that would be expected in a random distribution, Aurora, OR, 1971-1974.

					No. of doublets			
					Cumulative no.			
Year	Total plants tested (N)	Cumulative no. of infected plants[a] (M)	No. of plots (r)	No. of single plant plots (f)	Observed (T)	Expected[b] (μr)	Standard error of no. expected[b] (σr)	Standardized value[b] (Z)
1971	120	3	26	0	0	0.04	0.20	−0.20
1972	120	35	26	0	9	7.89	2.11	0.53
1973	109	69	33	7	35	30.29	2.81	1.68
1974	98	86	37	12	50	46.91	2.06	1.50

[a]Yearly cumulative total number of plants found to be infected with mild yellow-edge virus, regardless of whether or not they adjoined infected plants.
[b]$\mu_T = A\gamma_1$ where $A = N\text{-}r$; and $\gamma_1 = M(M-1)/N(N-1)$;
$\gamma_2 = \gamma_1\,(M\text{-}2)/(N\text{-}2)$; and $\gamma_3 = \gamma_2\,(M\text{-}3)/(N\text{-}3)$
$\sigma_T = [A\gamma_1 + 2B\gamma_2 + [A(A-1) - 2B]\gamma_3 - \mu_T^2]^{1/2}$; where $B = N-2r+f$
$Z = (T - \mu_T)/\sigma_T$

$(T - \mu_T)/\sigma_T$ for the data from 1971, 1972, 1973, and 1974. If we assume that Z is approximately normal, then Z values are moderate in size even though our test is one-sided; ie, the alternative hypothesis is that of clumping, implying large values of Z. There appears to be no strong evidence for rejecting the null hypothesis of a random pattern of disease spread.

The data in Table 1 were subjected to analysis by the chi-squared analysis of Cochran (3) to detect evidence of clumping when spread was evaluated temporally instead of spatially. By Cochran's test, temporal clumping lacked statistical significance ($P = 0.05$) but approached significance as the data accumulated year by year. Because of the small size of the experiment, however, this should be regarded as only a preliminary study.

DISCUSSION

To deal with data from plots in several rows as van der Plank suggested (14), rows are combined to obtain one large row of N plants, and the randomness of the distribution is ascertained by using the doublet theory. Because the method of combining rows could influence the resulting doublet analysis, particularly when r is relatively large, we preferred the vacancy approach of Freeman (5) to obtain the doublet count in each plot and used the sum of these doublet counts to obtain a final doublet count.

When there is only a single plot, our expressions for μ_T and σ_T should agree with those given by van der Plank (1). There is a discrepancy between our standard error σ_T and that computed by van der Plank, which, in our notation, would be

$$\sigma_{Tv} = \sqrt{M(M-1)(N-2)/N^2}.$$

The reason for the discrepancy is that adjacent pairs are not independent, as assumed by van der Plank. Our standard error agrees with that computed by Iyer (6). Also, the standard error σ_{Tv} does not seem correct intuitively. As M approaches N (ie, the diseased plants saturate the plot), the standard error should approach zero, which does not happen with the expression σ_{Tv}. Our methods of calculation are applicable to one-dimensional patterns of disease spread, regardless of plot size.

In addition to deriving μ_T and σ_T for a single plot, Iyer (6) also showed that for large N the distribution of T is approximately normal unless M is very small, in which case a Poisson approximation is appropriate. Freeman (5) also implied that T is approximately normal as N gets large, provided that r remains finite. Thus, our use of the normal approximation for Z seems to be quite reasonable.

From studies of the pattern of movement of *C. fragaefolii* in England, Dicker (4) concluded that plant-to-plant movement of apterae was considerable. During a single season aphids generally did not move more than 2 m from the plants that were originally infested. Shanks (11) at Vancouver, WA, found that CV, MV, and MYEV were transmitted rapidly by *C. fragaefolii* over a short distance (3.7 m) in the field in one season to strawberry (cultivar Alpine) indicator plants. He reported that a few Alpine plants (6%) were infected 11 m from originally infected plants, but that no seasonal spread was detected by use of this method at greater distances, to 389 m. In both studies the data, although indirect, suggest that there should be a marked clumping of newly infected strawberry plants around existing infections. Our field observations in strawberry fields in the Pacific Northwest in 1976 and 1977 indicated that runs or clumps of weakened, presumably virus-infected plants, were common.

Direct measurement of the local pattern of mild yellow-edge virus spread in our test did not demonstrate the degree of clumping expected if MYEV had been spread predominantly between neighboring plants by apterous aphids. Therefore we assume that the MYEV infections that we detected occurred primarily by means of alate aphids, but we do not know whether these aphids originated mostly in the test planting or at a distance from it. The plots used in this study were small, probably reducing the sensitivity of the statistics used to detect differences between random and clumped patterns of virus spread. Research is needed involving the spread of MYEV in larger two-dimensional plots like those described by Freeman for hop viruses (5). Our study gave no strong evidence for rejecting the hypothesis that aphid-borne mild yellow-edge virus infections occurred at random.

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