Spatial Pattern of Downy Mildew in Hop Yards

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ABSTRACT

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Hop plants in hills in six hop yards in the Yakima Valley of Washington were assessed visually to determine the spatial and temporal pattern of hop downy mildew caused by *Pseudoperonospora humuli*. Hop shoots infected systemically with downy mildew were aggregated within the hill. Patterns of distribution were best described by the Negative Binomial distribution, and values of variance-to-mean ratio and Morisita's index were greater than one. Small clumps of hills with downy mildew were identified with doublet analysis in all yards. Evidence of clumps of diseased hills seemed to depend on the time since the yard was established. The youngest yards exhibited more aggregation of diseased hills than the older yards. Disease-incidence clumps consisting of a large number of hills were not found in any of the yards. There was no evidence for an increase in aggregation in the direction of the prevailing winds.

Pseudoperonospora humuli (Miy. et Tak.) Wils. causes one of the most serious diseases of cultivated hop (*Humulus lupulus* L.) in the Pacific Northwest of the United States. The pathogen, *P. humuli*, overwinters as mycelium in infected hop crowns in Washington state (22). Shoots growing from infected crowns in the spring may become invaded by the fungus. These systemically infected shoots are typically stunted, chlorotic, have down-curled brittle leaves, and are known as primary spikes. Sporangia are borne on the abaxial leaf surfaces of the primary spikes and infect the apical meristem of healthy hop shoots when environmental conditions are favorable (19,27). These are called secondary spikes and possess symptoms similar to primary spikes, except plant tissues below the infected area remain normal in appearance.

Epidemics of downy mildew in the Yakima Valley of south central Washington are usually of short duration because favorable weather in May is generally followed by unfavorable hot, dry conditions in June (12). Severe epidemics have occurred in the Yakima Valley an average of one in three years (12). The greatest losses in the Yakima Valley occur from crown infections that eventually result in death of crowns and stand reductions. Losses also result from reduced yields due to infections of main shoots, lateral shoots, flowers, and cones (19).

Disease assessment in yards and a disease-forecasting system are used to help manage hop downy mildew in Washington state (11,23). A knowledge of the pattern of downy mildew occurrence in hop yards would increase sampling efficiency for estimation of disease incidence (3,17). Hop is a perennial plant and is grown in hills placed in a regular, square pattern with a distance of approximately 3.13 m between hill centers. This common spacing in most hop yards should be beneficial in determining the pattern of downy mildew infection by taking into account the actual location of the sampling sites in each test yard. The objectives of this study were to determine the spatial and temporal pattern of downy mildew in hop yards and to show the benefit of using several spatial analysis methods.

MATERIALS AND METHODS

Six hop yards (5–10 ha each) of the cluster cultivars L-1 and Early Cluster, which are very susceptible to downy mildew, were chosen for study. Yards were located throughout the hop-growing area of the Yakima Valley of Washington state. Three yards were near the town of Moxee (Moxee I, II, III), one near Sunnyside (Sunnyside I), and two near Mabton (Mabton I and II). All hills in a rectangular or square section (2.9–4.1 ha) of each yard were assessed one to four times at 10- to 25-day intervals during May and June 1985 (Table 1) to determine the number of primary and secondary spikes at each hill. Many shoots arise from the perennial crown (19) and numerous axillary buds from lateral shoots on the four to eight main shoots selected to grow (19) up two to three strings 5.5 m high. All of these may potentially become infected and produce spikes. However, in 1985 not more than 15 spikes per hill were observed.

When a yard had less than a 102-hill width, the entire width was included in the study area. Otherwise, a width of 90 hills (190 m) was used. The length of the study section in five yards was 92 hills (194 m), and in the sixth yard it was 80 hills (169 m). The study section of each yard was selected by choosing a hill randomly to begin observations that would include the desired length and width. Dimensions of study sections in the yards were 90 × 92 hills at Moxee II and Mabton II, 80 × 80 hills at Moxee I, 85 × 92 hills at Moxee III, 88 × 92 hills at Sunnyside I, and 101 × 92 hills at Mabton I.

Disease incidence counts were combined into frequency categories (e.g., 0, 1, 2, 3, etc.). Adjacent classes that had small expected frequencies were pooled until the cumulative frequency exceeded one. A FORTRAN program developed by Gates and Ethridge (7) was used to calculate the chi-square goodness-of-fit statistic of the data to the Negative Binomial, Neyman Type A, Poisson, Poisson-Binomial, Poisson with Zeros, and Thomas Double Poisson distributions (13). For data fitting the Negative Binomial distribution, a method suggested by Cliff and Ord (5) may indicate the mechanism underlying the pattern of clumping of disease incidence observed. The basis of the Cliff-Ord procedure is to estimate the values of the parameters p and k of the Negative Binomial distribution for counts obtained for a series of successively doubled areas, in our case starting with one hill and ending with an area of 128 hills. The parameter p will remain constant in the case of true contagion (clustering), whereas the parameter k will remain constant in the case of apparent contagion (heterogeneity). True contagion could result from a process in which cluster centers are randomly located, and the disease incidence counts in each cluster are independent observations from some specified distribution. Apparent contagion could result from a Poisson (random) process in which the intensity of disease incidence varies across the hop yard. It is not possible to distinguish between these two processes by observing the distribution of the disease incidence counts, unless it is assumed that true contagion implies small clusters and apparent contagion implies clusters of disease incidence much larger than the individual hill (5).

Several indices of dispersion were used to provide measures of spatial clustering and estimates of the size of disease incidence clumps (26). The value of the parameter k of the Negative Binomial distribution generally decreases as aggregation increases. The ratio of the variance to the mean is a simple index to calculate and can be used to indicate a departure from randomness in a spatial pattern. The ratio is expected to be less than one for a uniform spatial pattern, equal to one for a random spatial pattern, and greater than one for a spatially aggregated population. The ratio generally increases as aggregation becomes more intense.

Morisita's index of dispersion was computed for counts obtained by grouping hills as was done for the Cliff-Ord procedure (16,20). Values of Morisita's index, compared to one, correspond to uniform, random, and aggregated distribution in the same manner as the variance-to-mean ratio. An index of clumping (IC) was computed by dividing Morisita's index for an area of × hills by Morisita's index for an area of 2× hills. The index of clumping was computed for the whole series of areas and plotted against area. The area at which IC is a maximum provides an estimate of the number of hills in a disease incidence clump.

A related method for identifying the size of clumps was suggested by Greig-Smith (9). The procedure amounts to the construction of a hierarchical analysis of variance in which the total variation is apportioned to the component variations at each area in the series of successively doubled areas. The sum of squares may be expected to reach a peak at the area corresponding to the number of hills in a clump. The peak will be maintained for larger areas provided that the clumps are not regularly spaced. An approximate F-test was used to indicate evidence of existence of clumps (25). Data for all dates at a location were combined for analyses to determine the pattern and spread of downy mildew through time.

A distance method, semi-variogram graphs, was used to measure the degree of correlation between population counts from spatially contiguous hills at successively larger distances (14). The distance over which the semi-variogram continues to increase indicates the range of influence of diseased hills on nearby hills. Thus, the semi-variogram could indicate the spread of infections and the direction of spread.

Doublet analysis was also used to examine aggregation of diseased plants and to indicate disease spread (4,8). In doublet analysis the observed number of adjacent diseased plants is compared with the number expected if the disease were randomly distributed in the field. If the observed number is greater than the expected number, contagion within the field is suspected. Doublet analysis was used to examine direction of spread in the direction of each axis of the rectangular hop yards, in the direction of the prevailing wind, and in the direction perpendicular to the prevaling wind.

RESULTS

Weather in 1985 favored development of a mild epidemic (12) of hop downy mildew in the Yakima Valley. Total rainfall at Sunnyside was 5.8 mm with two rainy days in May and 11.9 mm with six rainy days in June.

Disease progress curves for four yards are shown in Figure 1. Yards Moxee III and Mabton II were not included because disease observations were made only once and twice, respectively. Yard Moxee III had a disease incidence of 0.58%; disease incidence in yard Mabton II increased from 0.39% to 0.43%. Disease incidence increased with time at Sunnyside I, increased and then decreased because of mortality of infected shoots (19) in two yards, and decreased in the fourth yard (Fig. 1). The incidence of primary spikes decreased in the four yards. However, the incidence of secondary spikes increased in the yards (Fig. 1). The percentage of secondary spikes that occurred in hills without primary spikes was 14 at Mabton I, 29 at Mabton II, 42 at Sunnyside I, 35 at Moxee I, and 43 at Moxee II. The patterns of infected hop plants with primary, secondary, and both primary and secondary spikes in five yards are illustrated in Figure 2.

The goodness of fit of each probability distribution to the incidence of primary disease of the first sampling date at each location and the combined primary and secondary disease

TABLE 1. Summary of indices for incidence of primary and secondary infections of *Pseudoperonospora humuli* in hop plants in six locations at various dates

Location	Date[a]	Best-fitting distribution[b]	*P*-value[c]	Cliff-Ord index[d]	K	Var./mean	Index of clumping[e]	Greig-Smith peak[e]
Sunnyside I	5/15*	NTA	0.577		0.006	2.22	8H, 16V, 64H, 64V	64H, 64V
	5/28	NB	0.725	T	0.005	2.42	32V	32V
	6/10	NB	0.528	T	0.004	1.60	32H	**
	6/21	NB	0.104	T	0.007	5.31	8H, 4V, 32H	8H, 4V, 32H, 64V
	All	NB	0.293	T	0.011	4.73	4H, 8V	8H, 32H, 4V
Mabton II	5/14*	NTA	0.553		0.004	2.69	**	**
	5/24	NB	0.006		0.004	4.39	**	**
	All	NB	0.004		0.006	3.90	**	**
Moxee III	5/10*	NB	0.589	N	0.006	2.61	32H, 16V	32H, 16V
Moxee II	5/13*	NB	0.827	T	0.011	2.04	**	**
	5/31	NB	0.113	T	0.011	2.72	**	**
	6/19	PB	0.469		0.013	1.54	4H, 16H, 32V	4V, 16H, 32V
	All	NB	0.272	T	0.026	2.34	**	**
Mabton I	5/7*	NB	0.356	N	0.003	4.42	**	**
	5/22	NB	0.003		0.003	5.15	64V	128H, 64V
	6/5	NB	0.551	T	0.003	3.78	**	**
	All	NB	0.354	T	0.006	4.89	**	**
Moxee I	5/9*	NB	1.000	N	0.012	2.84	**	32H, 128H
	5/23	NB	0.004		0.009	4.35	**	**
	6/17	NB	0.806	T	0.009	3.02	**	**
	All	NB	0.007		0.018	3.97	**	**

[a] All primary data.

[b] Distribution that best fits the data according to the chi-square goodness-of-fit test. NTA = Neyman Type A; NB = Negative Binomial; and PB = Poisson-Binomial.

[c] *P*-value for the chi-square goodness of fit of the data.

[d] Cliff-Ord index applied only when data conforms to the Negative Binomial distribution ($P > 0.10$). Type of contagion identified as true (T), apparent (A), or neither (N).

[e] Number of hills in a disease incidence clump consisting of 4, 8, 16, etc. hills. H = horizontal orientation used for rectangular blocks consisting of contiguous hills; V = vertical orientation used for rectangular blocks consisting of contiguous hills; and ** = no indication of clumps of diseased plants for the block sizes examined.

incidence of the other sampling dates indicated that the Negative Binomial provided a better model than the other distributions considered for most locations and dates (Table 1). The Cliff-Ord procedure indicated that true contagion appeared likely in many of the data sets examined (Table 1). True contagion for data fitting the Negative Binomial distribution ($P > 0.10$) would indicate a Poisson process generating a random distribution for location of diseased hills with disease incidence in a hill modeled by a logarithmic series distribution (5). For the logarithmic series distribution, the probability of occurrence of a particular clump size was inversely related to the size of the clump. The variance-to-mean ratio was significantly ($P \leqslant .01$) greater than one for the incidence of primary disease of the first sampling date at each location and the combined primary and secondary disease incidence data from the other locations and dates (1). For dates combined, the ratio was lower for Moxee II and III and higher for Moxee I, Sunnyside I, and Mabton I and II. The estimated values for the Negative Binomial parameter k were consistently small for all locations and dates (Table 1). Morisita's index was numerically larger than one for all locations and dates.

Peaks in the value of IC and the Greig-Smith sum of squares as well as approximate F-tests (25) indicated the clump size at Sunnyside I for all of the dates and dates combined (Table 1). The size of the clumps ranged from 2× 2 blocks of 4 hills to 8× 8 blocks of 64 hills. Clumping was also indicated for one date for each location except Mabton II. With the exception of block size 64 for the first date at Sunnyside I, the appearance of clumps depended on whether a horizontal (H) or vertical (V) orientation with respect to axes of the rectangular hop yards was used for combining hills into rectangular blocks.

Semi-variogram graphs were generally horizontal, indicating no spatial autocorrelation among disease incidence in contiguous hills. The semi-variograms for the combined primary and secondary data for all dates combined at Sunnyside I indicated some spatial autocorrelation and periodic behavior. The direction of spread could not be determined because the semi-variogram graphs appeared similar in all directions.

The doublet analysis indicated aggregation in most locations because the number of occurrences of two adjacent diseased plants was greater than the expected number (Table 2). An approximate z-test (4) indicated that there was significant aggregation in all four directions for Sunnyside I and Mabton I and significant aggregation in three directions for Moxee III. The other yards had significant z-values for one or two directions. There was no evidence of an increase in disease spread in the direction of the prevailing wind with the doublet analysis.

DISCUSSION

Spatial patterns of plant pathogens are seldom truly random or regular in nature (24). Diseased plants and pathogens are often found to be aggregated (2,3,6,18,20,21,24). The pattern of disease is not static; powdery mildew on wheat leaves changed from an aggregated to random pattern as the epidemic progressed through the growing season (18). Citrus canker changed from a nonaggregated pattern in the early stages of disease development to an aggregated pattern after secondary spread had occurred (6).

An aggregated, rather than random, pattern can be expected for many polycyclic plant diseases, such as hop downy mildew, because new infections are more likely to occur near an inoculum source (2,6,21). Royle and Kremheller observed in England and Germany that dispersal of primary inoculum of *P. humuli* in hop yards was short-range and that secondary infections began on leaves and shoots neighboring primary spikes (19). This is because primary spikes are close to the ground and often sheltered within a canopy of healthy basal shoots (19). We found aggregation of disease within the hill as shown by a good representation of the frequency distribution of the data by the Negative Binomial

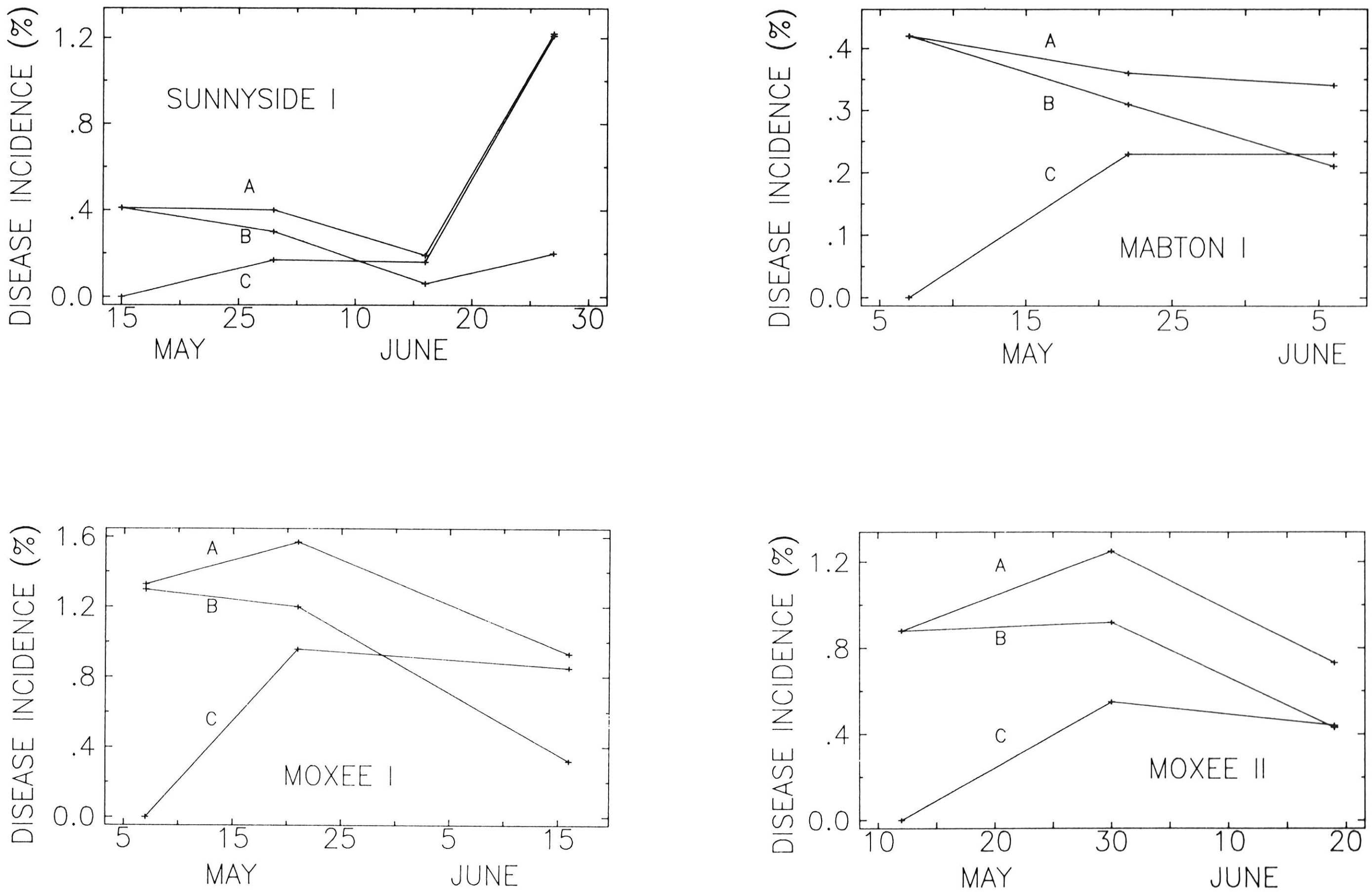


Fig. 1. Disease progress curves of hop downy mildew, caused by *Pseudoperonospora humuli*, in four hop yards in Washington State in 1985. Total disease incidence (curve A), incidence of hills with primary infections (curve B), and incidence of hills with secondary infections (curve C) are shown.

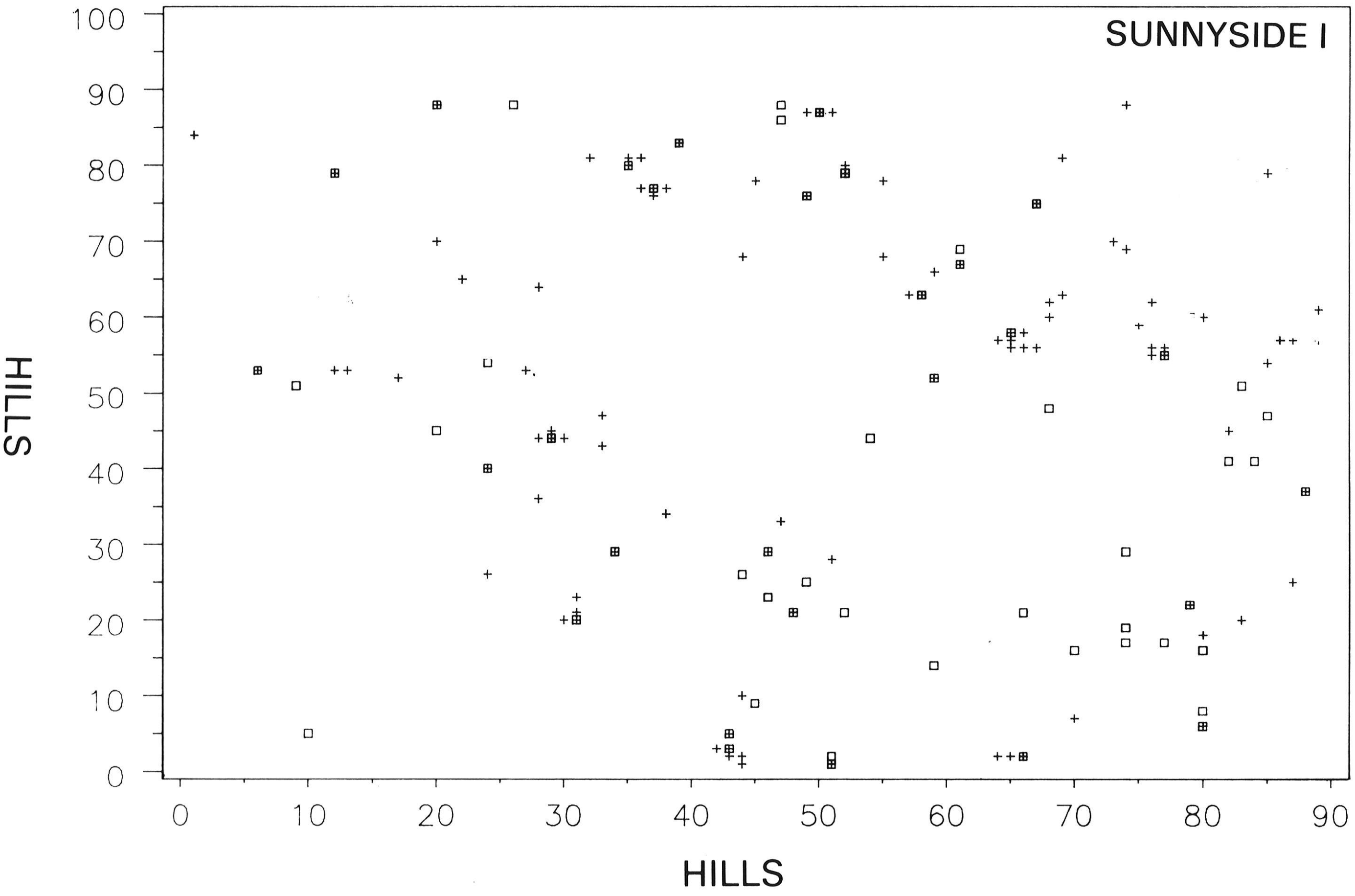
distribution, variance-to-mean ratios greater than one, and Morisita's index greater than one. The doublet analysis identified small clumps of diseased plants in all yards. Evidence of aggregation between nearby hills, as shown by the indices of dispersion, was seen at Sunnyside I and Moxee III. The periodic behavior of the semi-variogram for Sunnyside I could indicate a succession of diseased and nondiseased zones in the yard. This provides limited evidence of disease spread between hills, although the direction of spread could not be determined because the semi-variogram graphs appeared similar in all directions. The low rainfall that was unfavorable for rapid disease increase may have been partly the reason for not detecting more aggregation between nearby hills. We observed more secondary infections within hills that had primary spikes, but nearly 33% of the secondary spread was to hills without primary spikes. Spread of downy mildew during years with low rainfall is important in the seasonal carryover and buildup of initial inoculum of *P. humuli* for following years in the Yakima Valley (10,12).

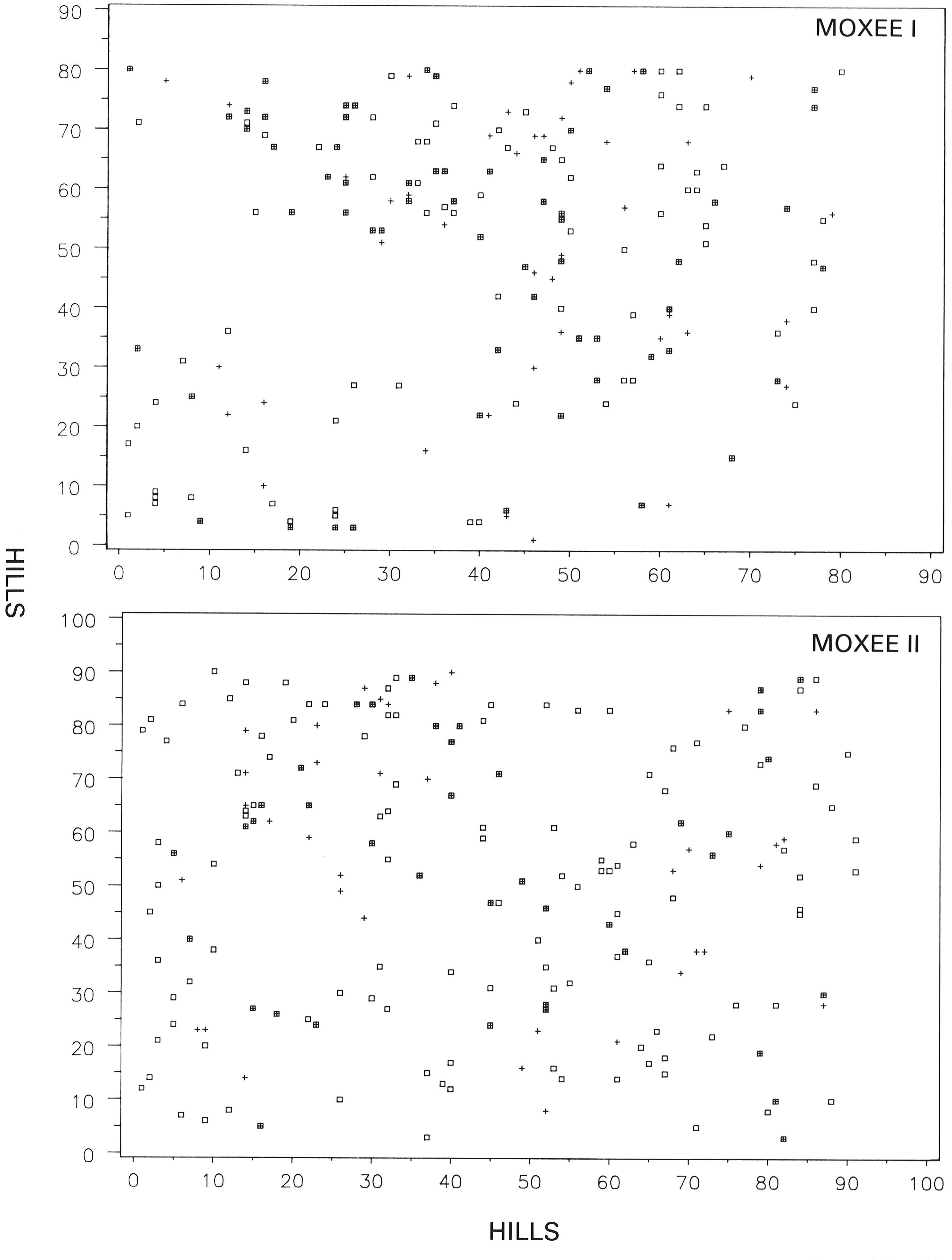
The time since the hop yard was established affected the pattern of downy mildew. Hop plants in yards Moxee III, Sunnyside I, and Mabton I had been planted for five, six, and 20 years, respectively. The other three yards had been in production for 25, 30, and 40 years. The three youngest yards had significant aggregation of adjacent diseased hills in at least three directions as shown by doublet analysis (Table 2), and aggregation of hills with disease was identified in the two youngest yards with the indices of clump size. Many cycles of infection would have occurred over time in the older yards. Depending on winter weather, usually several years pass before a crown dies after initial infection occurs. The dead plant is then replaced with a healthy plant. The older yards would have had more crowns die and be replaced over time than the younger yards, and a more aggregated pattern could have been changed to a less aggregated pattern.

There was no evidence for aggregation in the direction of prevailing winds, which are from the southwest. Sporangia of *P. humuli* are short-lived when detached from the sporangiophore, so sporangia that are carried in the prevailing winds to healthy foliage during dry weather soon die. The wind at ground level during rainfall in the spring in the Yakima Valley can be from any direction (Claude B. Graves, Jr., *personal communication*). Therefore, an aggregation in the direction of the prevailing winds would not be expected.

In summary, there appeared to be aggregation of disease incidence within the hills for nearly all yards for all dates. However, evidence of clumps of diseased hills seemed to depend on the age of the yard. The youngest yards exhibited more aggregation of nearby hills than the older yards. Clumps consisting of large numbers of hills with disease were not found in any of the yards. The use of several statistical methods allowed a more complete interpretation of the spatial pattern of disease than would have been possible with fewer methods of analysis.

The incidence of hop hills with downy mildew was relatively low in the six yards sampled, ranging from 0.2% to 1.6%. Knowing the pattern of small populations of diseased plants, especially of a very susceptible cultivar, is important in making disease management decisions and in increasing sampling efficiency for a polycyclic disease like hop downy mildew, which can rapidly increase. Initial inoculum of *P. humuli* is a major factor in development of severe epidemics in the Yakima Valley partly because the duration of epidemics are relatively short due to the environment (11). Hop plants with disease and small clumps of diseased plants were scattered throughout all the yards (Fig. 1). A scattered arrangement of disease foci or inoculum sources throughout the yard creates a high potential of rapidly infecting a large proportion of the healthy hop plants when environmental conditions become favorable for disease spread because of the relatively close proximity of inoculum to most healthy plants in the yard. It is most profitable to monitor hop yards with relatively low incidence of downy mildew so that controls may be applied before additional spread occurs. As few as one spike in 500 hills, or 0.2% incidence, can produce sufficient sporangia and subsequent infections during favorable weather to cause considerable damage (15).

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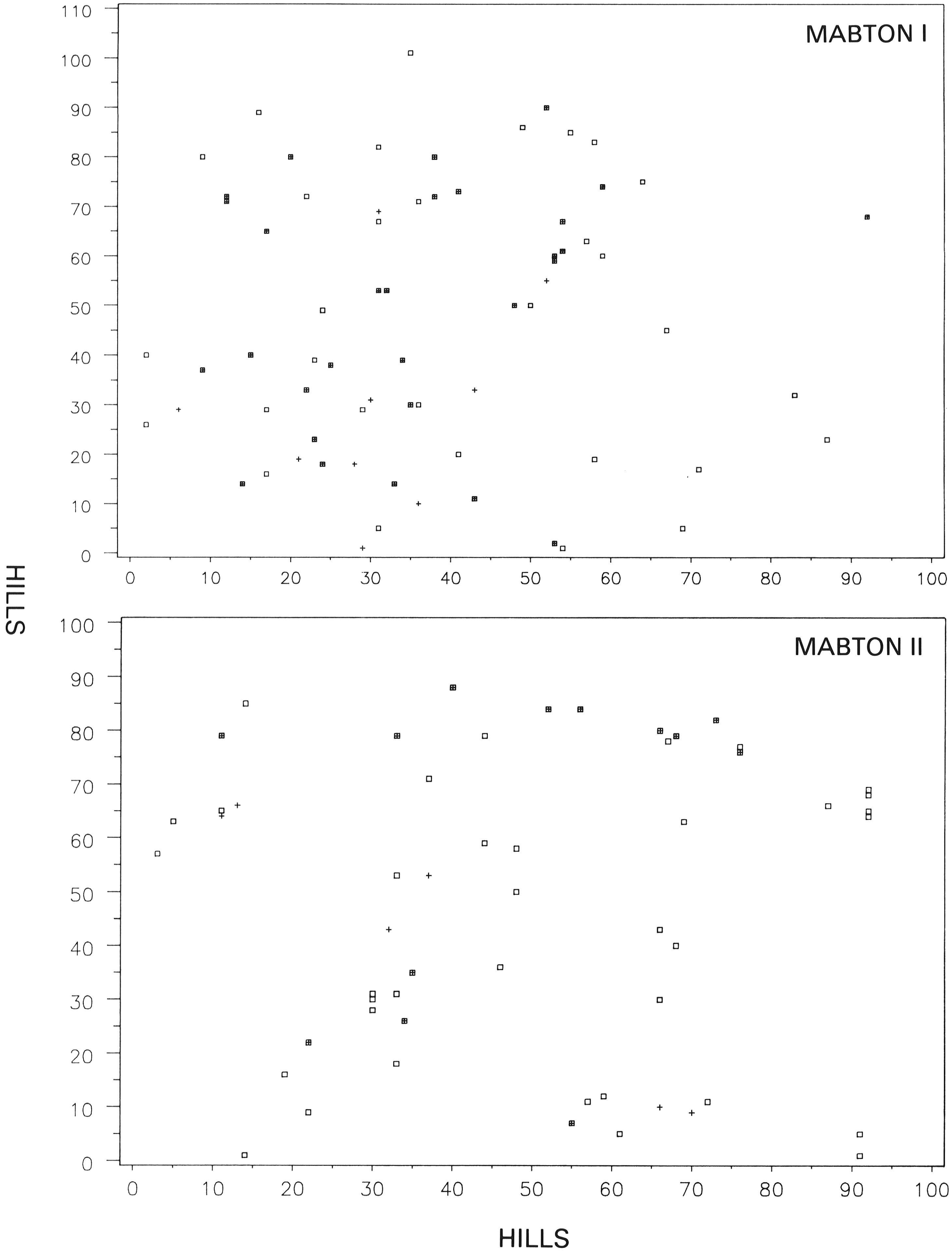

Fig. 2. Pattern of hop plants systemically infected with *Pseudoperonospora humuli* at five locations. Hop hills with primary (□), secondary (+), and both primary and secondary infections (⊞) are shown.

TABLE 2. Doublet analysis of hop plants with primary and secondary infection of *Pseudoperonospora humuli* in six hop yards

	Number of doublets				
	Expected	Observed			
Field	All directions[a]	Y	X	Diag.[b]	Diag.[c]
Sunnyside I	2.1	12[d]	22[d]	9[d]	11[d]
Mabton II	0.4	5[d]	0	1	0
Moxee III	0.3	2[d]	2[d]	3[d]	0
Moxee II	3.8	4	8[d]	5	10[d]
Mabton I	0.5	2[d]	2[d]	2[d]	2[d]
Moxee I	4.6	8	8	11[d]	3

[a] The expected number of doublets was the same for the four directions when values were rounded to the nearest tenth.

[b] Doublets in predominant wind direction.

[c] Doublets in direction perpendicular to predominant wind.

[d] Significant aggregation ($P = 0.05$) according to approximate z-test.

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