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Spatial Pattern of Verticillium Wilt in Commercial Mint Fields

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ABSTRACT

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Spatial patterns of mint plants with symptoms of Verticillium wilt caused by *Verticillium dahliae* were characterized in 10 commercial mint fields in Washington using several spatial analysis methods. The disease was assessed in 0.76-by-0.76-m quadrats (width of mint rows was 0.76 m) in randomly selected study sections varying in size from 5 to 76 m wide by 57 to 396 m long. The variance-to-mean ratio identified aggregation of diseased stems within quadrats even though probability distributions associated with cluster data did not fit the data well. Generally, there was more clustering within than across rows according to both doublets and runs analyses. Total number of wilt foci ranged from 5 to 170 per field, and mean size of foci ranged from 1 to 2.7 quadrats. In one field observed repeatedly, total foci increased from 24 to 104, and the mean size of foci increased from 1.0 to 1.3 quadrats in the same section of the field from one year to the next. Size of foci increased to 2.7 quadrats in a third year of sampling the same field. Mean focus size was larger within than across crop rows in 10 of 13 field-sampling occasions. The proximity index ranged from 0.88 to 1.00, indicating highly compacted disease foci. The statistical methods employed were useful in describing, quantifying, and visualizing spatial patterns of infected mint in commercial fields. Verticillium wilt spread during the life of the perennial mint crop. Inoculum for much of the secondary increase likely did not directly originate from microsclerotia present in soil before the crop was planted or from infected rhizomes that originally were planted.

Additional keywords: *Mentha × piperita*

Verticillium wilt, caused by the vascular wilt pathogen *Verticillium dahliae* Kleb., is a limiting disease of cultivated peppermint, *Mentha × piperita*, and Scotch spearmint, *M. × gracilis* (*M. cardiaca*), two of the three main mint species produced commercially for essential oils in western and midwestern states. The third mint species, Native spearmint (*M. spicata* L.), can be infected by *V. dahliae* but is less susceptible to this fungus than the other two mint species (20,21). Verticillium wilt of mint is characterized by chlorosis, stunting, asymmetric development of apical leaves, shortening of internodes, and plant mortality (3,13). Initial inoculum for the disease consists of microsclerotia present in soil before the crop is planted and infected rhizomes used for planting (9,25,33). Microsclerotia persist in the soil for about 13 years and function as primary inoculum (33).

Verticillium wilt is managed by planting *V. dahliae*-free mint rhizomes in nonin-

fested soil (9). Growers in the Columbia Basin attempt to obtain pathogen-free rhizomes by growing cuttings from pathogen-free plants in clean potting mix in the greenhouse, and then increasing plants in the field on ground that has not been used previously to produce mint. Limited tillage (27), soil fumigation (15), crop rotation (10), and deep plowing (8) are only partially or temporarily effective in managing Verticillium wilt.

Patterns of diseased plants and pathogens often are aggregated (1,18,32) and the degree of aggregation and amount of change in aggregation over time is a property of populations in an agroecosystem (39). Microsclerotia of *V. dahliae* were aggregated in soil in commercial potato fields in Ohio and Oregon (22,36). Microsclerotia of *V. dahliae* in cauliflower fields in California usually had a low degree of aggregation, but infected cauliflower plants had a random pattern (40). Disease patterns of Verticillium wilt in mint fields have not been characterized.

Statistical analyses of spatial pattern data of diseased plants are often complex (7,30); however, a simple alternative to the currently available analyses was proposed by Nelson (30). The latter method describes and summarizes populations of disease foci from mapped data. Computer software, called FOCI, is available for analysis but is limited to a disease cluster of 400 (e.g., 20-by-20 matrix) or fewer

plant positions. However, it often is desirable to characterize relatively larger sections of fields (18,19,40), and many more points would be required. Nelson's method may be used as a direct supplement to 2DCLASS, a software program to perform two-dimensional (2D) distance class analysis for characterizing spatial relationships of diseased plants (7,31).

Mint is a perennial crop and Verticillium wilt increases annually within mint fields. For example, the number of Verticillium wilt clusters (groups of infected stems/100 m of transect that were counted while walking through commercial fields of Scotch spearmint from 1999 to 2003) was 0.14, 1.00, 2.10, 19.9, and 26.1, respectively, during 5 years in a field by Royal City WA, and 1.1, 7.1, 53.0, 63.4, and 107.0, respectively, during 5 years in a field by White Swan, WA. (17). The source of inoculum for the annual increase is thought to be microsclerotia present in soil prior to planting the crop. However, this has not been demonstrated and is not supported by the characteristic rapid increases of Verticillium wilt observed in mint fields after the second year of crop growth (17). Spatial analysis of disease patterns would help to provide a better understanding of the source of inoculum and how Verticillium wilt spatially spreads within mint fields. Control practices then could be directed or devised to restrict spread of the disease within fields. The objective of this study was to characterize the spatial pattern of mint plants with symptoms of Verticillium wilt in commercial fields to aid in understanding the nature of the spread of the disease. Statistical analyses designed to assess spatial associations of diseased plants within quadrats, in adjacent quadrats, and in sequences of quadrats were used. In addition, populations of disease foci were described and summarized using Nelson's methods (30), with expanded software developed to handle large disease clusters.

MATERIALS AND METHODS

Ten mint fields, ranging in size from 9 to 50 ha, were chosen for study (Table 1). Fields were selected to give a range of Verticillium wilt incidence observed in commercial fields. Fields were located near Prosser, Othello, Royal City, and George, WA. One field was planted to Native spearmint, three to Scotch spearmint, and six to peppermint cv. Black Mitcham. Wilt incidence data were collected in five fields in 1995, in five additional

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fields in 1996, and in one of the 1996 fields from 1997 through 1999, for a total of 13 data sets. All fields were furrow irrigated except for George I, which was sprinkler irrigated by a center pivot system. Except for Prosser I, Royal II, and Othello I, fields had not been planted previously to mint. Prosser I had been used to produce two previous mint crops, but had not been cropped to mint within 5 years of this study. Royal II had a mint crop more than 20 years prior to this study, and Othello I had a mint crop approximately 10 years prior to this study.

In 1995, 1996, and 1997, mint stems in rectangular sections (0.32 to 1.09 ha in size) of each field were assessed visually in June and July for symptoms of Verticillium wilt. Symptomatic plants showed severe chlorosis, crescent-shaped leaves, severe stunting, and stem necrosis. Diagnosis was confirmed by recovering *V. dahliae* from symptomatic stem tissue placed on moistened filter paper in petri dishes and on potato dextrose agar. Healthy mint plants were at least 35 cm tall. The study section of each field was selected by choosing randomly a row and distance from the edge of the field to begin observations that would include the width and length of the study section. In all directional analyses, degree zero was defined as parallel to the first crop row of the sampled area. The baseline was perpendicular to the first row. Distances between pairs of infected plants and between clusters of infected plants were determined with a tape measure within each row of the studied areas. Each row was divided into multiple quadrats that were 0.76 m in length and 0.76 m in width (standard row width for mint in Washington). The dimensions of studied sections varied from 5 to 76 m wide (6 to 100 quadrats) by 57 to 396 m long (75 to 521 quadrats), depending on incidence of the disease (Table 1). The plot location and the number of infected plants

in each quadrat were recorded. Verticillium wilt incidence was recorded as the percentage of quadrats containing diseased stems.

Disease incidence data were collected in the same section of Royal II in 1996 and 1997. Because of a high incidence of Verticillium wilt in this field in 1998 and 1999, the studied section in Royal II was divided into thirds and data were collected from two adjacent rows in each third in 1998 and 1999, except for the first third in 1998, wherein four adjacent rows were used. The subsections of adjacent rows were selected randomly.

The data collected from each field were analyzed by multiple methods, which are introduced in this paragraph and described in detail in following paragraphs. Distribution fitting and variance-to-mean ratios were used to assess spatial patterns within quadrats (29). Both doublet and runs analyses (2,6) were used to search for clumps of two to three quadrats containing one or more diseased stems (diseased quadrat). In an earlier study, doublets analysis was more sensitive than runs analysis at detecting aggregation of adjacent quadrats (18). Runs analysis is designed to be more sensitive for larger sequences of quadrats. The Greig-Smith quadrat variance analysis was used to search for larger clusters of diseased quadrats. Additional methods were used to characterize populations of disease foci and to perform a 2D distance class analysis of the spatial arrangement of diseased plants (7,31). Counts of infected stems in quadrats were used for calculating variance-to-mean ratios and distribution fitting. Binary data for the presence or absence of mint stems with Verticillium wilt symptoms in quadrats were used for doublets, runs, Greig-Smith quadrat variance, disease focus, and 2D distance class analyses. The observed data commonly are referred to as spatial point patterns data in geostatistical literature. The aforementioned

methods were applied to detect and describe the departure of spatial point patterns from randomness.

The index of dispersion, defined as the variance to mean ratio \times one less than the number of quadrats (n), has an approximate χ^2 distribution with $n - 1$ degrees of freedom under the assumption of randomness. Therefore, values of the index of dispersion greater than the critical χ^2 value indicate that the variance to mean ratio is significantly larger than 1 and, thus, indicate aggregation (38).

A FORTRAN program developed by Gates and Ethridge (5) was used to calculate the χ^2 statistic to evaluate goodness-of-fit of the data to the Poisson, negative binomial, Thomas double Poisson, Neyman Type A, and logarithmic with zeroes distributions to frequencies of diseased quadrats in each field (23). The negative binomial, Thomas double Poisson, Neyman Type A, and logarithmic with zeroes distributions may be used to indicate aggregation in spatial data whereas the Poisson indicates a random spatial pattern. The program pooled adjacent categories that had small expected frequencies until the cumulative frequency exceeded 1.

Doublet analysis (2) compares the observed number of pairs of adjacent diseased quadrats with the number expected if disease were distributed randomly along a transect of quadrats. If the observed number is greater than expected, a clustering of diseased plants is suspected. The expected number of doublets and the standard deviation of the total number of doublets were corrected to allow adjacent transects of quadrats to be combined into a single long sequence of quadrats (2). Doublet analysis was used to examine spread in the 0 and 90° directions.

Runs analysis (6) was used to examine the spatial association between Verticillium wilt-infected plants in nearby quadrats. A runs analysis was used to examine

Table 1. Field location, mint species, year sampled, age of crop, dimensions of sampled sections, total number of quadrats in sampled area, and incidence of Verticillium wilt in 10 commercial mint fields used to analyze spatial patterns of mint infected with *Verticillium dahliae*

Field	Species	Year sampled	Crop age (years)	Dimensions ^a		Total quadrats	DI (%) ^b
				Distance (m)	Quadrat		
Prosser I	Scotch	1995	1	156 × 42	205 × 55	11,275	0.95
Royal I	Scotch	1995	5	396 × 27	521 × 36	18,756	0.46
Othello I	Native	1995	1	156 × 42	205 × 55	11,275	0.09
Othello II	Peppermint	1995	1	204 × 46	268 × 60	16,080	0.23
Othello III	Peppermint	1995	4	57 × 57	75 × 75	5,625	3.88
George I	Peppermint	1996	5	103 × 76	135 × 100	13,500	2.06
Othello IV	Peppermint	1996	1	131 × 43	173 × 56	9,688	1.32
Othello V	Peppermint	1996	2	150 × 27	196 × 36	7,056	1.23
Othello VI	Peppermint	1996	2	164 × 41	216 × 54	11,664	1.09
Royal II	Scotch	1996	2	122 × 47	160 × 62	9,920	0.24
Royal II	Scotch	1997	3	122 × 47	160 × 62	9,920	1.31
Royal II	Scotch	1998	4	122 × 6	160 × 8 ^c	1,280	13.52
Royal II	Scotch	1999	5	122 × 5	160 × 6 ^d	960	15.63

^a Distance in meters (m) and number of quadrats (quadrats) for the length and width of sampled section.

^b DI = disease incidence: percentage of quadrats containing plants with symptoms of Verticillium wilt.

^c Study section used in 1996 and 1997 was divided in thirds and four adjacent rows were sampled in the first third, two adjacent rows the second third, and two adjacent rows in the last third of the field.

^d Study section used in 1996 and 1997 was divided in thirds and two adjacent rows were sampled in each third.

aggregation of quadrats with diseased plants compared with the mixing of quadrats with and without diseased plants (designated diseased and healthy quadrats, respectively) that would be expected for a random pattern of disease. A run was defined as a succession of one or more healthy or diseased quadrats followed and preceded by a quadrat of the other disease status, or no quadrat at all (boundary of study area). Few runs occur if there is an aggregation of diseased or healthy quadrats, and a large number of runs occur for a random distribution of diseased and healthy quadrats. Under the null hypothesis of randomness, the expected value and standard deviation of the total number of runs in each row may be obtained from equations presented by Madden et al. (26). All quadrats contained mint plants, so there were no missing values. The ordinary runs analysis (6) was modified to allow for multiple rows of observations (18). A row was defined as a transect of contiguous quadrats in the specified direction terminated by the boundary of the study section of the field. A test statistic with an asymptotic normal distribution will have a large negative number if there is clustering; therefore, the test for aggregation is left-tailed. The runs test was performed in the 0 and 90° directions of each field.

A method suggested by Greig-Smith was used to indicate the approximate size of disease clusters (12,29,34). The number of diseased stems in each quadrat was counted. Adjacent pairs of quadrats were then combined to give oblong, two-quadrat blocks that were twice as large and half as numerous as the original quadrats. Adjacent two-quadrat blocks were combined to give square four-quadrat blocks, and so on, up to blocks of 128 quadrats. The total variation about the mean for single-quadrat blocks then was apportioned in a hierar-

chical analysis of variance, and the components of variation were plotted against the number of quadrats in successively doubled block sizes. A peak in variation indicated a clump in diseased plants of size equal to the number of quadrats in the corresponding block. The peak will be maintained for larger blocks provided the clusters are not regularly spaced. An approximate *F* test (37) was used to indicate the existence of clusters by identifying the cluster size associated with the Greig-Smith value that first exceeded a 0.01 critical *F* value. Peak values that were greater than the *F* value and that were both preceded by smaller variation and followed by smaller or nearly constant variation values were interpreted as indicating a cluster.

The analysis of disease foci offers a simple and direct description of disease foci and reduces the complexity of mapped data (30). In this work, a focus was defined as a set of diseased units such that, for any unit in the set, one of its adjacent neighboring sites also is contained in the set, where an adjacent neighbor site can be an adjacent site on the left, right, up, down, or one of the four diagonally adjacent sites (30). The analysis of disease foci then consists of the calculation of focus number (*N*) and focus size (*s*). Focus size was defined as the number of diseased quadrats in a disease focus, the maximum row (*r*) and column (*c*) distances spanned by a focus, and the proximity index (*PI*), defined as *s/rc*. *PI* may be used as an indication of compactness of focus organization (30). For example, a *PI* value of 1 indicates that all sites in the rectangle of *r* rows and *s* columns are diseased, whereas a *PI* of 0.5 indicates that only 50% of the sites in the rectangle are diseased. Nelson's method (31) for the analysis of disease foci was implemented in S-Plus (Insightful Corp., Seattle, WA) to handle large datasets.

Distance class analysis (7,31) characterizes spatial relationships of diseased plants and is useful in detecting departures from spatial randomness. It utilizes binary data, which represent presence or absence of disease in a particular quadrat or lattice position within a rectangular field plot. It complements the previous four methods by quantifying the association between quadrats containing diseased stems of all distance separations. The distance separation between a pair is measured by the absolute differences between their two coordinates. A distance class, denoted by *X,Y*, consists of pairs that share the same distance separation *X* and *Y*. The count frequency of a distance class is the number of pairs in the distance class. This count frequency is standardized to adjust for the total number of pairs on the lattice. A random sampling scheme is used in the 2D class analysis to determine the critical value of the standardized count frequency (*SCF*) of a distance class, under the assumption that the disease occurred randomly. If the observed *SCF* of a particular distance class is greater than the critical value, the distance class is declared to be significant.

RESULTS

Percentage of quadrats containing plants with symptoms of *Verticillium* wilt ranged from 0.09 to 3.88 in the 10 fields sampled in 1995 and 1996 (Table 1). Incidence of wilt increased in Royal II from 0.24% in 1996 to 15.63% in 1999 (Table 1). The Native spearmint field, Othello I, had the lowest incidence of quadrats with infected plants (0.09%; Table 1).

The variance-to-mean ratio was significantly greater ($P < 0.01$) than 1.00 for wilt incidence in quadrats at all locations and in all years (Table 2). The goodness of fit of each probability distribution to disease incidence data indicated that none of the

Table 2. Summary of doublet and runs analysis in two directions, variance-to-mean ratio, and summary of wilt foci of mint infected with *Verticillium dahliae* in 10 commercial mint fields

Field (year)	Doublets Z values ^a		Runs Z values ^b		Var/mean ^d	Foci analysis ^c				
	0°	90°	0°	90°		n	s	c	r	PI
Prosser I (1995)	12.11	2.04	-7.23	0.26	12.27*	90	1.2 (0.5)	1.1 (0.3)	1.2 (0.4)	0.99 (0.08)
Royal I (1995)	25.14	14.07	-4.71	-3.33	11.50*	58	1.5 (1.3)	1.2 (0.6)	1.3 (0.8)	0.96 (0.13)
Othello I (1995)	33.60	22.52	-8.30	-3.81	8.37*	5	2.0 (1.0)	1.4 (0.5)	1.6 (0.1)	0.95 (0.11)
Othello II (1995)	20.64	3.23	-1.19	-0.11	4.90*	30	1.2 (0.5)	1.0 (0.2)	1.2 (0.5)	1.00 (0.00)
Othello III (1995)	15.41	6.39	-8.94	-4.74	10.26*	129	1.7 (1.3)	1.3 (0.6)	1.5 (1.0)	0.93 (0.17)
George I (1996)	10.87	10.46	-6.10	-8.88	8.06*	170	1.6 (1.0)	1.4 (0.7)	1.4 (0.7)	0.88 (0.21)
Othello IV (1996)	9.67	5.01	-4.54	-5.24	3.35*	103	1.2 (0.5)	1.1 (0.3)	1.2 (0.4)	0.98 (0.10)
Othello V (1996)	11.77	9.94	-6.36	-4.21	9.94*	55	1.6 (0.8)	1.3 (0.5)	1.4 (0.7)	0.91 (0.18)
Othello VI (1996)	4.87	4.92	-2.82	-2.09	6.87*	106	1.2 (0.5)	1.1 (0.4)	1.1 (0.4)	0.97 (0.13)
Royal II (1996)	-0.24	-0.23	-0.10	-1.41	7.59*	24	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.00 (0.00)
Royal II (1997)	10.41	2.62	-6.68	-2.24	7.31*	104	1.3 (0.7)	1.1 (0.3)	1.2 (0.4)	0.97 (0.11)
Royal II (1998)	-	-	-	-	7.17*	64	2.7 (3.5)	1.5 (0.7)	2.0 (1.9)	0.88 (0.19)
Royal II (1999)	-	-	-	-	-	82	1.8 (1.3)	1.3 (0.5)	1.6 (1.0)	0.94 (0.16)

^a Zero degrees was defined as parallel with the first agronomic row of the sampled area. Doublets: values greater than 1.645 indicate significant aggregation at $P < 0.05$.

^b Runs: values less than -1.645 indicate significant aggregation at $P < 0.05$.

^c n = Total number of wilt foci, s = mean number of quadrats with wilted plants per focus (standard deviation), c = mean maximum number of quadrats with wilted plants in focus column (standard deviation), r = mean maximum number of quadrats with wilted plants in focus row (standard deviation), and PI = proximity index, which is an indication of compactness of focus organization and is calculated as *s/rc* (standard deviation).

^d Variance-to-mean ratio; asterisk (*) indicates significantly greater than 1.0 at $P < 0.001$ according to approximate χ^2 test.

distributions provided a satisfactory fit to the data for any of the locations ($P < 0.001$), except for Othello II in 1995. At that location, the Neyman Type A ($P = 0.104$) distribution was not rejected as a possible model for the data.

Significantly more doublets were observed than expected for a random pattern within and across agronomic rows in all fields except Royal II in 1996 (Table 2). Significantly fewer runs were observed than expected for a random pattern within

agronomic rows in all fields except Royal II in 1996, and across rows except for Prosser I in 1995, Othello II in 1995, and Royal II in 1996. There was more clustering within than across rows, except for Othello VI using doublets and George I, Othello IV, and Royal II in 1996 using runs analysis (Table 2). Incidence of quadrats with wilt in Royal II increased from 1996 to 1997 and significant clustering was observed within and across rows in 1997 according to doublets and runs analyses

(Table 2). Doublet and runs analyses were not conducted for Royal II in 1998 and 1999 because the sample area was too narrow. Greig-Smith quadrat variance analysis provided no evidence of a consistent cluster size based on an examination of plots showing components of variation versus the number of quadrats in successively doubled block sizes, as described in the previous section (*data not shown*).

Total number of wilt foci ranged from 5 to 170 per field section, and mean size of

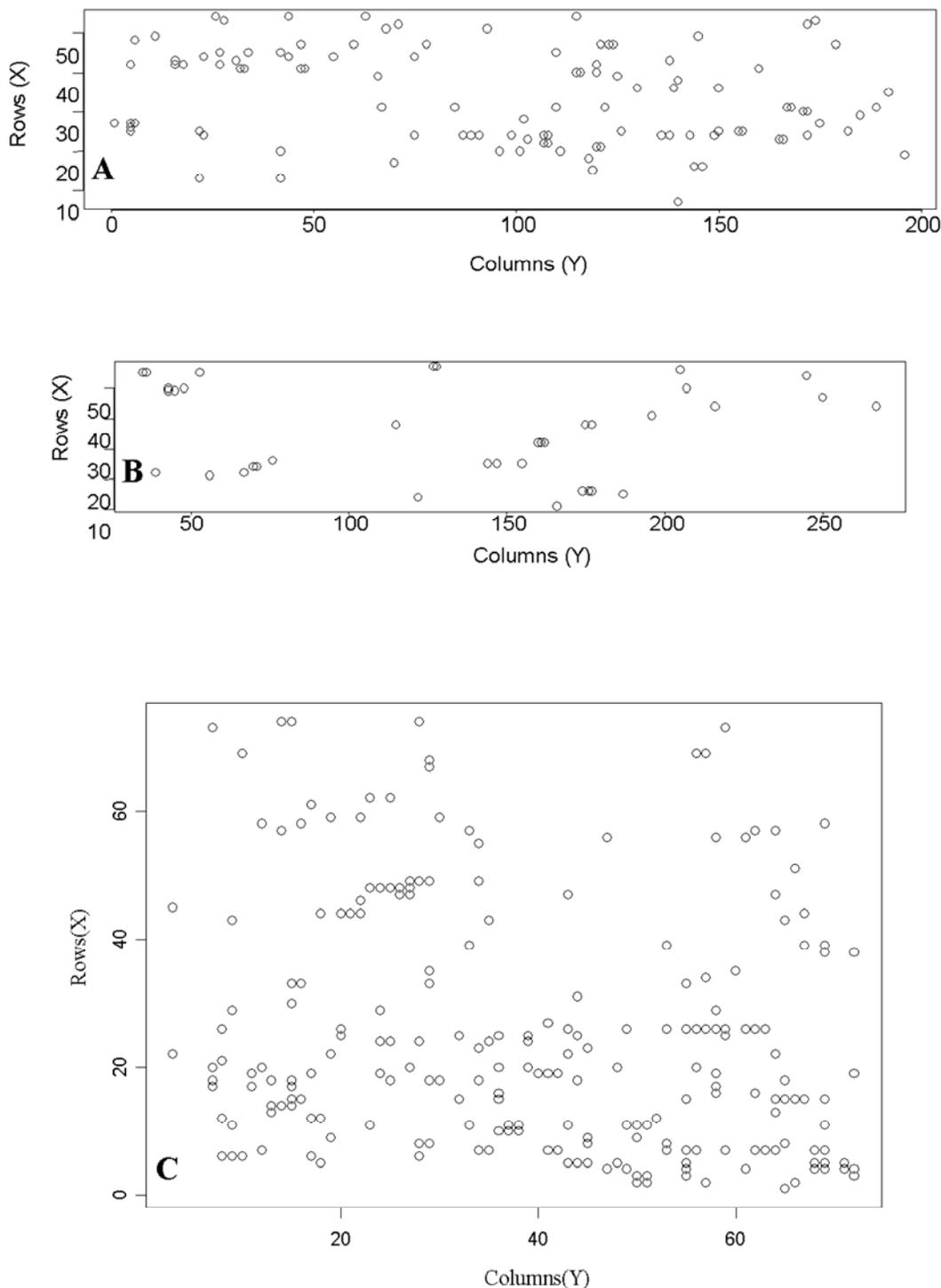


Fig. 1. Plots of diseased quadrats for six fields: **A**, Prosser I, **B**, Othello II, **C**, Othello III, **D**, Othello IV, **E**, Royal II in 1996, and **F**, Royal III in 1997. (*continued on next page*)

foci ranged from 1.0 to 2.7 quadrats (Table 2). Total number of foci increased from 24 to 104 at Royal II from 1996 to 1997, and the mean size of foci similarly increased from 1.0 to 1.3 in this study section. Size of foci increased to 2.7 quadrats in a subsection at Royal II sampled in 1998 (Table 2). Mean focus size was numerically greater for rows (within agronomic rows) than columns (across agronomic rows) for 10 of 13 fields. Values for the proximity index, a measure of cluster compactness,

ranged from 0.88 to 1.00 (Table 2), which means that 88 to 100% of all units in the rectangle that a focus spanned were diseased. Therefore, the disease foci were highly compact.

Clusters of quadrats with infected plants within rows and across rows generally were evident in study sections of most fields. The pattern of quadrats with *Verticillium* wilt-infected plants in Prosser I, Othello II, and Othello III in 1995; Othello IV and Royal II in 1996; and Royal II in

1997 are illustrated as examples of the aggregated disease patterns within and across rows observed in this study (Fig. 1).

Results of the distance class analysis are shown in Figure 2 for the same six fields shown in Figure 1. The two axes represent distance of separation of rows and columns. For a particular distance class X,Y, where X is the separation of columns and Y the separation of rows, a dot is plotted at the coordinate X,Y if the *P* value is less than 0.05. Therefore, each dot in Figure 2

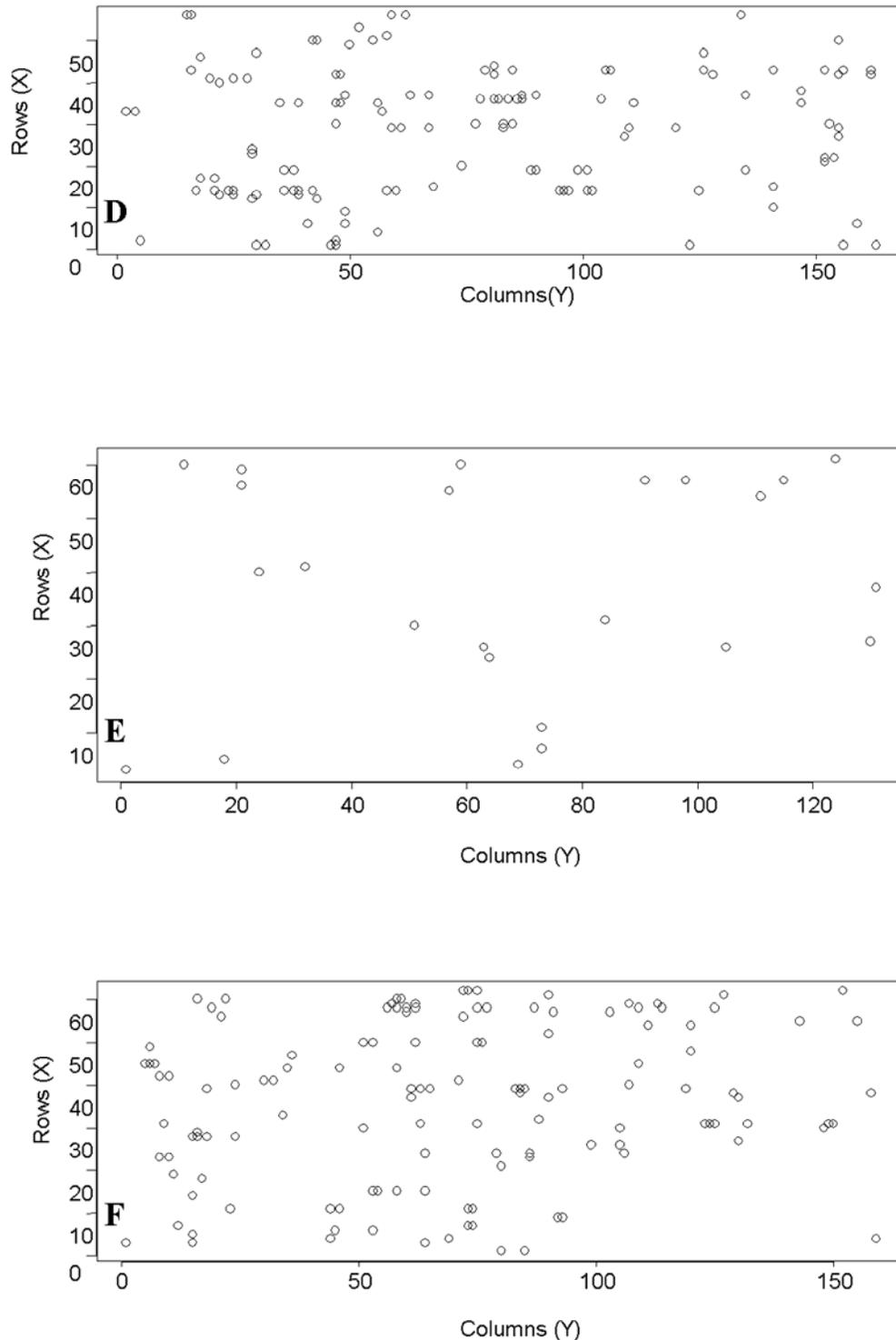


Fig. 1. (continued from previous page)

represents a distance class that was observed significantly more frequently than expected under the null hypothesis of a random pattern. Significant differences between observed and expected distance class frequencies were based on 400 simulations of random point patterns. If an observed SCF of the distance class is no less than 95% (380 of the 400 simulated SCFs of the distance class), it is significant at $P < 0.05$. Unlike Nelson (31), we do not show in Figure 2 those distance classes that have significantly lower SCFs because we were interested in distance separations that occurred more frequently.

For all fields except Royal II in 1996, there was a significant distance class of small distance separation, 0,1 or 1,0. This means that disease clusters occurred between two adjacent quadrats along the Y and X axes. This is consistent with the results of the doublet and runs analyses, both of which detected clustering within transects of quadrats. For Prosser I in 1995, and Othello IV and Royal II in 1996, most of the distance classes that occurred at significantly higher frequencies than expected were those representing greater separations between diseased quadrats, which indicated a lack of disease clusters.

For the other three fields shown in Figure 2, there are a number of distance classes of small separation that occurred at significantly higher frequencies than expected. For Othello III in particular, most distance classes of small separation are significant, which indicates the existence of a disease cluster.

Distance class analysis can reveal the geometry of disease patterns and complements the other analyses used. For example, both the doublets analysis and runs analysis showed strong anisotropy for Prosser I and Othello II: disease was more aggregated along the 0° line than along the

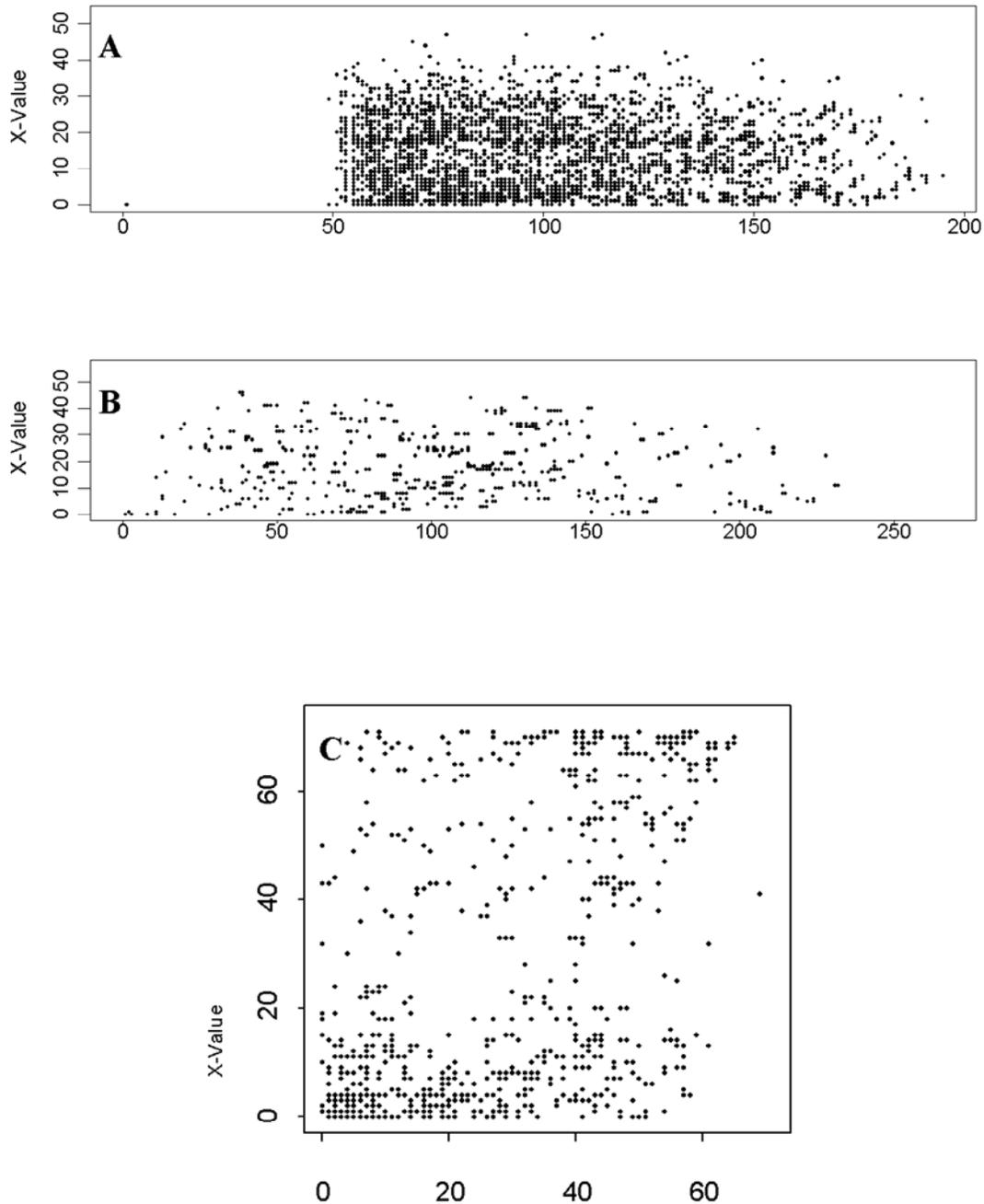


Fig. 2. Results of the two-dimensional class analysis for six fields: **A**, Prosser I, **B**, Othello II, **C**, Othello III, **D**, Othello IV, **E**, Royal II in 1996, and **F**, Royal III in 1997. The two axes represent distance of separation of rows and columns. For a particular distance class (X,Y), where X is the separation of columns and Y the separation of rows, a dot is plotted at the coordinate X,Y if the P value is less than 0.05. Thus, a dot represents a distance class X,Y with a standardized count frequency higher than expected ($P \leq 0.05$). (continued on next page)

90° line. The distance class analysis results not only confirmed what was found by the doublets and runs analyses but also uncovered a major difference between the disease patterns at these two sites. At Prosser I, the only significant distance class of small distance separation was 0,1, whereas a few significant distance classes of small distance separation existed at Othello II, indicating a disease cluster that contained more than just adjacent quadrats. Distance class analyses also complemented the doublets and runs analysis at Royal II in 1996.

Both the doublets and runs analyses revealed no aggregation along transects of quadrats. The distance class analysis showed that distance classes 0,1 and 1,0 were nonsignificant. However, there were a few significant distance classes of small distance separation (3,0, 4,0, 4,10, and 0,8), which indicated a degree of aggregation.

DISCUSSION

Aggregation of *Verticillium* wilt in mint fields was evident within and between

quadrats. More clustering was detected within than across crop rows, indicating the possibility of more spread of the disease within than between rows. Foci of infected quadrats were relatively small and compact. Aggregation was not detected in a 2-year-old Scotch spearmint field when disease incidence was low in 1966 (Royal II), with the exception of a significant variance-to-mean ratio. However, significant clustering was detected in this field after disease incidence had increased in 1997.

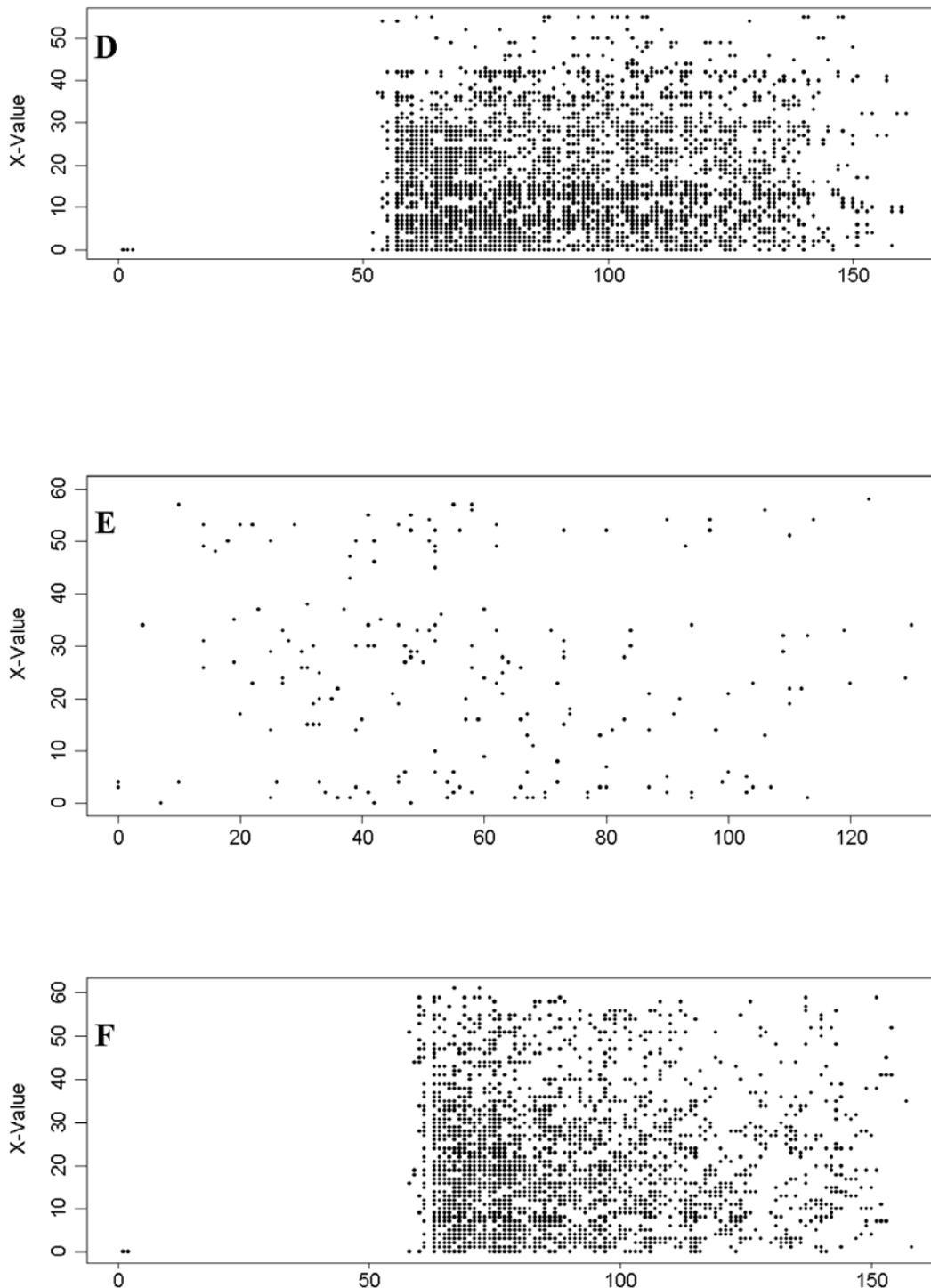


Fig. 2. (continued from previous page)

The spatial patterns of infected plants observed in this study provide information on the spread of *Verticillium* wilt in mint fields. The aggregated pattern of infected plants throughout the fields in their first year of mint production on ground without previous mint crops (Othello II and Othello IV) likely was due to the planting of infected rhizomes. This was due to the digging and planting process of rhizomes from source fields with aggregated wilt foci. Two mint rows at a time are dug systematically with a potato digger. The rhizomes are lifted from the soil, carried on a conveyor, and then dropped on a truck bed. They are transported to the field to be planted and loaded in bulk onto a planter bed about 3.2 by 6 m in size. The rhizomes then are compacted together and moved forward mechanically in bulk to where they are cut into 8- to 10-cm segments, dropped into a furrow in the ground, and then covered with soil. At no point in the process are the rhizomes mixed; therefore, neighboring rhizomes lifted from the source field are likely to be planted in close proximity. As a result, infected rhizomes would have been dug from disease clusters in the source fields as clusters of infected rhizomes, maintained as clusters of infected rhizomes in transport, and then planted in a clustered pattern throughout the field. Royal I served as the source of rhizomes for Royal II, and Othello VI was the source field for Othello IV. Aggregated patterns of infected plants in the first year of production in fields that previously were planted with mint (Prosser I and Othello I) likely resulted from microsclerotia present in the soil and from infected rhizomes that were planted originally.

Aggregated patterns of disease observed in fields 2 years and older (Royal I, Royal II, George I, Othello III, Othello V, and Othello VI) probably were the result of initial wilt foci present in the first year of production plus additional infections resulting from new roots contacting microsclerotia in the soil as the plants developed, and possibly from secondary spread. The rhizomes used to cultivate Royal II were obtained from Royal I, which had a low incidence of *Verticillium* wilt (Table 1). Royal II had been out of mint production for 20 years before the study crop was planted and the possible contribution of surviving microsclerotia is not known.

An increase in *Verticillium* wilt incidence was observed at Royal II each year from 1996 to 1999. Spread of *Verticillium* wilt within and across rows in fields cultivated with mint for two or more years was indicated from results of doublets and runs analyses, disease focus analysis, 2D distance class analysis, and mapping. Secondary spread may result from displacement of infected, dead mint stems short distances of several meters or less. Spread of disease across rows may have resulted from mechanical scattering of infected

mint debris during harvest. Disease spread within rows could be from the scattering of infected mint debris at harvest or the dispersal of inoculum in irrigation water. *V. dahliae* propagules can be carried in water and were detected in irrigation water at the ends of irrigation furrows but not at the beginning of irrigation furrows in potato crops (4). The form of inoculum is likely microsclerotia and possibly conidia produced on the dead, infested mint debris under humid conditions. Sporulation of *V. albo-atrum* was demonstrated on colonized tomato debris in soil (35) and on infected alfalfa stems in the field (16), and an increase in propagules of *V. dahliae* in fallow soil was shown to be due to sporulation on microsclerotia (28). Conidia of *V. dahliae* are capable of surviving up to 2 weeks in soil (11), which is sufficient time for dissemination and infection of nearby plants. Fewer aggregations within than across rows at George I, where the field was sprinkler irrigated, suggests that water running down crop rows in the furrow-irrigated fields may contribute to the movement of inoculum, resulting in disease aggregation.

Healthy mint plants adjacent to disease foci may become infected from roots contacting inoculum produced in the rhizosphere of infected plants. Soil populations of *V. dahliae* were shown to increase in the rhizosphere of infected mint (24), which could provide an infection source as roots of adjacent mint plants grow into the infested rhizosphere. In addition, mint roots contacting microsclerotia present in soil for two or more years after planting the crop also may account for the patterns observed. However, the magnitude of yearly increases in incidence of *Verticillium* wilt after the second year of production (17) and the size and shape of patterns of infected plots within and across rows suggest that secondary inoculum also is involved. Additional evidence for secondary spread of *Verticillium* wilt after the crop is planted comes from work by Horner and Dooley (14) and McIntyre and Horner (27), where they demonstrated a reduction in the spread of *Verticillium* wilt in fields by flaming the field after harvest to kill microsclerotia in infected mint debris.

Infected rhizomes are a major source of inoculum for *Verticillium* wilt in mint in the Columbia Basin, as shown in this study by doublets and runs analyses, maps, and initial incidences of disease in fields not previously used to grow mint. Therefore, growers need to further increase efforts to obtain *V. dahliae*-free planting stock. Additional research is needed to quantify the means of spread within and across crop rows and to develop more effective management practices to restrict this spread.

The statistical methods employed in this study were useful in describing and quantifying spatial patterns of *Verticillium* wilt in

commercial mint fields. The quantitative statistical methods and population foci analysis showed similar results for spatial structure of the disease at several scales. The variance-to-mean ratio identified aggregation of wilted plants within quadrats, even though probability distributions associated with clumped data did not fit the data well in most cases. Doublet and runs analyses usually were consistent in identifying aggregation of wilted plants along transects of quadrats. The few differences between results of the doublets and runs analyses likely reflect the lengths of aggregated sequences (18). The 2D distance class analysis also complemented these analyses by providing indications of cluster size and the geometry of disease patterns. Large-sized clusters were not present, as indicated by Greig-Smith analysis. The FOCI analysis identified that foci of infected quadrats were relatively small and compact, and helped the visualization of wilt foci and their spatial relationships.

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