Spatial Pattern Analysis and Sampling of Hypoxylon Canker in Naturally Occurring Clones of *Populus tremuloides*

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ABSTRACT

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Analysis of the spatial arrangement of trees with *Hypoxylon mammatum* stem canker in naturally occurring clones of *Populus tremuloides* determined that the degree of dispersion of diseased trees usually was not significantly different from random. Proximity of diseased trees does not always increase the chances of adjacent trees becoming infected. A random sample generally would be adequate for estimating the disease incidence in clones.

Spatial pattern analysis of disease incidence can show whether diseased individuals occur in clumped or randomly dispersed patterns (9,17,24,28). This information is useful for determining sampling strategies (10) and for evaluating the importance of internal or external sources of inoculum on disease spread.

There are several approaches to the spatial analysis of diseased plants. Frequency distributions of diseased individuals can be compared with an expected binomial distribution to test for randomness (11). In a regular arrangement of individuals, the observed number of pairs of diseased individuals, called doublets (29), can be compared with the number of doublets expected if all individuals have an equal chance of becoming diseased, i.e., that predicted from a binomial distribution (11,14,20). A modified method considers doublets linearly along, but not across, rows (12).

Populus tremuloides Michx. is a clonal tree species (6). Plots (2,3,5,7,18,19,23,25), transects (15,16,26), and censuses (8,13,15) have been used to assess the incidence of the stem canker pathogen *Hypoxylon mammatum* (Wahl.) Miller. None of these studies used spatial pattern analysis to determine the appropriate sampling scheme, although the clonal variation in field susceptibility to Hypoxylon canker suggests that the clone rather than the stand is the desired unit to sample (13).

This study uses the modified doublet method of Converse et al (12) and analysis of frequency distributions to test the hypothesis that trees with Hypoxylon canker are clumped in naturally occurring clones of *P. tremuloides*.

MATERIALS AND METHODS

Clone selection. Twenty-nine naturally occurring clones of *P. tremuloides* growing in central New York were used in this study. Bark color and texture, time of leaf flush, leaf color, and leaf shape were characteristics used to delineate clone boundaries. Age, largest diameter at breast height (dbh), and area for each clone are shown in Table 1.

Disease analysis. Every tree 2 cm dbh or greater in each *P. tremuloides* clone was examined for the canker caused by *H. mammatum* during the winter of 1984. Cankers occurring in the lower quarter of the main stem or on dead trees were noted separately from other cankers. Each clone was divided into octants based on eight compass points (N, NE, E, SE, S, SW, W, and NW) radiating out from the ortet or estimated oldest tree, which was usually near the center of the clone. The trees within each octant were examined from the center outward so that nearby trees were examined in a linear sequence. The first tree examined was the ortet, which was included in the north octant, that between N and NE. By use of these data in a spatial pattern analysis, neighboring trees were considered only along the linear sequence within each octant and not to neighbors in adjacent octants.

Spatial analysis. The observed number of doublets (pairs of diseased trees) was compared with the expected number of doublets to determine if trees with similar symptoms occurred in clumps in each clone. The expected number of doublets was calculated, assuming a random pattern of trees with the same symptoms, by the method of Freeman (14) as modified by Converse et al (12), which we call the Converse analysis. In the

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terminology of Converse et al (12) ("rows" being replaced by "octants"), if an octant contains n trees, the total number of trees N will equal $n_1 + ... + n_r$, where r is the number of octants sometimes less than eight. Let f equal the number of octants with only a single tree, M equal the number of trees with the same disease symptoms, and T equal the sum of the observed number of doublets in each of the octants. By joining the r octants together in a linear fashion and inserting a vacancy (a missing tree) at the end of each octant, the following formulas can be used to compute the mean μ_T (also the expected number of doublets), the standard error σ_T, and Z, which is the standardized value of T: $\mu_T = A\gamma_1$, $\sigma_T = (A\gamma_1 + 2B\gamma_2 + [A(A-1) - 2B]\gamma_3 - \mu_T^2)^{1/2}$, and $Z = (T - \mu_T)/\sigma_T$, where $\gamma_1 = M(M-1)/[N(N-1)]$, $\gamma_2 = \gamma_1(M-2)/(N-2)$, $\gamma_3 = \gamma_2(M-3)/(N-3)$, $A = N - r$, and $B = N - 2r + f$. If clumping occurs, the probability of obtaining a larger Z is less than 0.05, assuming that Z has an approximately normal probability distribution (12).

The presence of clumping also was tested by examining the frequency distributions of diseased trees in samples of two, three, four, or five adjacent trees. Such samples were drawn from each octant using the census data so that the maximum possible number of nonoverlapping samples of the same size was obtained from each clone. Within each octant those trees that could not be included, because the number of trees was not exactly divisible by the sample size, were excluded by a random process. In most cases, the samples were contiguous.

The frequency distributions were compared to an expected frequency that was calculated using a binomial function. This assumes a random pattern, with the clonal parameters used for the proportions with ($\hat{p}$) and without ($\hat{q}$) the disease trait. The goodness of fit of the observed to the expected frequency distribution was tested by the G test, adjusted to more closely fit the χ^2 distribution by the Williams correction. Adjacent cells of frequencies were pooled for expected frequencies (f) <5 when there were fewer than five cells of frequencies and if (f) <3 for five or more classes of frequencies, as recommended by Sokal and Rohlf (27). Clumping was detected if the standard deviation from the observed frequency distribution was greater than that from the expected binomial distribution and the probability of obtaining a larger G was less than 0.05 (27).

RESULTS

Table 1 shows the results of the Converse analysis on all 29 clones for the variables, cankers on the lower quarter of the main stem, incidence of Hypoxylon canker, and mortality caused by Hypoxylon canker. Four clones showed clumping of trees with lower quarter stem cankers, but clone A20 had small values for the observed (T) and expected (μ_T) number of doublets, and its significance is questionable; although $T = 1$ and $\mu_T = 0.1$ are significant statistically, such an expected number is not realistic and must be either 0 or 1. Incidence of Hypoxylon canker was clumped in six clones. Trees killed by Hypoxylon canker were clumped in five clones, although clone D30 had low numbers of observed (T) and expected (μ_T) doublets. Four clones showed clumping of trees with two of the disease variables. One clone showed clumping of trees for all three of the disease variables.

The same disease variables were examined for clumping by frequency distribution analyses, using samples containing two, three, four, or five trees, where the population sizes were sufficiently large to make this possible. Table 2 shows those clones found to have a frequency distribution different from

Table 1. The Converse analysis for comparison of the observed (T) number of doublets (pairs of diseased trees) with that expected (μ_T) from a random spatial pattern in *Populus tremuloides* clones of size N, where M trees have the indicated disease condition[a]

Clone no.	Age[b] (yr)	Largest dbh[c] (cm)	Area[d] (ha)		Lower quarter stem cankers			Hypoxylon incidence			Hypoxylon mortality		
				N	M	T	μ_T	M	T	μ_T	M	T	μ_T
A00	22	20	0.015	94	24	4	5.4	35	10	11.7	33	8	10.4
A10	41	34	0.085	219	3	0	0.0	21	5	1.9**[e]	10	1	0.4
A20	41	26	0.060	174	5	1	0.1**	39	11	8.2	18	4	1.7*
A30	39	26	0.041	61	2	0	0.0	22	8	6.7	7	1	0.6
A40	40	26	0.024	67	10	1	1.2	27	15	9.4**	21	7	5.6
A50	51	26	0.028	71	0	0	0.0	8	0	0.7	0	0	0.0
B00	36	21	0.053	144	20	2	2.5	67	38	29.2**	33	9	7.0
B10	36	22	0.030	119	14	0	1.4	28	8	6.0	18	3	2.4
B20	24	16	0.006	57	3	0	0.1	6	0	0.5	2	0	0.0
B30	21	12	0.010	58	12	4	2.0*	16	5	3.6	6	2	0.5**
B40	24	22	0.018	109	4	2	0.1**	17	5	2.3*	4	0	0.1
B50	20	11	0.015	102	2	0	0.0	12	1	1.2	2	0	0.0
B60	22	12	0.013	106	4	0	0.1	11	0	1.0	4	0	0.1
B70	36	29	0.015	116	19	5	2.8	33	10	8.5	22	5	3.7
B80	26	24	0.019	125	20	2	2.9	46	19	15.6	28	8	5.7
C00	59	44	0.133	224	16	1	1.0	141	95	85.4**	68	29	19.7**
C10	62	40	0.126	135	10	1	0.6	46	14	14.5	23	5	3.6
C20	48	28	0.047	263	43	12	6.7**	99	52	35.9**	56	24	11.4**
C30	18	14	0.017	83	24	6	6.3	48	25	25.9	27	9	8.0
C40	35	32	0.033	126	43	10	13.5	59	24	25.6	28	6	5.7
C50	28	22	0.026	225	44	9	8.1	93	39	36.8	48	13	9.7
C60	37	20	0.046	395	48	4	5.6	92	17	20.8	66	7	10.7
C70	35	30	0.060	290	66	16	14.4	104	40	36.0	68	16	15.3
D00	26	12	0.017	123	27	5	5.4	40	10	12.0	30	5	6.7
D10	32	18	0.036	68	22	6	6.1	37	16	17.5	21	5	5.5
D20	34	22	0.087	176	10	0	0.5	45	14	10.8	19	2	1.9
D30	44	30	0.053	139	1	0	0.0	15	3	1.4	5	1	0.1**
D40	35	30	0.023	43	0	0	0.0	4	0	0.2	1	0	0.0
D50	37	30	0.032	65	1	0	0.0	8	1	0.8	5	1	0.3

[a] Clumping of diseased trees (more doublets than expected) is indicated by large values of the standardized variate of T.
[b] Number of growth rings in an increment core (at 1.4 m) from the ortet or oldest tree with 4 yr added.
[c] Diameter at breast height (1.4 m above ground) of largest tree.
[d] Estimated from length to clone boundary from ortet in each of eight compass directions.
[e] Significant clumping at $P = 0.01$ (**) and 0.05 (*).

random expectations ($P = 0.05$). No clone showed clumping of trees with lower quarter stem cankers. Disease incidence was clumped in three clones; these also showed clumping by the Converse analysis. Two other clones had a distribution of disease incidence different from the binomial, but the distribution was only slightly clumped in one and was dispersed in the other (Table 2). Disease incidence in these clones was not clumped by the Converse analysis. Three clones showed clumping of trees killed by Hypoxylon canker. One of these also was clumped by the Converse analysis; in the other two, the significance was only 0.04 (Table 2).

These analyses do not consider dispersion biased in one direction, such as in the direction of the prevailing winds. To test this, the data were subdivided within each clone to compare clumping in octants parallel and in octants perpendicular to the prevailing wind direction, which is approximately southwest. The Converse analysis was used to examine the subdivided data. Clumping was detected as frequently in octants parallel to the southwest as in octants perpendicular to the southwest.

Table 2. Frequency distributions of groups of nearby diseased trees significantly different ($P = 0.05$) from those expected from a random pattern[a]

Disease variable	Clone no.	Sample size (no. of trees)	G[b]	$pr>G$	Δstd[c]
Hypoxylon incidence	B00	2	6.90	0.03	+0.11
		4	7.73	0.02	+0.30
		5	8.22	0.04	+0.44
	C00	2	6.60	0.04	+0.08
		3	8.66	0.01	+0.21
	C20	3	8.14	0.02	+0.20
		4	9.78	0.02	+0.25
		5	18.26	0.001	+0.42
	C60[d]	5	10.76	0.01	+0.01
	D00[d]	3	9.41	0.01	−0.18
Hypoxylon mortality	C20	3	12.95	0.002	+0.17
	C50[d]	4	6.21	0.04	+0.10
	C70[d]	5	8.38	0.04	+0.16

[a] Goodness of fit test by the G test with clumping detected by the difference (Δstd) between the standard deviation from the observed and the expected frequency distributions.
[b] With the Williams correction to more closely fit the χ^2 distribution.
[c] + = Clumping, − = dispersion.
[d] Not clumped by the Converse analysis.

DISCUSSION

Clumping of trees with Hypoxylon canker occurred in nine of the 29 clones examined, indicating that the spatial pattern of diseased trees was usually not significantly different from random. This suggests that proximity of trees to diseased trees does not always increase their chances of becoming infected. A temporal analysis would be needed to confirm this.

A previous study of disease distribution in an aspen plantation in central New York found a random pattern (22), whereas a study of aspen stands in the Lake states found higher disease incidence in edge trees than in interior trees (1). Regionwide differences in the spatial distribution of diseased trees may be due to differences in the infection courts. Insects, which prefer open stands and the edges of stands, appear to be more important in establishing wounds for infection in the Lake states (4) than in New York (21).

Because the spatial distribution of trees with Hypoxylon canker was usually not significantly different from random, a random sample would generally be adequate for estimating the disease incidence in *P. tremuloides* clones.

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