Ordination, A Multivariate Method for Estimating Relative Resistance of *Populus tremuloides* and Virulence of *Hypoxylon mammatum*

D. H. Griffin and P. D. Manion

Department of Environmental and Forest Biology, College of Environmental Science and Forestry, Syracuse, NY 13210.
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ABSTRACT

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An ordination technique using modified q-correlations as measures of similarity has been developed and applied to data from virulence testing with *Hypoxylon mammatum*, the causal agent of a stem canker on *Populus tremuloides*. The ordination produces linear vectors of the aspen clones or the fungal isolates that are correlated to the underlying measurement variables in a consistent fashion such that one pole of the ordination can be interpreted as representing susceptibility of the clones or low virulence of the isolates, and the opposite pole representing resistance of the clones or high virulence of the isolates. Intermediate positions in the vector represent intermediate levels of resistance or virulence proportional to their distance on the vector. The ordination process can use both quantitative and quantal variables and effectively summarizes the information contained in these variables into a unified scale of resistance or virulence. The complex of data for the host clones or pathogen isolates compared by the ordinations can be dissected to determine how the variables relate to the response gradient. Isolate specific responses of the aspen clones, vertical resistance, were indicated by reversals in the ordinations of the clones by different isolates. Some clones were consistently placed at either the resistant or susceptible ends of the ordination vectors of the various isolates, indicating horizontal resistance mechanisms as well.

Virulence testing to assess the relative virulence of strains of pathogens and the relative susceptibility of host clones involves several steps that can be standardized: preparation of the pathogen for inoculum, preparation of the host, and inoculation. However, observation and scoring the host responses, and coding or condensation of the response data into an estimate of the magnitude of the response involve considerable subjectivity in choice of the response measured and the coding of this response for comparisons. Ercolani (2) discussed these problems in relation to infectivity titration with bacterial pathogens and concluded that the complexity of the responses and interactions between host and pathogen leads to great difficulty in developing models to adequately explain susceptibility-virulence relationships. Another problem that has been encountered is the diversity of reaction types between various host cultivar and pathogen isolate interactions that makes data analysis difficult (7). The tendency to simplify the situation by using a single measure of the interaction, such as numbers of uredia produced by rust fungi (6), ignores other potentially useful information. Selection of a suitable multivariate method of analysis will help to overcome these problems.

Virulence and resistance testing in *Hypoxylon mammatum* (Wahl.) Miller has largely involved inoculations of stem wounds by mycelium from agar or grain cultures (1,3,5,11). Canker length was the response measured by earlier investigators (1,3), but Valentine et al (11) introduced callus formation and branch death as additional measures. Griffin et al (5) included all three measures and added time as a factor over a 16-mo period of examination. Significant differences among aspen clones, pathogen isolates and clone by isolate interactions were obtained in the latter study (5). However, it was difficult to compare relative resistance of aspen clones or relative virulence of *H. mammatum* isolates with these data, because of the differences among the three response measurements at the same time and the differences among times for each response. The relative orders of the clones (or the isolates) varied among the three measurements and with the time of measurement for each. Faced with this difficulty in analyzing the data, we searched for a multivariate method of comparing the responses that could use all of the data together, rather than only one response and time of measurement.

Ordination as a means of displaying vegetational relationships between plots in a study area has been used by ecologists for many years (4,8,12). Principal coordinates analysis, an ordination method, has been used to identify susceptibility relationships in cereal rusts (10). Ordination produces vectors of study plots in sequences with distances that indicate their relative similarities. It is based on an $N \times N$ matrix of similarity coefficients, which may be used as such, or converted to dissimilarity coefficients before the ordination (12). Several different types of similarity coefficients have been described that have different properties depending on the nature of the data and the ordination mathematics applied (4,10).

The purpose of this article is to present a novel method of ordination analysis and apply this to host-pathogen response data to identify response gradients among host clones or among fungal isolates.

MATHEMATICAL BASIS OF THE ORDINATION

The ordination procedure can be described as a series of steps: 1) standardization of the data, 2) calculation of the matrix of similarity coefficients, 3) calculation of the indices of similarity, 4) determination of the axes for ordination, and 5) calculation of the distances of the clones (or isolates) on the ordination vector. These steps will be described in turn.

Because of the disparate scales of the data (canker lengths ranging up to 200 mm, and callus and branch death as quantal characteristics scored as 0 and 1), a data centering technique was necessary to effectively use the variation present in each variable. All measurement variables were standardized to z-scores (8). After standardization, the means of clones by isolates for each variable were calculated. These means were used to calculate the similarity coefficient matrices for each ordination group.

Q-technique correlation coefficients are one measure of similarity that is commonly used, especially with principal coordinate analysis (8). Our similarity coefficient differs from q-correlation coefficient in that the sample centroid is substituted for

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the means of clones in the calculation of the covariances and standard deviations. Because the measurement variables have been previously standardized to z-scores, the sample centroid equals 0, and drops out of the calculation, equation 1.

$$Q_{ij} = \Sigma(x_i)(x_j)/(\Sigma(x_i^2)(x_j^2))^{0.5} \quad (1)$$

where Q_{ij} = the similarity coefficient of the ith clone with the jth clone, x_i and x_j = the individual observations of the variables clone i and clone j, respectively.

The indices of similarity are calculated as the row sums of the matrix of similarity coefficients. These are measures of the overall degree of similarity of a clone to the others. The similarity coefficients may take the values from 1, indicating identical responses, to -1, indicating disparate responses, and the index of similarity may range from $2-N$ to N, the number of clones being compared. With nine clones, for example, the maximum possible range is -7 to 9.

Two matrices of importance to this ordination method are represented in equation 1. The values of Q_{ij}, the similarity coefficients, form an $N \times N$ matrix (N = the number of clones). This matrix is used for determining the axes for the ordination plot. The numerator of equation 1 is also an $N \times N$, matrix analogous to the variance-covariance matrix of r-sense statistics. The calculation of this matrix is represented in terms of linear algebra as $\mathbf{X'X}$, where $\mathbf{X}$ is the $N \times p$ matrix of standardized data values arranged for q-sense calculations. The $\mathbf{X'X}$ matrix provides the values for the ordination plot from which the ordination distances are calculated.

The axes for the ordination are chosen to obtain the minimal similarity. These are: (a) the clone, y, with the lowest similarity index for the ordinate and (b) the clone, x, having the lowest similarity coefficient with clone y for the abscissa. These two clones, x and y, are used for the axes of the ordination plot. The positions of all clones on the plot are determined from the $\mathbf{X'X}$ matrix, the numerator in equation 1. The columns representing clones x and y, respectively, provide the coordinates for plotting the position of each clone, represented by the rows of the matrix.

Figure 1 shows a hypothetical ordination of three clones to illustrate the derivation of the formula for calculating the ordination distance. The plots of clones x and y form the poles of the ordination (points B and A, respectively). The line AB represents the ordination vector. The ordination distance (D) of each clone on this line is the distance from the end of the ordination vector (A) to the point of intersection of the perpendicular from the point of the clone (C) to the line AB (G in Fig. 1). D, the length of line segment AG (Fig. 1) can be determined by solving the triangles ACG and ABC from the coordinates of the points A, B, and C, which are obtained from the $\mathbf{X'X}$ matrix. The legs of the three right triangles AEC, AFB, and CHB can be determined directly from the coordinates of points A, B, and C. The sides of triangle ABC are the hypotenuses of these three right triangles and can be calculated by the Pythagorean theorem. With the three sides of ABC determined, the angle CAB is determined by the Law of Cosines, and line segment AG (the ordination distance, D) can then be calculated.

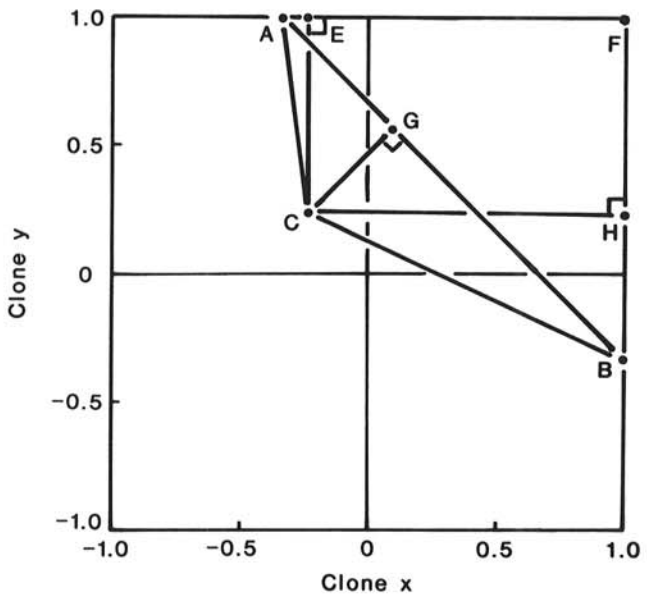

Fig. 1. Diagram for the ordination calculations for a plot of three clones. The axis clones y and x are represented by points A and B, respectively, and a third clone is represented by point C. The coordinates of the points A, B, and C are obtained from the columns A and B of the $\mathbf{X'X}$ matrix, where row A yields the coordinates for A, row B the coordinates for B and row C the coordinates for C.

The calculations were performed using computer programs provided by SAS version 82.3B (9) at the Syracuse University Academic Computer Service. The ordination calculations were made using a program written in the SAS Matrix language. An annotated program for the calculations using SAS procedures is available from the authors on request.

It can be proven mathematically that these plots form precisely straight lines with only two types of matrices (Ucci, *personal communication*). The matrices must be rank 1 (all rows or columns formed as linear combinations of a single arbitrary vector) or rank 2 (all rows or columns formed as linear combinations of two vectors) in which one of the vectors may be arbitrary, but the second vector must be composed of a constant. That is the vector

$$B = [\alpha_1, \alpha_2, \ldots \alpha_n]$$

where $\alpha_1 = \alpha_2 = \ldots \alpha_n$, for example $B = [1\ 1\ 1\ 1 \ldots 1]$. Matrices, $\mathbf{X}$, of rank 2 not formed of this special constant vector as one of its dimensions or matrices of higher rank will not form straight lines from plots of pairs of columns of the $\mathbf{X'X}$ matrix.

The arbitrary vector of the data matrix is determined by the response gradient of the host-pathogen interactions measured by the susceptibility testing data. Deviation from linearity will occur when the data contain two or more response gradients, or when the data deviate somewhat from their expected values as by random normal variation. Where more than one response gradient is suspected, the ordination can be obtained with selected data removed from the data matrix to determine the effect of these data on scatter in the plots.

In summary, pairs of columns of an $\mathbf{X'X}$ matrix form straight line plots when the $\mathbf{X'X}$ matrix is rank 1, or rank 2 with one of the dimensions a constant vector. Other rank 2 matrices or matrices of greater rank will form scatter plots rather than straight lines. The distances between points in the plot are related to the distances defined by the primary gradient vector of the $\mathbf{X'X}$ matrix. Random variation in the values of the primary gradient vector that is small relative to the values in the vector causes small deviations of the ordination plots from a straight line. This ordination method is a powerful technique for identifying response gradients in data matrices and identifying the related components responsible for the gradients.

APPLICATION TO EXPERIMENTAL DATA

The data used for this analysis is from a study of the development of cankers caused by *H. mammatum* on *Populus tremuloides* Michx., which was originally described by Griffin et al (5). These data showed significant differences among the aspen clones and the fungal isolates and among the clones by isolate interactions.

In the current analysis, four isolates were used because isolate 208-6 had lost its ability to produce cankers (5). The measurement variables, canker length, branch death, and callus formation were judged to have considerable though not complete independence as shown by the weak to moderately strong correlations between them (5). Ranking the aspen clones by these variables as measures of susceptibility produced different rankings depending on the variable and the time after inoculation the measurements were

TABLE 1. Similarity coefficients (Q) with the four virulent isolates pooled

Clone	Clone								
	208	209	210	211	604	608	609	610	611
208	1	0.33	0.39	0.23	0.54	0.29	0.38	0.44	0.71
209	0.33	1	−0.33	0.54	0.28	0.52	0.67	0.28	0.20
210	0.39	−0.33	1	−0.05	0.33	0.02	−0.12	0.20	0.36
211	0.23	0.54	−0.05	1	0.46	0.77	0.73	0.68	0.19
604	0.54	0.28	0.33	0.46	1	0.59	0.57	0.65	0.59
608	0.29	0.52	0.02	0.77	0.59	1	0.60	0.61	0.15
609	0.38	0.67	−0.12	0.73	0.57	0.60	1	0.63	0.44
610	0.44	0.28	0.20	0.68	0.65	0.61	0.63	1	0.42
611	0.71	0.20	0.36	0.19	0.59	0.15	0.44	0.42	1

TABLE 2. Comparison of indices of similarity (S) for ordinations with the isolates pooled and each isolate separately

Clones	Isolates				
	Pooled	209-5	209-6	605-1	608-7
208	4.31[a]	4.19	8.29[a]	−0.10	3.10
209	3.49[a]	−1.28[a]	7.93	1.08	4.89[a]
210	1.80[a]	2.11[a]	8.30	−0.06	−4.14[a]
211	4.56	2.11	8.36	2.73	4.80
604	5.01	3.39	7.72	2.22	5.69
608	4.55	2.65	7.56[a]	2.97[a]	4.67
609	4.89	1.44	8.37	2.81	5.21
610	4.91	3.35	8.13	1.76	5.30
611	4.06	2.93	7.97	−2.51[a]	5.03

[a] Polar clones chosen as the clone with the lowest index (above) for the ordinate and the lowest coefficient of similarity with the clone for the ordinate for the abscissa (cf. Table 1 for Pooled Isolates, the coefficients of similarity for the individual isolates are not shown).

made. With the ordination procedure we use all of these data and reduce it to a single scale of relative susceptibility or virulence.

Similarity coefficients. The matrix of similarity coefficients for the ordination of the clones by the pooled virulent isolates, 209-5, 209-6, 605-1, and 608-7, is shown in Table 1. Inspection of the similarity coefficients reveals certain patterns. Negative similarity coefficients do not mean opposite response as do negative correlation coefficients. They have been calculated from z-scores and response values below the mean will have negative z-scores which, when paired with observations greater than the mean (positive values), will result in a negative similarity coefficient. Thus, the negative values represent pairs that are on opposite sides of the mean response, but not necessarily having opposite responses. All negative coefficients are associated with clone 210 (210 with 209, 211, and 609), so it is not surprising that clone 210 has the lowest index of similarity with the isolates pooled (Table 2). The highest coefficient, 0.77, is between clones 211 and 608, but neither of these clones has the highest index of similarity.

Indices of similarity. The indices of similarity (Table 2) for the pooled isolates and for each isolate separately reveal other patterns not so readily evident from inspection of the similarity coefficients directly. The indices of similarity for the pooled isolates in Table 2 are the row sums of the coefficients in Table 1. The indices for each individual isolate are the row sums for matrices of coefficients (not shown) calculated with the subset of the data relating to each individual isolate. One striking feature of these indices is the consistently high level with isolate 209-6 (range 7.56–8.37), close to the maximum possible value of 9. Other isolates have much lower indices indicating greater differences in responses among the clones. Clone 210 has the lowest index of similarity with the pooled isolates and with isolate 608-7. Clone 210 is also chosen for one axis with isolate 209-5. Three of the five ordinations have the same axis clones, pooled isolates, 209-5 and 608-7. This indicates a consistency in the responses of the clones to these isolates.

Ordination plots. Plots of the ordinations showing the relative positions of each clone are shown in Figure 2. The line joining the polar clones is the ordination vector. In each ordination, the clones plot closely to the ordination vectors. This striking result indicates a particular relationship among the measurement variables defined

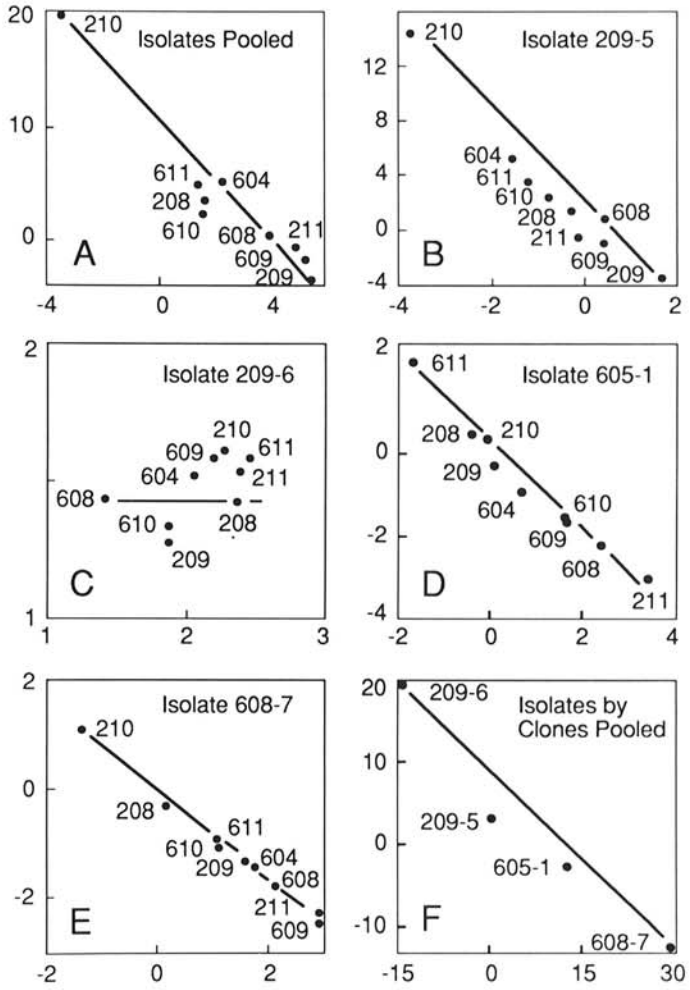

Fig. 2. Ordination plots for the clones based on (A) the four virulent isolates pooled; (B) isolate 209-5, (C) isolate 209-6, (D) isolate 605-1, (E) isolate 608-7; and (F) ordination plot for the isolates by the nine clones pooled.

by the primary response gradient vector discussed above. The ordination distances (D) for these vectors were calculated with the clone or isolate having the shorter cankers of the two poles taken as the origin of the vector. Note the variation in the lengths of the various plots (Table 4), which indicates the amount of covariation involved in each ordination. The ordination distance with isolate 209-6 is short, consistent with the high level of similarity among clones. The ordination distance with isolate 209-5 is large, which indicates that the pooled ordination is largely controlled by the data from this isolate.

Some clones tend to group together on several of the ordinations, for example clones 209, 211, 608, and 609 form one group and clones 208, 604, 610, and 611 form another, although these are not always distinct from each other (compare Fig. 2A, B, and E). Some of this grouping is retained with the ordination by isolate 605-1 (Fig. 2D), but some reversals are evident with this

TABLE 3. Ordination of aspen clones[a]

Ordination vector	Canker response: Canker length 4 mo	Canker length 12 mo	Canker length 16 mo	Callus formation 4 mo	Callus formation 12 mo	Callus formation 16 mo	Branch death 4 mo	Branch death 12 mo	Branch death 16 mo	Vector length (covariation units)
All variables:										
Clones by isolates:										
Pooled	0.82	0.82	0.76	0.00	0.89	0.84	0.24	0.36	0.23	24.8
209-5	0.84	0.76	0.84	0.00	0.82	0.83	0.20	0.42	0.54	18.6
209-6	0.44	0.24	0.00	0.00	0.00	0.37	0.00	0.62	0.32	1.0
605-1	0.46	0.59	0.13	0.52	0.41	0.36	0.51	0.92	0.88	6.5
608-7	0.92	0.77	0.13	0.00	0.46	0.53	0.28	0.63	0.37	5.9
Selected variables:										
Clones by isolates pooled:										
Canker length	0.86	0.95	0.66	0.01	0.72	0.82	0.22	0.39	0.22	12.5
Callus formation	0.49	0.61	0.36	0.12	0.77	0.83	0.23	0.40	0.42	2.3
Branch death	0.27	0.18	0.09	0.04	0.21	0.22	0.68	0.88	0.95	5.7

[a] Coefficients of determination (r^2) of the ordination distances (D) of the ordinations of the clones with the canker response measurements (5).

TABLE 4. Indices of similarity (S) and ordination distances (D) of the fungal isolates

Isolate	S	D
209-5	1.40	1.05
209-6	0.39	0
605-1	1.59	1.64
608-7	1.06	2.22

isolate, for example clones 209 vs. clones 604 and 610 and clone 611 vs. 604 and 610 (Fig. 2B and D).

Ordination of fungal isolates. Ordination of the isolates was done in the same manner as described for the clones with the standardized data arranged with each isolate as a variable and the measurements of the canker responses for each clone as the observations. The indices of similarity and the ordination distances (Table 3) show isolate 209-6 as the most disparate, and the ordination vector between 209-6 and 608-7 is shown in Figure 2F. This ordination vector indicates a response gradient between the polar isolates. The length of the vector indicates the considerable differences among the isolates (Table 5).

INTERPRETATIONS OF THE ORDINATIONS

To compare the ordination vectors derived from the canker data and to see what response gradients in the host-pathogen system they represent, we have calculated the coefficients of determination (r^2) between the canker development variables and various ordination vectors obtained from these data, Tables 4 and 5. In these tables, the ordination vectors were calculated using the entire data matrix (Table 4; all variables, isolates pooled), or selected parts of it as indicated. For the entire data matrix there is a strong relationship of the ordination vector to canker length and callus formation, except the 4-mo callus response, and a weak relationship to branch death (Table 4). In general, callus formation at 4-mo was not related to any of the ordinations. This could be due to the low response level obtained, which could mean either that random variation was large relative to the response level, or that this early response was unrelated to the longer term responses measured at 12 and 16 mo.

Examining the clonal responses using only the data for each type of measurement variable, i.e., canker length, callus formation, or branch death, Table 4, we see that the ordination by canker length alone shows a similar response pattern to that of the whole data matrix. The ordination by callus formation alone shows a much weaker relationship to canker length and no greater relationship to callus formation than either of the ordinations with all data or with canker length. We note that the vector lengths of these ordinations indicate that the largest amount of interclonal covariation is accounted for by canker length and very little is accounted for by callus formation, indicating that callus formation as measured in this study (5) did not contribute greatly to the measurement of host-pathogen interaction. Because callus formation was highly correlated to the canker length ordination, we may conclude that this measurement is redundant to canker length. The length of the branch death vector is intermediate between the canker length and callus formation vectors (Table 4) and shows a relatively weak relationship to the other variables. This suggests that branch death has a component independent of the canker length-callus formation vector.

The ordinations of the clones by each isolate individually also show some interesting relationships (Table 4). The length of the vector by isolate 209-5 is the greatest of the four isolates, nearly as long as that of the isolates pooled, indicating that isolate 209-5 accounts for most of the covariation of the clones. Thus, it is not surprising to see that this vector has similar relationships to the measurement variables as the pooled isolates vector.

The shortness of the vector by isolate 209-6 and its lack of consistent correlation to the measurement variables is consistent with the random scatter in the ordination plot, Figure 2C. The consistently high indices of similarity of the clones with this isolate, Table 2, is also indicative of the lack of differential behavior of the clones to this isolate. We conclude that this ordination only represents random variation from various sources and no meaningful biological relationships can be inferred from it.

It is interesting that the vector by isolate 605-1 shows a strong relationship to branch death, and weaker relationships to canker length and callus ordination. This relationship and the concept that the branch death variable has a component independent of the canker length-callus ordination vector are related to the reversals evident in the visual comparison of the 605-1 ordination to the others (Fig. 2). We also see a moderately strong relationship of the ordination by isolate 608-7 to branch death, although this vector is most strongly associated with canker length up to 12 mo.

In addition to the reversals noted above, we see that the extremes of clonal variation differ among the isolates (Fig. 2). Note especially that clone 210 is the most resistant clone to isolates 209-5 and 608-7 and clone 209 is the most susceptible, or nearly the most susceptible with these isolates. Clones 210 and 209 are about equally susceptible to isolate 605-1, but near the resistant pole of this ordination. These differences suggest vertical resistance factors operating in this system. The consistency of clone 210 being at or near the resistant extreme of these ordinations suggests that it may carry important horizontal resistance as well.

The strong correlation of the ordination by pooled isolates to both canker length and callus formation is probably the result of the dominant relationship of isolate 209-5 in this ordination. In contrast, the ordinations of the isolates by the clones show a more evenly distributed relationship among the clones than was seen among the isolates, Table 5. No single clone accounts for the bulk of the covariation among isolates as isolate 209-5 accounted for most of the covariation among the clones. Clone 208 has a short

TABLE 5. Ordination of hypoxylon isolates[a]

Ordination vector	Canker response									Vector length (covariation units)
	Canker length			Callus formation			Branch death			
	4 mo	12 mo	16 mo	4 mo	12 mo	16 mo	4 mo	12 mo	16 mo	
Clones pooled	0.92	0.76	0.53	0.00	0.03	0.72	0.00	0.67	0.17	54.3
Clones:										
208	0.42	0.57	0.66	0.00	0.04	0.00	0.35	0.29	0.00	1.5
209	0.39	0.65	0.97	0.13	0.34	0.05	0.32	0.51	0.31	3.9
210	0.09	0.00	0.17	0.90	0.99	0.85	0.12	0.02	0.00	11.9
211	0.92	0.91	0.61	0.09	0.01	0.29	0.93	1.00	0.68	11.0
604	1.00	0.91	0.51	0.26	0.09	0.44	1.00	0.91	0.43	7.5
608	0.04	0.19	0.59	0.59	0.78	0.35	0.02	0.16	0.26	5.1
609	0.96	0.99	0.73	0.09	0.01	0.22	0.94	0.95	0.49	11.2
610	0.95	0.88	0.52	0.17	0.05	0.39	0.97	0.97	0.62	4.5
611	0.85	0.90	0.70	0.12	0.00	0.16	0.79	0.68	0.15	7.0

[a] Coefficients of determination (r^2) of the ordination distances of the isolates by clones with the canker response measurements (5).

vector indicating that it distinguished poorly among the isolates as isolate 209-6 distinguished poorly between the clones. However, isolate 209-6 was the least virulent isolate as shown by the ordination, and its lack of differentiation among the clones can be attributed to its generally low virulence. Clone 208 was neither the most resistant clone nor the most susceptible; its lack of differentiation of the isolates cannot be attributed to either generally high or generally low susceptibility. Clone 208 might best be described as lacking any strong vertical resistance factors for any of the tested isolates and being moderately susceptible in horizontal resistance characteristics. Clone 209, which was one of the most susceptible clones, also has a relatively short ordination vector indicating similarity of reaction to the isolates and general susceptibility. The three clones that produced the greatest covariation among the isolates, 210, 211, and 609, ranged from the least susceptible, clone 210, to the most susceptible, clone 211. These clones would be excellent test clones for surveying virulence with large numbers of isolates, because of this ability to differentiate among isolates.

The association of variables with the ordination vectors is different for these ordinations than the ordinations of clones by the isolates, Table 5. It is interesting that among these vectors, canker length and branch death are commonly associated, i.e., clones 211, 604, 609, 610, and 611. Callus formation is not associated with another variable in any of these vectors. Only two clones show a strong relationship to callus formation, 210 and 608. These differences again suggest genetic distinctiveness of the responses of the clones, but the uniform association of branch death to canker length among the aspen clones is intriguing, especially in view of the lack of association of these responses among the hypoxylon isolates.

CONCLUSIONS

We conclude that these ordinations represent the relationships between aspen clones and isolates of *H. mammatum* governed by the underlying measurement variables. These ordinations make effective use of both quantitative and quantal variables. The ordination distance of each clone (or isolate) on the ordination vector is a measure of its relative susceptibility (or virulence) as measured by the inoculation bioassay. The ordination is an effective way of reducing the complex relationships of a number of measurement variables into a single index retaining the information content of the measurement variables.

The technique presented here does not depend on the assumption of multivariate normal distribution of the variables, which is the common assumption of other multivariate analytical techniques such as factor analysis, principal component analysis, canonical correlation, and multivariate analysis of variance (8). Ordination is a technique of visualizing relationships in a multivariate system of interactions among various biological units. Because it does not involve multivariate normal distributions, calculations of probabilities and hypothesis testing are not possible. Much is left to the judgement of the investigator. The importance of this ordination technique is not that it provides evidence for specific relationships, but that it provides insight into these relationships, permitting the development of hypotheses for testing by other means.

The ordinations of the aspen clones showed that canker lengths and callus frequencies were very closely related to each other, with the two variables defining essentially the same response vector. We may conclude that canker length and callus frequency were redundant, and only one of the measures would suffice. Branch death frequency was independent of the canker length response vector, but did not contribute sufficient variation to introduce much scatter into the ordination. However, reversals in the ordination, suggestive of vertical resistance factors, were primarily related to the fungal isolate most strongly associated with branch death. This variable contributed useful information beyond that contained in canker length, and its use should be retained.

LITERATURE CITED

1. Bagga, D. K., and Smalley, E. B. 1974. The development of hypoxylon canker of *Populus tremuloides*: Role of interacting environmental factors. Phytopathology 64:658-662.
2. Ercolani, G. L. 1984. Infectivity titration with bacterial pathogens. Annu. Rev. Phytopathol. 22:35-52.
3. French, J. R., and Manion, P. D. 1975. Variability of host and pathogen in hypoxylon canker of aspen. Can. J. Bot. 53:2740-2744.
4. Grieg-Smith, F. 1983. Quantitative Plant Ecology. third ed. University of California Press, Berkeley. 359 pp.
5. Griffin, D. H., Manion, P. D., Valentine, F. A., and Gustavson, L. 1984. Canker elongation, branch death, and callus formation as resistance or susceptibility responses in *Populus tremuloides* and virulence or avirulence characteristics of *Hypoxylon mammatum*. Phytopathology 74:683-687.
6. Heather, W. A., Chandrashekar, M., and Sharma, J. K. 1980. Forms and degrees of resistance in *Populus* spp. to the *Melampsora* leaf rusts in Australasia. Aust. For. 43:52-57.
7. Ou, S. H. 1980. Pathogen variability and host resistance in rice blast disease. Annu. Rev. Phytopathol. 18:167-187.
8. Pimentel, R. A. 1979. Morphometrics. The Multivariate Analysis of Biological Data. Kendall/Hunt Publ. Co. Dubuque, Iowa. 276 pp.
9. Ray, A. A., ed. 1982. SAS Users Guide. Statistical Analysis Systems Institute, Inc., Cary, NC.
10. Thompson, J. P., and Rees, R. G. 1979. Pattern analysis in epidemiological evaluation of cultivar resistance. Phytopathology 69:545-549.
11. Valentine, F. A., Manion, P. D., and Moore, K. E. 1975. Genetic control of resistance to *Hypoxylon* infection and canker development in *Populus tremuloides*. Pages 132-146 in: Proc. 12th Lake States Forest Tree Improvement Conf. USDA Forest Service.
12. Whittaker, R. H. 1973. Ordination and classification of communities. Handbook of Vegetation Science Vol. 5. Junk, The Hague. 737 pp.