A Multivariate Analysis of Pathogenic Variation in *Colletotrichum gloeosporioides* Infecting the Tropical Pasture Legume, *Stylosanthes scabra*

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ABSTRACT

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Multivariate statistical analysis was used to characterize and classify pathogenic variation in isolates of *Colletotrichum gloeosporioides* that cause anthracnose disease of the tropical pasture legume, *Stylosanthes scabra*. A total of 182 isolates collected from field sites in Queensland, Australia, over the past 15 years were tested for pathogenic variation on six differential genotypes of *S. scabra* using a seedling bioassay. Four reference isolates, representing the four pathogenic races, were included in the bioassay for comparison. Data on the disease severity of 172 field and four reference isolates (set 1) were used to classify the reference isolates into races and to determine if the field isolates belonged to an existing or new race. Linear discriminant functions were developed to classify the four reference isolates, and a cross-validation procedure was used to test the classification success of placing these isolates into the four races. Isolate sr4 was classified 76% of the time as race 1 and 17% of the time as race 4, isolate sr24 was classified 88% of the time as race 3, and isolates wrs20 and wrs32 were mainly classified as either race 4 or 4a. With one small cluster of weakly virulent isolates and the prior expectation of the four races, the field isolates were classified into five virulence groups using cluster analysis. Three of these clusters were associated with the existing races: race 1 in cluster 3, race 3 in cluster 1, and races 4 and 4a jointly in cluster 2. Cluster 1 isolates were avirulent on the differential cultivar Seca, cluster 3 isolates were virulent on 'Seca', and isolates in clusters 2 and 4 were virulent on accessions 36260 and Q10042. For an independent evaluation of the discriminant analysis, additional data were obtained on 14 isolates (set 2), of which eight had been previously classified. The five set 1 clusters were used to develop linear discriminant functions to classify the isolates in set 2. Five of the eight isolates common to both data sets were correctly classified; while isolates wrs20 and wrs32, previously in cluster 2, were classified in cluster 4 in set 2. However, clusters 2 and 4 were close neighbors with no striking differences in the overall disease severity levels on the six differentials for the isolates. In future analyses, three races, represented by the reference isolates sr4, sr24, and wrs20 and/or wrs32, may be used to account for the existing range of pathogenic variation. The usefulness of the multivariate approach to classify field isolates into races, in order to ascertain if isolates belong to an existing or novel race, was discussed.

Additional keywords: pathogen diversity, race composition, virulence analysis.

Species of *Stylosanthes*, native to South and Central America, are among the most important pasture legumes for the tropics and subtropics. Because of their tolerance of acidic and infertile soils, they have been used to improve native pastures or as a forage crop in Australia and several countries in the Americas, Asia, and Africa. In Australia since the early 1960s, species of *Stylosanthes* have been introduced because of their adaptation to the low phosphorus soils of tropical and subtropical Queensland and the Northern Territory, with 12 cultivars released for commercial use (15).

Since it was first reported on *S. guianensis* and *S. humilis* in 1937 at Deodoro in Brazil (3), anthracnose disease, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., has become widespread in most countries where *Stylosanthes* is grown. In Australia, anthracnose seriously damaged an estimated 500,000 ha of *S. humilis* pastures in the 1970s and continues to damage most of the commercial cultivars grown today.

There are two types of anthracnose diseases recognized in Australia. Type A, that attacks almost all species of *Stylosanthes* and produces discrete lesions on all aerial plant parts, is economically more important than type B. Type B infects mainly *S. guianensis* to produce a blight of the terminal shoots (18). This grouping could also apply to anthracnose in almost all other countries. Over 96% of isolates from *Stylosanthes* spp., other than *S. guianensis*, produced typical type A symptoms, and all of the 34 isolates from *S. guianensis* produced type B symptoms in an international study of isolates from Southeast Asia, Africa, Australia, and South America (14).

Pathogenic specialization has been reported in *C. gloeosporioides* causing type A anthracnose (12,18) and four races have been identified on *S. scabra* Vogel in Australia (6). New pathogenic races have arisen in Australia following the release of new cultivars such as *S. scabra* 'Seca' (12). An understanding of the extent of pathogenic variation and of the mechanisms that direct this variation is essential if anthracnose management through the stable genetic resistance of its host is to be achieved.

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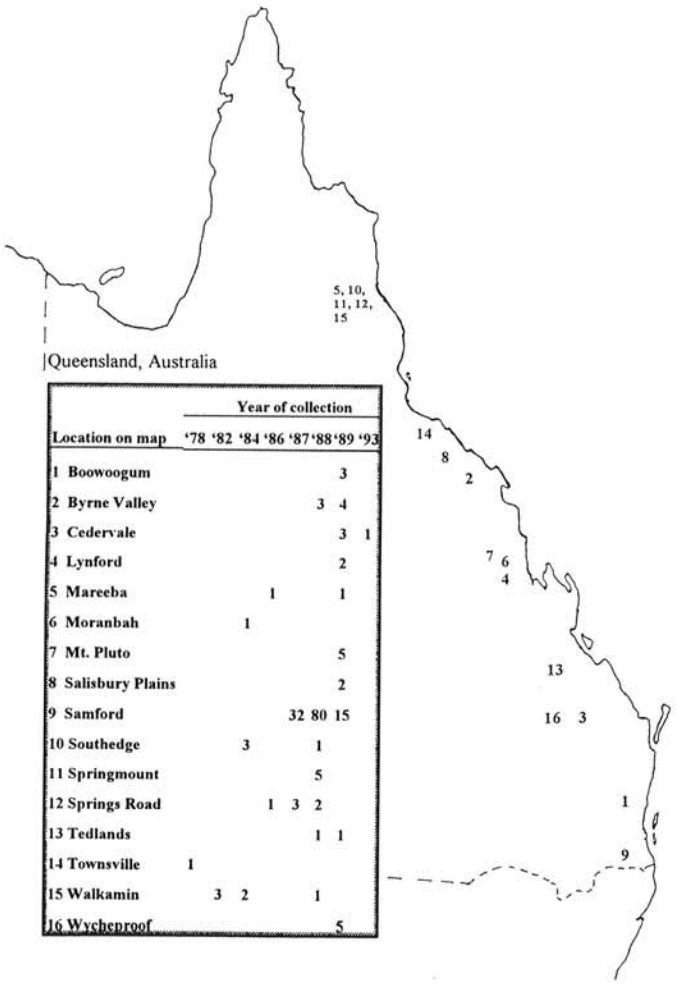
Assessment of pathogenic variation in the type A anthracnose pathogen involves inoculation of a set of six to eight accessions and cultivars of *Stylosanthes* showing moderate to high levels of resistance in a bioassay. Seedlings are scored using a visual disease rating scale (5) to estimate the percentage of tissue damaged, and then qualitatively classified as resistant or susceptible based on an arbitrarily selected threshold value (6,11,12,18). For a pathogen isolate, the bioassay determines the virulence or avirulence on a given host differential that is then used for race assignment. Pathogen virulence also measures relative aggressiveness of an isolate. Therefore, race assignment of an isolate is complicated by the variation that exists in the bioassay. Most of the host differentials produce a mesothetic response, that is a mixture of different infection types on the same leaf (6). Failure to sporulate on a lesion, that is often designated as avirulence (4), cannot be readily used in the case of anthracnose of *S. scabra*.

The Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Queensland Department of Primary Industries conduct regular surveys of *Stylosanthes* pastures that cover over 700,000 ha in the state of Queensland. These surveys are designed to monitor anthracnose severity and provide an ongoing record of the appearance of new pathogenic variants of *C. gloeosporioides*. Field isolates collected in these surveys are assessed for virulence in a seedling bioassay (7) on a set of six host differentials. Four reference isolates, representing four pathogenic races on *S. scabra*, are included in these bioassays to enable comparisons between geographical regions and years.



Location on map	Year of collection '78	'82	'84	'86	'87	'88	'89	'93
1 Boowoogum						3		
2 Byrne Valley					3	4		
3 Cedervale						3	1	
4 Lynford						2		
5 Mareeba				1		1		
6 Moranbah			1					
7 Mt. Pluto						5		
8 Salisbury Plains						2		
9 Samford					32	80	15	
10 Southedge			3			1		
11 Springmount						5		
12 Springs Road				1	3	2		
13 Tedlands						1	1	
14 Townsville	1							
15 Walkamin		3	2			1		
16 Wycheproof							5	

Fig. 1. Number of *Colletotrichum gloeosporioides* isolates obtained from samples of *Stylosanthes scabra* grown in different regions in Queensland, Australia, during 1978 to 1993.

The aim of this work was to develop analytical procedures to classify field isolates into existing or new races. This was achieved in three distinct steps. In step one, analytical procedures were developed to consistently classify the reference isolates into races based on their disease severity scores on the six differentials. If it is not possible to recognize the reference isolates using the severities on the differentials, there is little point in further work. The second step was to classify field isolates into natural virulence groups and ascertain if these virulence groups were associated with particular races. The third step was to evaluate the methodology on an independent data set.

MATERIALS AND METHODS

Source of isolates and bioassay for pathogenic variation. A total of 182 isolates collected between 1978 and 1993 from surveys of *S. scabra* accessions grown at different field sites in Queensland, Australia (Fig. 1), were used. Two *S. scabra* cultivars ('Fitzroy' and 'Seca') and four accessions (36260, 55860, Q10042, and 93116) were used as host differentials. Four isolates (sr4, sr24, wrs20, and wrs32), that represent the four existing pathogenic races of *C. gloeosporioides* on *S. scabra* in Australia (8), were used as reference isolates. All four races were virulent on 'Fitzroy' and avirulent on 93116 (Table 1). Race 1, represented by the reference isolate sr4, was only virulent on 'Fitzroy'. Race 3 (isolate sr24) was the only race virulent on 'Seca'. Races 4 (wrs32) and 4a (wrs20) were most severe on 36260. Difference in susceptibility to races 4 and 4a in Q10042 was quantitative and, in some tests, similar disease severity levels on Q10042 have been recorded with both races (6).

All other isolates from the field survey (field isolates) were purified by single spore isolation and stored as lyophilized cultures. Isolates were screened for pathogenic variation using a seedling bioassay (7), that consisted of growing seedlings of each of the six differentials in a sandy loam soil in 40-mm^2 peat pots in a greenhouse for 6 weeks. Peat pots were randomized in a tray containing four replicate seedlings of each of the host differentials. Each tray was inoculated with a single isolate by spraying seedlings to runoff with a conidial suspension of 10^6 conidia/ml, and then maintained at near 100% relative humidity at 25°C for 48 h. Ten days after inoculation, plants were visually assessed for disease severity using a 10-point rating scale (0 = disease free, 1 = 1 to 3% leaf area diseased, 2 = 4 to 6% leaf area diseased, 3 = 7 to 12% leaf area diseased, 4 = 13 to 25% leaf area diseased, 5 = 26 to 50% leaf area diseased, 6 = 51 to 75% leaf area diseased, 7 = 76 to 87% leaf area diseased, 8 = 88 to 94% leaf area diseased, and 9 = 95 to 100% leaf area diseased).

Description of data sets and statistical analysis. Data on the disease severity of 172 field and four reference isolates for the six host differentials were obtained from the seedling bioassay (set 1). A lyophilized culture of an isolate was grown on oat meal agar to inoculate host differentials in a tray. Because of limited greenhouse space, only a small number of isolates could be screened on any given date. At each inoculation, one or more of the reference isolates were included for comparison. A previous study had shown that the coefficient of variation decreases with an increase in the number of times a bioassay is repeated (7). Therefore, to increase precision, more than one tray was inoculated with some of the reference and field isolates. Consequently, in set 1, data for the reference isolates sr4, sr24, wrs20, and wrs32 originated from 34, 17, 20, and 24 trays, respectively. Set 1 was used to develop linear discriminant functions to classify the reference isolates into races and to determine if the field isolates belonged to an existing or new race.

To provide an independent evaluation of the discriminant analysis, the seedling bioassay was repeated twice with 10 field isolates, of which four had been previously classified, and the four reference isolates. At each inoculation, lyophilized cultures

of these isolates were grown as before and inoculated onto three to six replicates each of the six differentials. Data from these two inoculation dates (set 2) were used to evaluate the classification scheme.

Before conducting detailed statistical analysis, the multivariate response across differentials was explored using principal component analysis. Data from set 1 were averaged over replicates and dates to form a mean value for each isolate. Scores on each differential were considered as separate variates and data were log transformed to stabilize variance. This generated a data matrix with 176 observations (172 field isolates plus four reference isolates) and six variates (differentials). Principal component scores were calculated from the data matrix. An eigenvalue, associated with each component, represented the variance of the component, and the sum of the eigenvalues was equal to the sum of variances of the original six differential scores. An eigenvector was also calculated for each principal component, representing the loading on each variable used to generate the principal component scores. This analysis sought to determine the number of independent dimensions that were needed to account for variation in the response profile. This was useful to interpret the contribution of these dimensions. The SAS (20) and S-plus (22) software packages were used for this analysis.

Discrimination and cross-validation of reference isolates. A discriminant function analysis was used to assign the reference isolates into races. In this analysis, a training set of data on individuals with known group membership was used to develop linear discriminant functions. These functions were then used to classify individuals of unknown grouping. In this case, the data in the training set consisted of disease severity on each of the six differentials for each isolate. We expressed the severity for each isolate as a row vector x. The races were characterized by their location and their spread in the multidimensional space of the variables (disease score on the six differentials). The location of the ith race was given simply by the race mean x_i. Each race was assumed to have the same spread given by the pooled within-group covariance matrix V. To classify an isolate of unknown race with severity vector x_{new}, the linear discriminant analysis first calculated the distance of this isolate from each race. Distance from race I was defined as $d_i = \sqrt{[(x_{new} - x_i)V^{-1}(x_{new} - x_i)^T]}$, in which x^T represents the transpose of the vector x. This distance measure took into account the spread of the races, giving, for example, more weight in directions in which the within-race variation was small. The isolate was then simply classified as belonging to the nearest race, i.e., the race having the smallest d_i. Details of the procedure are given by Anderson (1).

Data on the reference isolates in set 1 contained 95 observations on each of the six host differentials. Because the data were skewed towards low scores on the 9-point scale, a $\log_e$ transformation was necessary to stabilize variance.

One way to test the discriminant rule was to use the data as a training set to produce the linear discriminant functions and then use these functions on the same data to generate predictions. However, this method provided a naive estimate of classification success, that was known to be overoptimistic. Instead, estimates of classification success were obtained using a cross-validation technique (23). This technique was used to obtain an unbiased estimate of the prediction error and should not be confused with a related procedure called 'jackknifing', that is concerned with obtaining unbiased parameter estimates (19). The cross-validation technique operates as follows: observations corresponding to the first inoculation date are dropped from the data set; linear discriminant functions are developed using the remaining data as the training set; the linear discriminant functions are then used to predict race assignment for the dropped observations; observations from the dropped inoculation date are reinstated and observations corresponding to the second inoculation date are dropped; this process is repeated until all the inoculation dates have been excluded in turn. Classification success for an isolate is the percentage of observations (trays) correctly classified into the race corresponding to that isolate. At the end of the process, all of the dates have been used to develop and test the classification success of the linear discriminant functions. The process provides a severe test of the stability of the discriminant rule across inoculation dates.

Classification of field isolates. Data on all isolates in set 1 were averaged over replicates and inoculation dates to form observations representing a mean value for each isolate. This generated a data matrix with 176 observations and six variates. Data were log transformed and subjected to cluster analysis using a complete linkage algorithm (16). In order to ascertain the number of clusters required, the cluster tree was examined, with the number of clusters varying from 2 to 10. The proportional reduction in residual sum of squares (R^2) was plotted against the number of clusters.

The clusters existed in a six-dimensional space corresponding to the severities on the six differentials. To display the clusters, we applied the dimension reduction technique of canonical covariate analysis. As the axes of the canonical covariate coordinate system were the eigenvectors of the matrix $W^{-1}B$, in which W is the within-cluster covariance matrix and B is the between-cluster covariance matrix, the first two canonical covariance coordinates provided the optimal projection of the clusters. The eigenvectors were ordered by decreasing eigenvalue.

Testing the classification of isolates using new data. Linear discriminant functions were developed with set 1 data as the training set and used to classify isolates in set 2. This way, the training set was independent of the evaluation set. Instead of using the reference isolates, the set 1 clusters were used by the linear discriminant functions to define races and membership of the set 2 isolates. Data were log transformed and averaged across replicates and inoculation dates before allocation to races.

RESULTS

The eigenvalues for the principal components of isolates in set 1 showed that the first three components explained 80% of the total variance, indicating that three dimensions were required to explain the variation among the six differentials. The first eigenvector had a positive weight on each component (Table 2). Variation in this component reflected changes in the overall level of severity over all differentials, rather than variation in the pattern of severity across the differentials. The second eigenvector had a large negative coefficient for 36260 and a large positive coefficient for 'Seca', that was indicative of the differential susceptibility of 36260 and 'Seca' to the isolates. Similarly, the third eigenvector contrasted responses obtained on 36260 and 'Seca' with the responses on Q10042.

TABLE 1. Average disease severity[a] of four reference isolates of *Colletotrichum gloeosporioides* inoculated on a set of differential genotypes of *Stylosanthes scabra*

Differential cultivar or accession	Isolate[b]			
	sr4 (race 1)	sr24 (race 3)	wrs32 (race 4)	wrs20 (race 4a)
'Fitzroy'	57 (24)[c] +[d]	97 (0) +	25 (6) +	93 (2) +
'Seca'	0 (0) –	19 (9) +	0 (0) –	0 (0) –
36260	2 (0) –	25 (6) +	97 (0) +	97 (0) +
Q10042	3 (1) –	5 (2) –	9 (4) –	33 (14) +
55860	3 (0) –	12 (3) +	3 (0) –	5 (0) –
93116	0 (0) –	0 (0) –	0 (0) –	0 (0) –

[a] Percent leaf area diseased.
[b] Source of data is Chakraborty et al. (7).
[c] Figure in parenthesis is the standard error of mean.
[d] Indicates susceptible (+) or resistant (–) based on >10% leaf area diseased.

Discrimination and cross-validation of reference isolates. Linear discriminant functions were developed and used to classify the reference isolates into races. The classification success was determined using the cross-validation procedure of removing data for one inoculation date at a time. Isolate sr4 was classified 76% of the time as race 1 and 17% of the time as race 4. Isolate sr24 was classified 88% of the time as race 3 (Table 3). Isolates wrs20 and wrs32 were mainly classified as either race 4 or 4a. Discrimination between these two isolates was less successful, and the two could not be distinguished from each other on more than 50% of instances.

Classification of field isolates. Kmeans and complete linkage algorithms (16) were used in an exploratory analysis to establish the existence of clusters in the set 1 data. Both techniques showed a gradual proportional reduction in residual sum of squares with increasing number of clusters. There are no satisfactory methods for determining the number of clusters for any type of cluster analysis. We presented results of the complete linkage method (Fig. 2), with the coefficient of determination (R^2) increasing with an increase in the cluster number from 2 to 10. For all numbers of clusters between 2 and 10, four avirulent or weakly virulent isolates were grouped into a separate cluster of their own. Therefore, with the prior expectation of four races and allowing for the additional cluster of weakly pathogenic isolates, the five-cluster solution was selected as the best to represent the number of natural virulence groups in set 1 data. These clusters accounted for 53% of the variation. In the five-cluster solution, cluster 1 contained 53 isolates, including the reference isolate sr24; cluster 2 contained 55 isolates, including reference isolates wrs20 and wrs32; and cluster 3 contained 16 isolates, including the reference isolate sr4. The two remaining clusters did not contain any reference isolates. Cluster 4 contained 48 isolates and cluster 5 contained the four avirulent or weakly virulent isolates.

Although clusters existed in a six-dimensional space corresponding to the severities on the six differentials, the first two canonical covariate coordinates explained 95% of the variation among clusters. A projection of these canonical covariates showed that groups of isolates represented by clusters 1, 3, and 5 were easily separated (Fig. 3), but clusters 2 and 4 were difficult to separate. The joint clustering of isolates wrs20 and wrs32 was consistent with our inability to distinguish them using the discriminant analysis.

The overall mean severity of the set 1 isolates lay at the origin of the canonical coordinates (Fig. 3). To relate the original log-transformed scores on the differentials to the two canonical coordinates on the plot, we projected the original axes of the six differentials onto the plot, centered at the overall mean. The length of arrows demonstrated a change in scores on the canonical coordinate given a unit change from the mean score for each of the six differentials. Cluster 1 isolates were separated from the others by their virulence on 'Seca'. The remaining isolates tended to lie along an axis corresponding to virulence on 36260 and 'Fitzroy'. Virulence on these differentials increased from cluster 5 through 3 and 4 to cluster 2, that contained isolates that were most virulent on these differentials. The other three differentials had small contributions in the first two canonical coordinates and, therefore, were less important in explaining variation.

The characteristics of isolates in the five clusters could be summarized from their disease severity on the six differentials (Table 4). Cluster 1 contained isolates virulent on 'Seca', and cluster 3 contained isolates that were avirulent on 'Seca'. Isolates in clusters 2 and 4 were virulent on 36260 and Q10042, in addition to 'Fitzroy', with cluster 2 generally showing a higher disease severity towards Q10042 than those in cluster 4.

Testing the classification of isolates using new data. The five clusters in set 1 data were used as the training set to develop linear discriminant functions and these were used to classify the 14 isolates in set 2. Of the four field isolates that were represented in both data sets, three (cs205, cs239, and cs282) were correctly classified into cluster 1; cs289, that was in cluster 1 in set 1, was misclassified in cluster 4. Among the reference isolates, sr4 and sr24 were correctly classified in clusters 3 and 1, respectively

TABLE 2. Eigenvectors for the first three principal components from an analysis of anthracnose severity on six differential genotypes of *Stylosanthes scabra* inoculated with 176 field and reference isolates of *Colletotrichum gloeosporioides* in data set 1

Differential cultivar or accession	Principal component 1	2	3
'Fitzroy'	43[a]	32	16
'Seca'	20	73	−49
36260	59	−53	−59
Q10042	47	−15	54
55860	31	21	31
93116	34	9	7
Percent cumulative variation explained	42	68	80

[a] Values have been multiplied by 100 and rounded to the nearest integer, and values greater than the root mean square coefficient are underlined.

TABLE 3. Classification of reference isolates of *Colletotrichum gloeosporioides* in data set 1 into the four races using linear discriminant functions developed from the severity of these isolates on a set of six differential genotypes of *Stylosanthes scabra*

Isolate	No. of observations	Classified as sr4 (race 1)	sr24 (race 3)	wrs32 (race 4)	wrs20 (race 4a)	Success (%)[a]
sr4	34	26	1	6	1	76
sr24	17	1	15	1	0	88
wrs20	20	0	0	11	9	45
wrs32	24	2	3	10	9	41

[a] Classification success for an isolate is the percentage of observations (trays) correctly classified into the race corresponding to that isolate. Classification is based on a cross-validation technique that operates as follows: observations corresponding to the first inoculation date are dropped from the data set; linear discriminant functions are developed using the remaining data as the training set; the linear discriminant functions are then used to predict race assignment for the dropped observations; observations from the dropped inoculation date are reinstated, and observations corresponding to the second inoculation date are dropped; this process is repeated until all the inoculation dates have been excluded in turn.

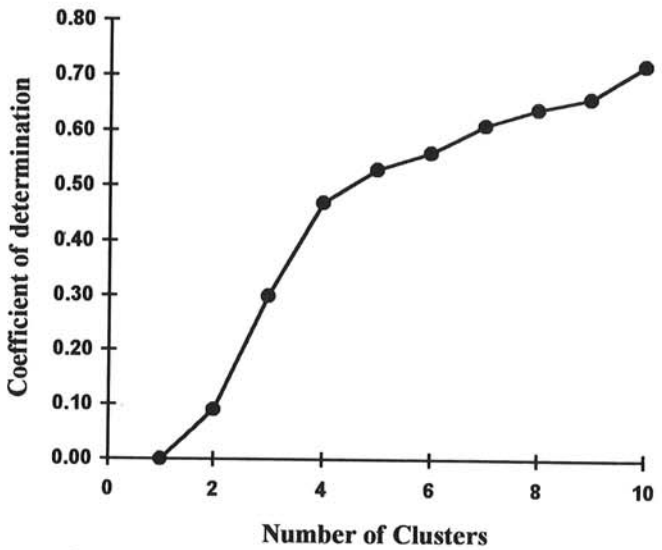


Fig. 2. Coefficient of determination (R^2) for two to ten clusters of isolates of *Colletotrichum gloeosporioides* causing anthracnose on six differential host genotypes of *Stylosanthes scabra* in a data set comprising of 176 reference and field isolates.

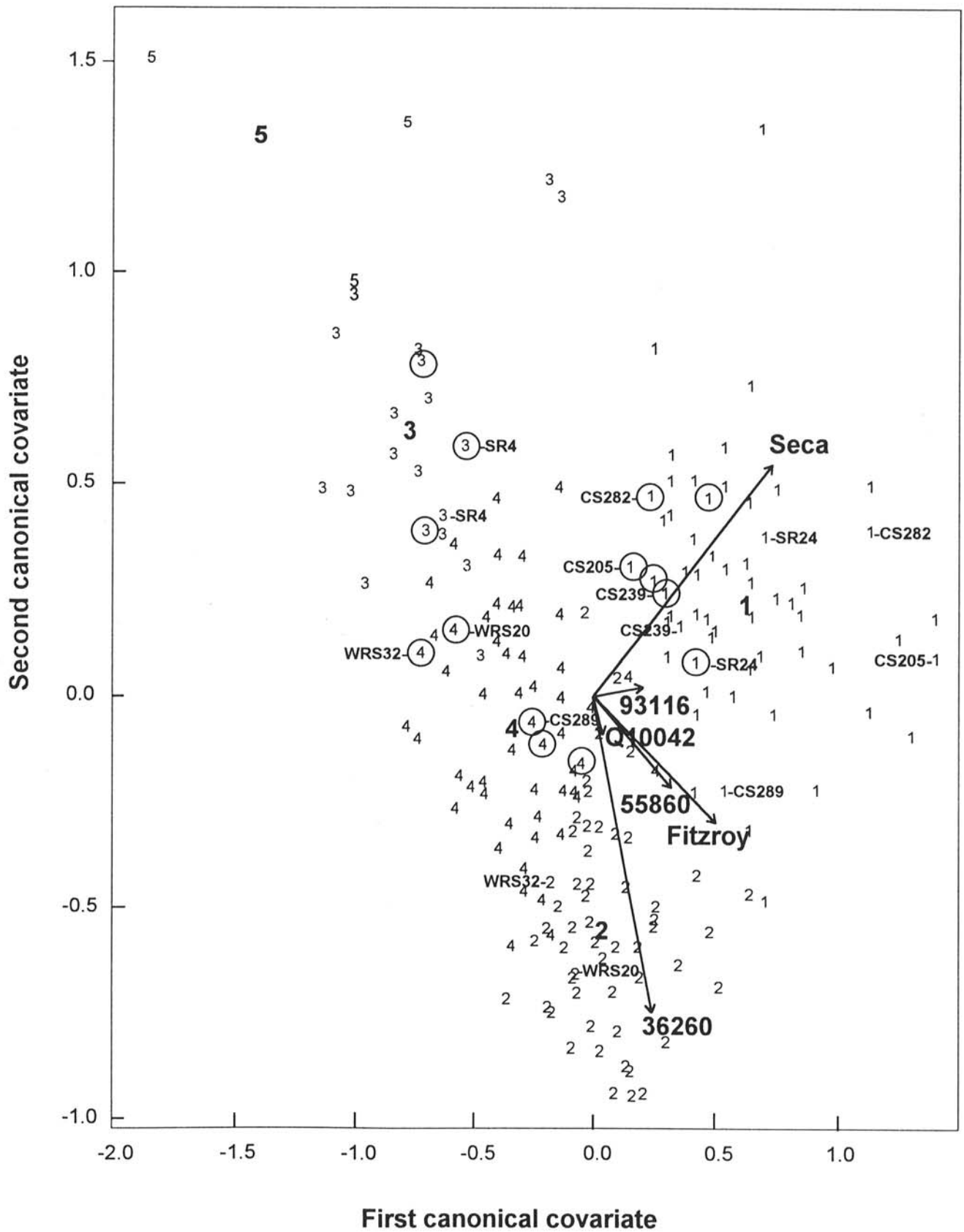


Fig. 3. Canonical covariate coordinates representing mean anthracnose severity for 182 isolates of *Colletotrichum gloeosporioides* inoculated onto six differential host genotypes of *Stylosanthes scabra*. The six original axes (arrows) have been projected onto the plot to relate the log-transformed scores on the differentials to the two canonical coordinates. The arrows originate from the point representing the overall mean severity of the set 1 data. The length of each arrow represents the change in canonical coordinates given a unit change from the mean score for each differential. The center point of each cluster is shown as a large numeral. Isolates from set 2 have been circled. For clarity, only the eight isolates common to both data sets 1 and 2 have been labeled.

(Fig. 3). Isolates wrs20 and wrs32, previously classified in cluster 2 in set 1, were classified into cluster 4. However, based on the level of anthracnose severity on the host differentials for clusters 2 and 4 (Table 4), there was very little difference between these two clusters. Of the six field isolates not present in set 1, two each were classified in clusters 1, 3, and 4.

DISCUSSION

We used multivariate techniques to characterize and classify pathogenic variation in isolates of *C. gloeosporioides* infecting *S. scabra*. A set of 176 isolates were grouped into five clusters to represent the existing range of virulence. Discriminant functions were developed using the five clusters and applied to classify an independent set of data on 14 isolates. Race assignment was known for eight of these isolates. Of these, five were classified correctly and one was misclassified. Although the remaining two isolates were classified into a different cluster compared with their earlier classification, these two clusters were near neighbors with little difference in anthracnose severity on the host differentials. This showed that the multivariate techniques were useful to characterize pathogenic variation in the field isolates when variability within the bioassay would have made it cumbersome to use simple techniques based on thresholds. In the future, a similar analysis can be used to classify field isolates into one of the established races or into an unclassified group. Further analysis of isolates in the unclassified group may then be carried out to test for new races.

The principal component analysis provided a measure of an overall usefulness of the six host differentials used in characterizing the isolates. The first eigenvector was positive on all differentials and may be interpreted as a measure of the relative susceptibility of the genotypes without consideration of the differential interaction. With regard to interactions, the susceptible 'Fitzroy', the resistant 93116, and the partially resistant 55860 did not help in the discrimination of the isolates. Of these, the universal suscept and the resistant 93116 were necessary for a differential set. The accession 55860 could probably be eliminated from the host differential set.

Cluster analysis of set 1 suggested the existence of four or five clusters. Three of these could be associated with the existing races: race 1 (sr4) in cluster 3, race 3 (sr24) in cluster 1, and races 4 (wrs32) and 4a (wrs20) jointly in cluster 2. Cluster 4 was very similar to cluster 2 in its level of severity on the host differentials, and the small difference between these two clusters may not be biologically relevant. Cluster 5 consisted of four nonpathogenic or weakly pathogenic isolates. The grouping of isolates wrs20 and wrs32 into one cluster confirmed earlier observations (6,7) and brought into question the need to create two separate races based on these two isolates as opposed to a single race. In future analyses, three races, represented by the reference isolates sr4, sr24, and wrs20 and/or wrs32, may be used to account for the existing range of pathogenic variation.

TABLE 4. Mean, standard deviation in parentheses, and median of log-transformed anthracnose severity ($\ln[x + 1]$) on a set of six host differential genotypes of *Stylosanthes scabra* for all 182 reference and field isolates of *Colletotrichum gloeosporioides* in sets 1 and 2 contained in five groupings derived by cluster analysis

Differential cultivar or accession	Statistic	Cluster 1	2	3	4	5
'Fitzroy'	Mean	2.1 (0.2)	1.9 (0.2)	1.4 (0.4)	1.7 (0.3)	0.4 (0.5)
	Median	2.1	1.8	1.4	1.7	0.4
'Seca'	Mean	1.0 (0.3)	0.3 (0.2)	0.1 (0.2)	0.1 (0.2)	0.2 (0.3)
	Median	0.9	0.0	0.0	0.0	0.1
36260	Mean	1.2 (0.3)	1.7 (0.4)	0.5 (0.3)	1.2 (0.3)	0.1 (0.2)
	Median	1.2	1.8	0.5	1.2	0.1
Q10042	Mean	1.1 (0.4)	1.4 (0.2)	0.8 (0.3)	0.9 (0.4)	0.2 (0.2)
	Median	1.1	1.4	0.8	0.9	0.1
55860	Mean	0.9 (0.3)	0.9 (0.3)	0.5 (0.3)	0.6 (0.2)	0.2 (0.2)
	Median	0.9	0.9	0.5	0.6	0.2
93116	Mean	0.5 (0.3)	0.6 (0.2)	0.1 (0.1)	0.3 (0.2)	0.1 (0.2)
	Median	0.5	0.7	0.0	0.2	0.0

In plant pathology literature, both the concept and nomenclature of pathogenic variation are subjects of intense scientific debate (2,17,21). In this work, virulence was defined as 'the relative amount of damage caused to a host by a given pathogen' (21). In genetically well-defined host-pathogen combinations, such as the cereal rusts and mildews, analysis of virulence using infection type data is relatively clear-cut with the availability of near-isogenic lines as host differentials (24). This is not the case with other less-studied host-pathogen interactions, such as the one studied here, in which a basic knowledge of the nature of variation in the host-pathogen association was necessary before any detailed analysis of pathogenic variation could be made. For example, it is necessary to establish if the association is nondifferential, in that, regardless of the host genotype, a pathogen of one genotype always produces more disease; or differential, in that a particular combination of hosts and pathogens results in greater or lesser development than would be predicted from the overall performance of either partner (9). The analytical approach used here could be used to demonstrate the differential nature of a host-pathogen interaction.

Previous studies on pathogenic variation in *C. gloeosporioides* causing type A anthracnose of *Stylosanthes* spp. in Australia have all used thresholds to convert disease severity ratings into virulence or avirulence categories (13). The number of virulence groups identified using a threshold changed from seven to sixteen for isolates collected from a field site in Queensland within a period of 2 years (11). Using different thresholds on the 0 to 9 disease rating data on the 182 isolates used in the present study, we could potentially generate between one and twenty-two virulence groups. Use of thresholds is not satisfactory because of large experimental errors in bioassays used to characterize pathogenic variation in *C. gloeosporioides* (7).

The rather limited range of pathogenic variation that could be classified in the three races was partly a reflection of the range of resistance factors represented in the six host differentials. If each differential carried a unique resistance factor, up to 2^6 virulences could be differentiated. It appeared that differentials other than 'Seca', 36260, and 'Fitzroy' (Fig. 3) may not have been useful in differentiating the isolates. Work on the development of a better host differential set must be a priority and any new differential must only include lines that are potential sources of resistance in a breeding program. Otherwise, an expanded differential set may simply characterize 'unnecessary' virulence carried by the pathogen that is not required to facilitate pathogenicity on either the cultivar of origin or cultivars commonly grown in the area (10). The current differential set represents some of the resistances in use in the commercial cultivars; although a clear understanding of the genetics of resistance is lacking. An improved knowledge of the genetics of anthracnose resistance in *S. scabra* must be a prerequisite to the development of a better differential set. Developing a less variable bioassay for the study of pathogenic variation must be the other priority. In the meantime, the multivariate approach used in this work could be used to classify field isolates of *C. gloeosporioides* infecting *S. scabra* into existing or potentially new pathogenic races.

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