Field Evaluation of Quantitative Resistance to Anthracnose in *Stylosanthes scabra*

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ABSTRACT

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A field study was conducted for two successive growing seasons at the Southedge Research Station, Queensland, Australia, to evaluate and characterize the expression of a quantitative resistance in seven accessions of the tropical pasture legume *Stylosanthes scabra* to anthracnose caused by *Colletotrichum gloeosporioides*. These accessions had shown moderate to high levels of resistance to four isolates of the pathogen in earlier glasshouse studies. Five of the seven accessions showed moderate to high levels of resistance in the field. Resistance was generally consistent in the two seasons, despite a high level of initial inoculum in 1989. A "broken stick" model, fitted using a weighted linear regression, best summarized the disease progress in most accessions. Parameter estimates of the logistic model were not precise enough to be useful in comparing accessions. Two separate rates were used as alternatives to the apparent infection rate. Rate of early anthracnose progress was lower in most accessions than in Fitzroy. Of the eight attributes used, area under the disease progress curve (AUDPC) provided the best measure of resistance in both seasons. A significant rank correlation between AUDPC, spore production in the field, and daily rate of sporulation showed that, in association with other attributes, spore production in the field can be used in evaluating quantitative resistance. Performance of accessions was effectively and simply depicted by combining rankings based on the various attributes in a star plot.

Species of the tropical pasture legume *Stylosanthes* are used for pasture improvement in semiarid tropics and parts of subtropical Australia (32). In the state of Queensland, *Stylosanthes* species are capable of adapting to 18.3 million ha (30). Of the four commercial species, *S. humilis* Kunth, *S. hamata* (L.) Taub., *S. guianensis* (Aubl.) Sw., and *S. scabra* Vogel, the most widely used are *S. scabra* 'Seca' and *S. hamata* 'Verano'. Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc in Penz. has been a major limiting factor in the persistence and productivity of *Stylosanthes*-based pastures in Australia. Two types of anthracnose have been recognized (10). Type A causes discrete lesions with grey centers and dark brown margins on all aerial plant parts in all species of *Stylosanthes* and is economically more important than the type B disease (10). Up to 80% loss in seed and herbage yield due to the type A disease has been recorded in susceptible cultivars (7). Type B, mainly infecting *S. guianensis*, causes a general necrosis of the terminal shoots with the affected stems and leaves turning black to give a blighted appearance (10).

New pathogenic races, able to attack previously resistant cultivars, have arisen rapidly in Australia. *S. scabra* 'Fitzroy', released in 1979 for use in drier areas with average annual rainfall of less than 700 mm, has been discarded due to severe damage from highly virulent strains of the type A pathogen (5). At the time of its release in 1976, the cultivar Seca was highly resistant to all known strains of *C. gloeosporioides* in Australia (10). By 1982, a new race virulent on Seca was identified (5). Another race, virulent on *S. scabra* accessions 36260 and Q10042, was recorded from North Queensland (6) after these accessions were grown at field sites for 3 yr. Four races have now been identified within the Type A disease-causing group in Australia (11). This includes the one virulent on *S. viscosa* Sw. accession 33941 (10).

To control this variable pathogen, mixtures of genotypes, carrying resistance to a number of races, are currently being tested. Another way to combat a variable pathogen is by utilizing a broad-based resistance effective against all or most variants or races of the pathogen. Terms such as horizontal resistance (29), partial resistance, and durable resistance have been used to describe different features of what may or may not be similar forms of quantitatively expressed resistance. In many cases, this form of resistance allows some disease development but at a rate slower than that in a more susceptible host (15,18,29). Rate-reducing resistance such as slow rusting (18) and slow mildewing (21) operates by reducing one or more components of the epidemic cycle.

Some accessions of *S. scabra* have shown a quantitative resistance to anthracnose in seedling assays (3). These accessions increased the duration of incubation period and reduced infection efficiency of *C. gloeosporioides*. Resistance was expressed consistently under different concentrations of inoculum and at four different day-night temperatures (2). The purpose of the present study was to evaluate the effectiveness of this quantitative resistance in selected accessions of *S. scabra* under field conditions over two growing seasons. In addition, rate of disease progress, area under the disease progress curve (AUDPC), lesion types, and spore production in the field were measured to characterize the expression of quantitative resistance.

MATERIALS AND METHODS

Selection of host accessions. We used seven accessions of *S. scabra* (Table 1) which have shown low levels of anthracnose with three races of *C. gloeosporioides* in previous glasshouse trials (3; Chakraborty, *unpublished*). Fitzroy and accession 93116 were used as susceptible and resistant controls, respectively. A 'disease-free' Fitzroy, maintained by spraying with benomyl (Benlate; DuPont Australia Ltd., Sydney, NSW 2060) every 2 wk and the cultivar Verano of *S. hamata* were planted as two additional controls.

Field plots. Plots were established in December 1987 at the Southedge Research Station (17° 0′S; 145° 20′E) of the Queensland Department of Primary Industries. The site, with an average annual rainfall of 1,112 mm and a granitic sandy-

loam soil, is typical of large areas of Cape York Peninsula where *Stylosanthes* spp. have a useful role in improving native grass pastures. The area had been used for *Stylosanthes* evaluation in previous years, and all known pathogenic races had been recorded (6).

Single seedlings were raised in a 3:2:1 (loam/sand/peat) mixture in 4 × 4 cm 'rite gro' (Cheetham Plastics Ltd., Brisbane, Australia) pots in a glasshouse. Seedlings were fertilized, as required, with a 0.8 g/L solution of a mineral fertilizer ('Aquasol', Hortico, Sydney, Australia) containing 23% N, 4% P, and 18% K as well as the trace elements. Six-wk old seedlings were transplanted into 4.5 × 4.5 m field plots on 16 December 1987. Between and within row spacings of 50 cm accommodated 100 plants per plot. After transplanting, plots were sprayed with the pre-emergent herbicide oryzalin (Surflan; Elanco Products Co., Australia). The 11 treatments were arranged in a randomized complete block with three replications. Plots were separated from each other on all sides by a 5-m fallow to reduce interplot interference.

A group of three Fitzroy plants was planted in the center of each plot and an anthracnose epidemic was initiated by inoculating each of these plants with a different isolate of *C. gloeosporioides*. Isolates NQ 135, SR 24, and WRS 20, representing races 1, 3, and 4a, respectively, were grown for 5–7 days at near-UV light with a 12-hr photoperiod. Races of *C. gloeosporioides* were identified using a host differential set consisting of cultivars Fitzroy and Seca and accessions 36260, 55860, and Q10042 (5). Plants were inoculated between 4 and 6 p.m. on 12 January 1988, by spraying a conidial suspension of 10^6 conidia/ml to incipient runoff and then covered with a reflective plastic bag (Agricultural Plastics, Sydney, Australia) for about 20 hr to provide the necessary leaf wetness (4).

At the end of the growing season (June 1988), all plants were mowed to a height of 10 cm with a reciprocating mower. Plots in 1989 mainly consisted of plants which regrew in spring. Plants which did not survive the winter and/or anthracnose attack were replaced with 6 wk-old glasshouse-grown seedlings in early summer (December 1988). At the time of this planting, the transplanted seedlings were at the vegetative growth stage 12, according to the 'Winch' system of legume growth stage keys (12) and similar in size to the surviving second year plants. Infected plant residues served as the source of primary inoculum in 1989.

Disease and other assessments. In 1988, starting 4 wk after inoculation, all 20 plants along the two diagonals in each plot were assessed on eight occasions at nominal 1–2 wk intervals. Proportion of leaf area diseased (y) was estimated from the top 10–15 cm length of a randomly selected branch for each plant using a ten-point visual assessment key (2) based on the Horsfall and Barratt (9) scale. In 1989, 20 plants per plot, selected at random, were assessed for y on seven occasions, and all 100 plants per plot were assessed on one occasion.

Lesion type and spore production were assessed for each accession on 30 May 1988 and 6 February 1989. Lesion types were determined on three infected young leaves for each of 10 randomly selected plants per plot in both years. In assessing spore production, all 10 plants per plot were used in 1988, whereas only five plants per plot were used in 1989. Four different lesion types were recognized: 1) minute brown/dark-brown specks similar to a hypersensitive reaction (highly resistant); 2) lesion <0.5 mm in diameter with a dark margin and grey center (susceptible); 3) lesion 0.5–1 mm in diameter with a dark-brown margin and grey center (susceptible); and 4) lesions >1 mm in diameter with dark-brown margin and grey center (highly susceptible). As most accessions produced a mesothetic reaction (a mixture of lesion types on a single leaf), weighted lesion types (y_w) were calculated by ranking lesion types according to their relative frequencies (26). The highest and lowest rankings, respectively, were assigned to the most and least prevalent lesion types and y_w was calculated using the following formula:

$$y_w = [\Sigma \text{ (rank} \times \text{lesion type code)}]/ \Sigma \text{ rank}.$$

After recording the type and number of lesions, all three leaves from a plant were immersed in 5 ml of sterile distilled water in a screw cap container and shaken for 2 hr on an orbital shaker (13). The number of spores present in the suspension (n_o) was counted using a hemocytometer. This provided a measure of inoculum production on each accession under prevailing field conditions. To estimate the production of secondary inoculum (spore production per day, n_d) under a more favorable condition, leaves were removed from the suspension and incubated on a moist, sterile filter paper in a petri dish for 24 hr at 25 C. Leaves were then resuspended in 5 ml of fresh sterile distilled water and shaken, and spore numbers were obtained from the suspension. If not counted immediately after shaking, 0.02 ml of lactophenol per ml of suspension was added to inhibit spore germination (8), and the suspension was stored at 4 C.

Leaf area was determined using a Delta-T area meter (Delta-T Devices Ltd., Burwell, Cambridge, England). For lesion types 1 and 2, diameters of 150 lesions each were measured using a stereo microscope; and mean lesion areas of 0.105 and 0.313 mm^2, respectively, were determined assuming a circular outline. Lesion area of types 3 and 4 were measured individually by superimposing a transparent acetate sheet divided into one-sq-mm grids. Both n_o and n_d were expressed on a per unit lesion area basis.

Statistical analysis. Rate of disease progress, AUDPC, y_w, time required for disease severity to reach 10% (t_{10},23), terminal severity (y_t), n_o, and n_d were analyzed using analysis of variance and regression techniques. Data on y for each year were treated separately, and disease progress curves were generated for each accession. AUDPC was calculated using the trapezoid rule where the area is approximated by joining the points at the observed times by line segments and calculating the area of the resultant trapezoids. Data were transformed, where necessary, to correct for heterogeneous error variance. The SAS (20) and Genstat 5 (19) software packages were used for analysis.

RESULTS

Disease progress. Symptoms of anthracnose developed on the three central Fitzroy plants within a week of inoculation with the three races. A few plants in one Fitzroy plot showed some disease from the background inoculum. Spread of anthracnose from the central source was very rapid in the susceptible Fitzroy and 55803. In other accessions, the spread was slow, and in 93116 and the benomyl-sprayed Fitzroy only a few plants became infected. From a low level in early 1988, the y of Fitzroy increased rapidly between day 46 and 71; and, thereafter, no further increases in y were noted (Fig. 1). There was little increase in y of the highly resistant 93116 or benomyl-sprayed Fitzroy (data not shown), although it increased steadily in 55803 and 92873 following a slow start.

TABLE 1. Origin of *Stylosanthes scabra* and *S. hamata* accessions used for anthracnose field trial at the Southedge Research Station in 1988 and 1989

CSIRO accession no.	Species	Country of origin	Latitude	Longitude
38842[a]	*S. hamata*	Venezuela	10.36 N	71.42 W
40205[b]	*S. scabra*	Brazil	12.40 S	39.00 W
55803	*S. scabra*	Brazil	12.32 S	39.32 W
55860	*S. scabra*	Brazil	12.30 S	38.31 W
92873	*S. scabra*	Brazil	14.24 S	49.12 W
92918	*S. scabra*	Brazil	12.48 S	49.12 W
93037[c]	*S. macrocephala* & *S. scabra*	Brazil	21.36 S	51.36 W
93055	*S. scabra*	Brazil	17.48 S	44.48 W
93099	*S. scabra*	Brazil	15.24 S	43.00 W
93116	*S. scabra*	Brazil	...[d]	...

[a] Cultivar Verano.
[b] Cultivar Fitzroy.
[c] Accession containing a mixture of *S. macrocephala* and *S. scabra*.
[d] Information not available.

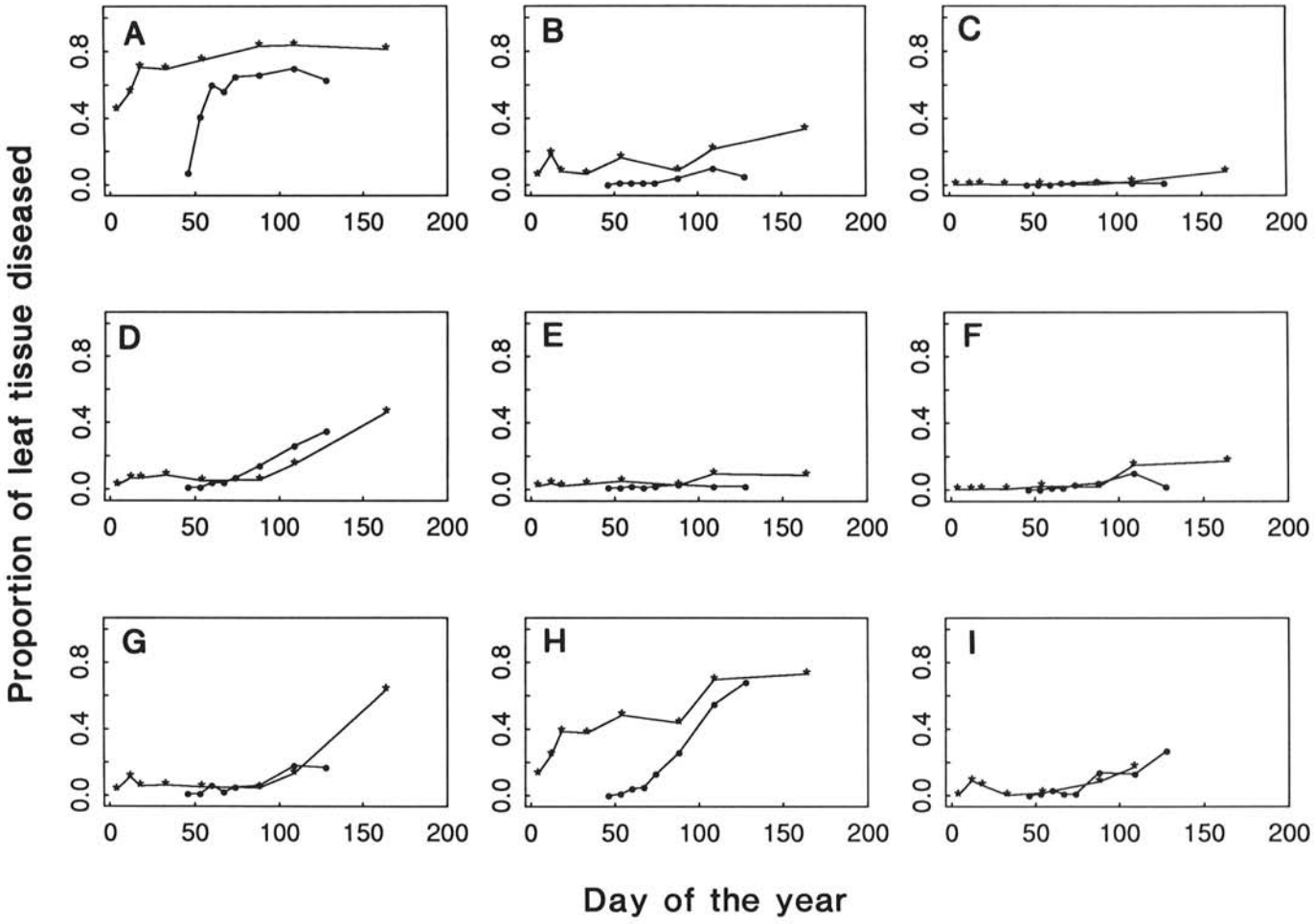

Fig. 1. Anthracnose development on *Stylosanthes scabra* **A,** 'Fitzroy'; **B,** accessions 55860; **C,** 93116; **D,** 92873; **E,** 93099; **F,** 93055; **G,** 92918; **H,** 55803; and **I,** *S. hamata* 'Verano' during 1988 (•) and 1989 (*) at Southedge Research Station. The epidemic was initiated in 1988 on 12 January by inoculating Fitzroy seedlings planted in the center of each plot. In 1989, the epidemic developed from infected plant residues in each plot.

TABLE 2. Early (r_e) and late (r_l) rates of anthracnose progress in accessions of *Stylosanthes scabra* and *S. hamata* in field plots in 1988 and 1989

	Rate of anthracnose progress ($\times 10^2$)			
	1988		1989	
Accession	r_e[a]	r_l[b]	r_e	r_l
Fitzroy	2.30 (0.27)[c]	−0.14 (0.16)	0.37 (0.07)	−0.07 (0.10)
55803	0.23 (0.15)	1.20 (0.12)	0.38 (0.08)	0.26 (0.11)
92873	0.21 (0.01)	0.48 (0.07)	0.00 (0.03)	0.53 (0.05)
92918	0.13 (0.06)	0.24 (0.06)	−0.06 (0.05)	0.79 (0.06)
93055	0.09 (0.04)	0.06 (0.03)	0.07 (0.03)	0.18 (0.04)
Verano	0.05 (0.07)	0.36 (0.07)	0.03 (0.05)	0.54 (0.21)
55860	0.01 (0.02)	0.12 (0.02)	0.03 (0.07)	0.29 (0.09)
93099	0.06 (0.01)	0.00 (0.00)	0.03 (0.01)	0.05 (0.02)
93037	...[d]	...	0.71 (0.08)	0.28 (0.11)
93116	0.03 (0.01)	0.00 (0.00)	0.00 (0.00)	0.10 (0.02)

[a] r_e = Represents increase in anthracnose severity per unit per day during the initial time period, between day 46 (15 February) and 71 (11 March) in 1988 and day 4 (4 January) and 88 (29 March) in 1989, when y of Fitzroy increased rapidly.

[b] r_l = Represents increase in anthracnose severity per unit per day in the later part of each season, after day 71 in 1988 and day 88 in 1989, when y of Fitzroy did not increase.

[c] Figure in parenthesis is the standard error ($\times 10^2$) of the estimated regression coefficient.

[d] Not assessed.

In 1989, the Fitzroy and 55803 plots, with high initial inoculum reserves, recorded a high y from a very early part of the season (Fig. 1). Among the other accessions, 92918 and 55860 showed increased y toward the end of the season. Anthracnose progress curves in all other accessions were of similar shapes in both years. Accession 93037 was only assessed in the second year after replacing all contaminant *S. macrocephala* Ferr. et Costa plants with *S. scabra*-type plants from the 93037 collection. This accession showed a high degree of susceptibility (data not shown). There was considerable anthracnose development in the benomyl-sprayed Fitzroy plots in 1989 due to the occurrence of benomyl-resistant strains in the pathogen population (Boland and O'Brien, *unpublished*).

Rate of disease progress. The logistic model of Verhulst, as used by VanderPlank (28), was fitted to the data and either the nonlinear estimation procedure (19) failed to converge, or the resulting parameter estimates had very large standard errors. However, in almost all cases the logistic curve gave a reasonable fit to the data, that is, it provided a reasonable way of smoothing the data. The conclusion to be reached from this analysis is that the estimated logistic parameters for progress curves gave a very poor way of comparing accessions because of the imprecision of the estimates. We considered an analysis which would give more precise estimates and therefore more precise comparisons.

The progress curves for Fitzroy in both years display a rapid, early increase in disease followed by a later period of little change in disease amount. We have taken the disease progress for Fitzroy to define two distinct periods, 'early' and 'late', with day 71 being the break for 1988 and day 88 for 1989. We modeled the disease progress curve for each season by a straight line over the early phase and a straight line over the late phase, ensuring that the two lines meet at the time of transition from the early phase to the late phase: the so-called "broken stick" model.

The slopes of the two straight lines estimate the rate of disease progress in the early (r_e) and late (r_l) phases. If the disease progress

TABLE 3. Time (days from first observation) needed to reach 10% severity (t_{10}) and area under the disease progress curve (AUDPC) for accessions of *Stylosanthes scabra* and *S. hamata* 'Verano' at Southedge Research Station in 1988 and 1989 (t_{10} values for 1988 are averages of plant times, and values for 1989 are averages of plot times)

	t_{10}		AUDPC[a]	
Accession	1988	1989	1988	1989
Fitzroy	8 (100)[b]	0 (100)	49.9 (7.5)	123.3 (5.9)
55803	38 (98)	0 (100)	24.1 (0.7)	84.9 (9.3)
92873	41 (95)	17 (100)	12.5 (1.6)	24.2 (1.4)
Verano	67 (88)	87 (100)	7.9 (2.1)	5.9 (1.6)
92918	71 (75)	42 (100)	7.3 (0.9)	27.8 (2.5)
55860	159 (46)	19 (100)	3.5 (0.7)	27.8 (4.7)
93055	264 (27)	105 (100)	3.3 (0.5)	11.6 (0.4)
93099	264 (26)	133 (67)	1.7 (0.1)	9.3 (2.5)
93116	636 (12)	160 (67)	0.8 (0.05)	3.2 (0.7)
Control[c]	1174 (7)	22 (100)	0.7 (0.1)	43.5 (13.3)
93037	...[d]	15 (100)	...	83.6 (4.4)

[a] AUDPC = Calculated using proportion of tissue diseased.
[b] For t_{10}, figure in parenthesis is the percentage of plants and plots which reached a 10% severity level in 1988 and 1989, respectively. For AUDPC, figure in parenthesis is the standard error of the mean.
[c] Control = Fitzroy that received a spray of benomyl every 2 wk in an attempt to control anthracnose.
[d] Not assessed.

TABLE 4. Spore production (n_o) and rate of sporulation per day (n_d) by *Colletotrichum gloeosporioides* on accessions of *Stylosanthes scabra* and *S. hamata* 'Verano' under field conditions in 1988 and 1989

	n_o[w]		n_d[w]	
Accession	1988	1989	1988	1989
Fitzroy	2.02 A[x]	3.45 A	2.18 A	3.48 A
55803	0.82 B	1.20 C	1.77 AB	1.67 B
92873	0.62 B	0.00 E	1.19 BC	0.00 E
Verano	0.16 C	2.14 B	0.00 E	2.98 A
92918	0.40 BC	0.00 E	0.75 CD	0.37 DE
55860	0.40 BC	0.19 DE	0.41 DE	1.02 BCD
93055	0.00 C	0.00 E	0.59 CDE	0.00 E
93099	0.13 C	0.33 DE	0.43 DE	0.54 CDE
93116	0.00 C	0.00 E	0.00 E	0.00 E
Control[y]	...[z]	0.72 CD	...	1.15 BC
93037	...	3.54 A	...	3.33 A

[w] Data were $\log_{10}(n_o + 1)$ and $\log_{10}(n_d + 1)$ transformed.
[x] Within a column, values followed by the same letter do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.
[y] Control = Fitzroy that received a spray of benomyl every 2 wk in an attempt to control anthracnose.
[z] Not assessed.

for a given accession is the same over the two phases, then the slopes of the two estimated straight lines will be approximately the same. These same 'early' and 'late' phases were used to model disease progress of all other accessions. Data from each plot were used; and to allow for between-block variation, a different intercept for the broken stick model was used for each plot. The variation of proportion of disease varied with mean proportion of disease, $\bar{y}$, such that it was well approximated by $\bar{y}(1-\bar{y})$. The broken stick model was therefore fitted using weights given by the reciprocal of $\hat{y}(1-\hat{y})$ where $\hat{y}$ was the fitted value obtained by smoothing the data using the logistic model.

As expected, Fitzroy had the highest r_e and lowest r_l in both years (Table 2). In 1988, 55803 had a r_l which was five times higher than its r_e. As a result of this, y increased rapidly from a very low level to a y_t which was not significantly ($P < 0.05$) different from that of Fitzroy. Apart from the highly susceptible 93037, the r_e-reducing characteristic of all other accesions was evident in both years and r_e of most accessions was not significantly ($P < 0.05$) different from zero. However, only 55860, 93055, 93099, and the resistant 93116 were able to maintain a low r_l in both years.

t_{10}, y_t and AUDPC. In 1988, there was a five- to sevenfold difference in t_{10} between Fitzroy and other accessions. While all Fitzroy plants reached 10% severity by the end of the season, only 12% of 93116 and 7% of benomyl-sprayed Fitzroy plants ever reached this level. Therefore, the time to 10% y for 1988 could not be calculated on a plot or block average basis. Instead it was calculated on a plant basis and a correction used for those plants not reaching 10% y. The corrected t_{10} is an estimate of the time to 10% y based on the incomplete or censored sample. The correction was

$$\text{Corrected } t_{10} = \text{average } t_{10} \text{ for plants which reached 10\% } y + (x_0/x_p)t$$

in which x_0 = number which never reached 10% y, x_p = number which reached 10% y, and t = time of last observation. The formula is based on the assumption that t_{10} has an exponential distribution taking into account that some times are censored at the last observation. In 1988, this gave a somewhat unrealistic t_{10} for benomyl-sprayed Fitzroy because only a small number of plants ever reached 10% y (Table 3). This simple correction should only be used for a probable ranking of times rather than the estimate of absolute times because of the weakness of the exponential assumption.

AUDPC were higher in 1989 than in 1988 because of generally higher y and an extended period of disease assessment (Table 3). Fitzroy had the maximum AUDPC in both years. Compared

TABLE 5. Spearman's rank correlation between attributes used to characterize quantitative resistance in *Stylosanthes scabra* to *Colletotrichum gloeosporioides* during 1988 and 1989

	1988								1989						
	y_w	n_o	n_d	AUDPC	y_t	r_e	r_l	t_{10}	y_w	n_o	n_d	AUDPC	y_t	r_e	r_l
1988															
n_o	0.9**[a]														
n_d	0.9**	0.8**													
AUDPC	0.8**	0.9**	0.9**												
y_t	0.8*	0.9**	0.9**	0.9**											
r_e	0.8*	0.7*	0.9**	0.9**	0.8*										
r_l	0.1	0.2	0.2	0.3	0.5	0.2									
t_{10}	0.9**	0.9**	0.9**	0.9**	0.9**	0.8*	0.3								
1989															
y_w	0.8**	0.6	0.6	0.5	0.5	0.5	−0.1	0.6							
n_o	0.7*	0.6	0.5	0.5	0.5	0.4	−0.3	0.6	0.8*						
n_d	0.7	0.7*	0.5	0.6	0.6	0.4	−0.1	0.7	0.7	0.9**					
AUDPC	0.7*	0.9**	0.8*	0.9**	0.9**	0.7	0.2	0.9**	0.4	0.6	0.8*				
y_t	0.8*	0.9**	0.9**	0.9**	0.9**	0.8*	0.3	0.9**	0.4	0.5	0.6	0.9**			
r_e	0.4	0.3	0.4	0.3	0.3	0.4	−0.2	0.3	0.4	0.8*	0.6	0.4	0.3		
r_l	−0.1	0.1	0.0	0.1	0.2	−0.1	0.8*	0.1	−0.3	−0.5	−0.3	0.1	0.2	−0.6	
t_{10}	0.8*	0.9**	0.9**	0.9**	0.9**	0.7*	0.4	0.9**	0.5	0.6	0.6	0.9**	0.9**	0.4	0.1

[a] Level of significance: ** = $P < 0.01$; * = $P < 0.05$.

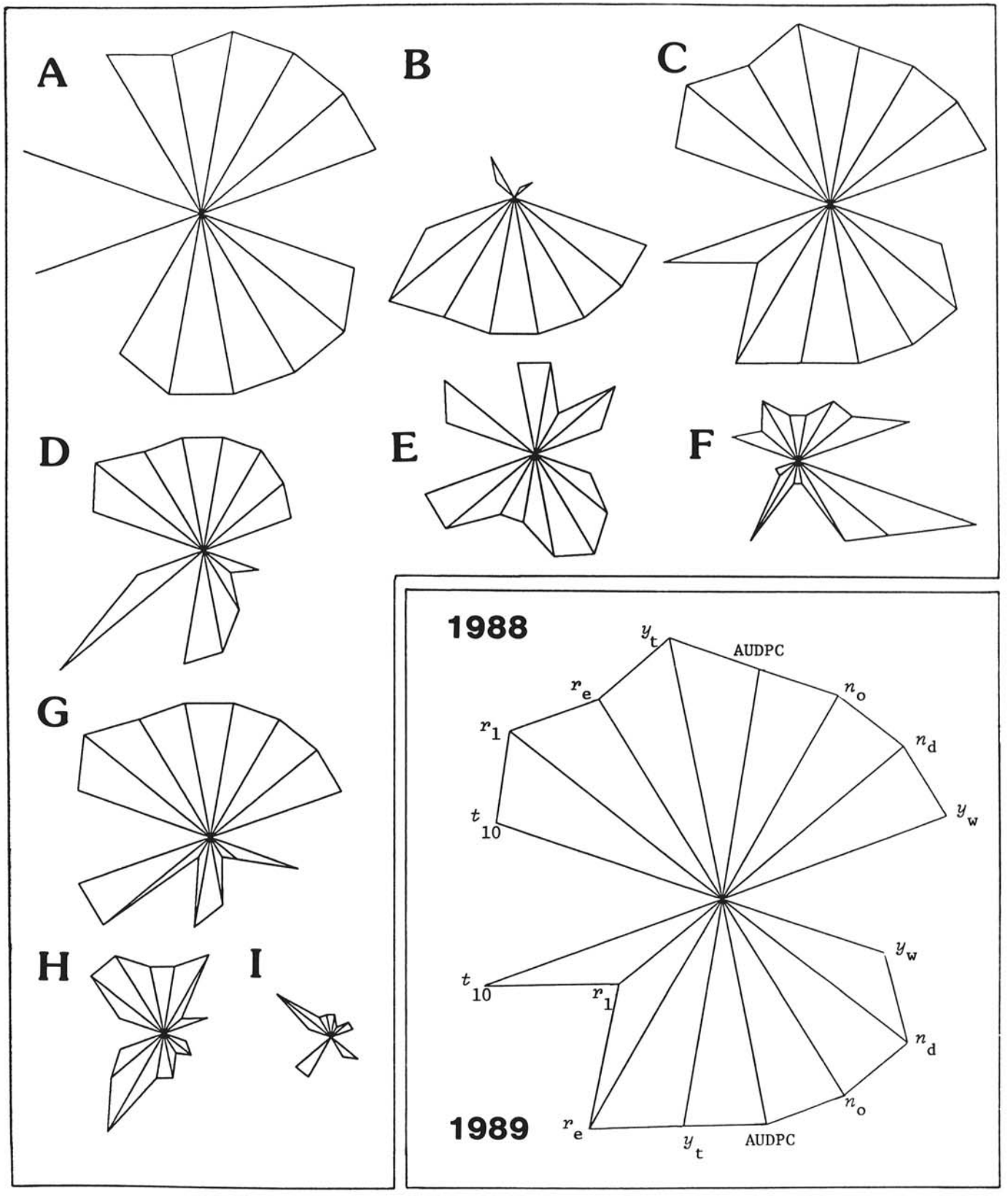

Fig. 2. Star plot for *Stylosanthes scabra* **A,** 'Fitzroy'; **B,** benlate-sprayed Fitzroy; **C,** accessions 55803; **D,** 92918; **E,** 55860; **F,** 93099; **G,** 92873; **H,** 93055; and **I,** 93116. Plots show accession rankings based on weighted infection type (y_w), spore production in the field (n_o), spore production per day (n_d), area under the disease progress curve (AUDPC), terminal severity (y_t), early (r_e) and late (r_l) rates of anthracnose progress, and the time required for disease severity to reach 10% (t_{10}) during 1988 (top half of each star) and 1989 (bottom half of each star) at the Southedge Research Station. The length of each radius in a star is proportional to the accession rank for a given attribute; greater length indicates higher susceptibility. Inset: The order in which these attributes appear in each plot.

with the AUDPC of benomyl-sprayed Fitzroy, accessions with putative quantitative resistance produced 2–17 times more disease in 1988. Increases for Fitzroy and 55803 were 71 and 34 times, respectively. AUDPC of 93055 and 93099 was less than 10% of that of Fitzroy in both years; for accessions, 92873, 92918, and 55860, this was between 14 and 25%. AUDPC of 55803 was 48 and 68%, respectively, of that recorded for Fitzroy in 1988 and 1989. Excluding the benomyl-sprayed Fitzroy and Verano, most accessions maintained their relative rankings for AUDPC in the 2 yr. Low AUDPC for Verano was caused by the lack of data on y toward the end of 1989, as severe defoliation due to natural senescence made it difficult to assess anthracnose severity. Since assessments did not continue over as long a period, defoliation was less of a problem in 1988.

Data on y_t showed similar trends to those on AUDPC, and details on the performance of individual accessions are not presented for this attribute.

***y_w, n_o,* and *n_d*.** Although all accessions produced mesothetic reactions, the proportion of each lesion type varied with the accession. Very few susceptible lesions were recorded on 93116, 93055, or 93099 (data not presented).

C. gloeosporioides failed to sporulate on the resistant 93116 in both years (Table 4). Higher n_o and n_d for Verano in 1989 was due to an earlier assessment than in 1988. At the time of assessment in 1988, Verano had lost most of its leaves, and lesions on senescent leaves failed to sporulate after incubation. With the exception of Verano in 1988 and 93037 in 1989, values for n_d in all accessions were higher than their respective n_o in both years.

To examine the relationship between the eight attributes, r_e, r_l, t_{l0}, AUDPC, y_t, y_w, n_o, and n_d, relative rankings of the eight *S. scabra* accessions were used to calculate Spearman's coefficient of rank correlation (25) for each year. Benomyl-sprayed Fitzroy, which no longer served as a "disease-free" control in 1989, and 93037, which was only assessed in the second year, were excluded. The number of significant correlations between attributes was greater in 1988 than in 1989 (Table 5). There was no significant ($P < 0.05$) correlation between r_l and any other attribute in either year, although the correlation between r_l-based rankings for the 2 yr was significant. Attributes AUDPC, y_t, and t_{l0} in 1989 were significantly correlated with most attributes of the previous year.

Performance of eight accessions of *S. scabra*, common to both years, was summarized by plotting accession ranks for the eight attributes for each year using a 'star plot' (1, Fig. 2). The length of each radius in a star is proportional to the accession rank for a given attribute: the longer the radius the higher the rank, and, hence, the level of susceptibility. The 1988 values are plotted in the top half of the star, and the 1989 values are plotted in the bottom half, so that if ranks for each attribute were identical in the 2 yr, then the lower half would be a mirror image of the upper half. The star plot for Fitzroy had the largest radii with the bottom being almost a perfect reflection of the top half (Fig. 2). This was followed by 55803 in size. The star plots of 55860, 93055, and 93099 were intermediate in their size with reasonably consistent patterns for the two years. As expected, star plot of benomyl-sprayed Fitzroy had a much larger lower half than the upper half.

DISCUSSION

Results of this study demonstrate the effectiveness of quantitative resistance of *S. scabra* to *C. gloeosporioides* under field conditions. Five of the seven chosen accessions of *S. scabra* showed moderate to high levels of resistance to *C. gloeosporioides*, with accessions 93099 and 93055 the most resistant. Accessions 55803 and 93037 were susceptible. The relative levels of resistance are similar to those found in earlier glasshouse studies for all accessions other than 92918 and 93099 (3; Jamieson and Chakraborty, *unpublished*). Disease rating in the field was higher than in a glasshouse for 92918, and 93099 gave a lower rating in the field. These results show that effective screening for quantitative resistance can be done in the glasshouse.

As a breeding strategy, combining apparently different forms of resistance may provide a relatively long-term protection against the anthracnose-causing pathogen. In the glasshouse, accessions 92873, 92918, and 93055 expressed uniform levels of resistance to races 1, 3, and 4a of *C. gloeosporioides*, while 55860 and 93099 showed higher susceptibility to race 3 and 4a, respectively (3). Early assessment of n_o in 1989 failed to detect sporulation in 92873, 92918, and 93055. Whether this is due to long latent periods needs to be determined. While sporulation was detected under field conditions for both 92873 and 92918, in 93055, spores were only produced after 24-hr incubation in conditions highly conducive to sporulation. This may suggest a different resistance mechanism for 93055. Genetics of inheritance and components of resistance are currently being studied to understand the operation of this resistance. Whether the quantitative resistance will confer an enduring and stable protection against all races of the anthracnose pathogen can only be determined from further long-term field studies.

We used a "soft-testing" approach (33) to evaluate quantitative resistance in *S. scabra*. The provision of autoinfection at the exclusion of alloinfection was met first by introducing comparable amounts of inoculum at the center of each plot in 1988 and, second, by physically isolating the plots to reduce interplot interference. The size and distance between plots were considered adequate for a splash-dispersed pathogen such as *C. gloeosporioides*. In the second year, variable amounts of residual inoculum survived in different plots. Weather conditions were generally more favorable for anthracnose development in 1989 than in the previous year with higher rainfall distributed throughout the season (Fig. 3). A combination of this and a high initial inoculum resulted in higher disease levels for all accessions in 1989. The disease progress curve of Fitzroy followed a more-or-less sigmoid shape in 1988, but in 1989 the curve was much flatter with high levels of y from a very early part of the year. Both seasonal variations (13) and inoculum level (24) have been shown to influence components of a quantitative resistance, such as latent period, for the splash-dispersed *Septoria nodorum* (Berk.) Berk. in Berk. and Broome in wheat.

Single disease assessments made early in the season underestimated the susceptibility of 55803 in 1988 and of 92873 and 92918 in both years. As seen with Verano during 1989, assessments made toward the end of a season are confounded

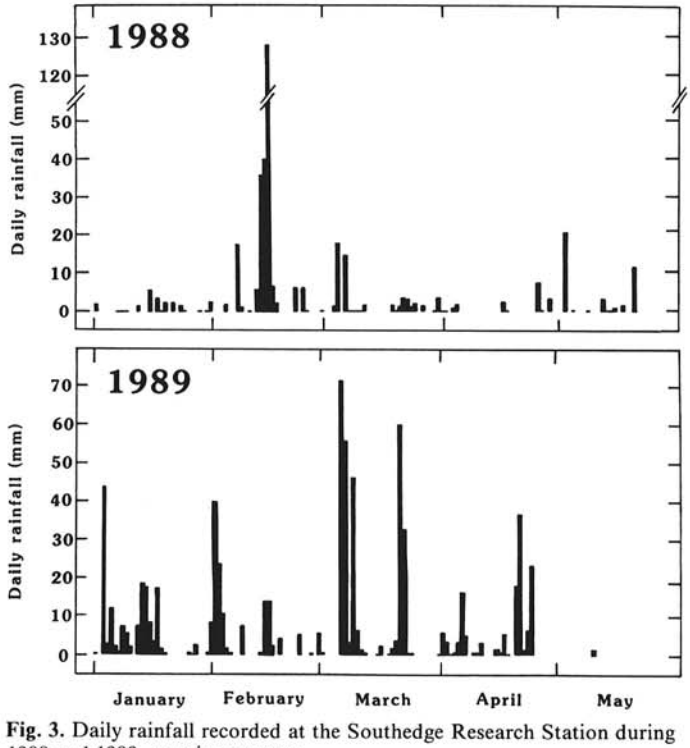

Fig. 3. Daily rainfall recorded at the Southedge Research Station during 1988 and 1989 growing seasons.

by natural senescence which may be indistinguishable from symptoms caused by a disease. Alluding to these and other difficulties, Shaner and Finney (23) recommended against measurement of resistance levels based on single observations.

Of the eight epidemiological parameters considered, AUDPC proved to be the best measure of resistance in both years. This is similar to findings on wheat slow-mildewing (23), barley slow-rusting (14), and rate-reducing resistance to *Phytophthora* in soybeans (27), among others. However, to make a valid comparison between treatments, AUDPC has to be calculated from a common time base (23). This was evident from the low AUDPC for Verano in 1989. Measurement of resistance based on t_{10} presents some difficulties. For t_{10} to be meaningful, the time of commencement of an epidemic needs to be determined. This may not be an easy task even with a splash-dispersed pathogen which has limited long distance dispersal. Some Fitzroy plants became infected with background inoculum before the epidemic was initiated by artificial inoculation. Also, t_{10} offers no advantage over AUDPC (23) and is complicated by the fact that not all plants of a resistant line may reach 10% severity.

The star plot was useful in combining accession rankings for a number of attributes in a simple and meaningful way. Such a combination of rankings is especially useful when the relationships between the various attributes are unclear. By plotting ranking of attributes associated with the level of host resistance, the star plot can be used as an index of resistance. Separate plots would need to be drawn for individual host accessions/genotypes for each season/treatment.

In our studies, a weighted linear regression using a 'broken stick' model best described anthracnose progress in most accessions. Parameter estimates of the logistic model were not precise enough to allow comparison between accessions. The logistic transformation of $\ln[y/(1-y)]$ tends to magnify minor differences in early severity; and, hence, the usefulness of apparent infection rate as a statistic for studying rate-reducing resistance has been questioned by other researchers (17,23,31). The two rates of disease progress, r_e and r_l, were more realistic than the apparent infection rate; and all accessions had low r_e in both years, despite the high level of initial inoculum in 1989. However, the poor correlation for r_e-based accession rankings between the two years suggests that r_e alone may not provide a reliable measure of resistance.

Spore production in hosts with quantitative resistance has generally been studied following artificial inoculation (8,14,22). In such studies, inoculum load, leaf age, lesion age, and environment are controlled to obtain a reproducible and meaningful measure of sporulation. As rain disperses conidia of *C. gloeosporioides* held in a mucilaginous matrix (16), our assessment of n_o essentially measured the cumulative spore production since the last rain. The attribute n_d was, therefore, used as a standardized (per day) production rate. While there was very little difference between n_o and n_d for Fitzroy, spore production of all other accessions, except 93116, generally increased following the 24-hr incubation on moist filter paper. This demonstrates the influence exerted by the environment on the expression of quantitative resistance (23). Despite significant correlations with AUDPC, usefulness of n_o and n_d as a simple measure of quantitative resistance may be limited. However, the significant correlations highlight the dependence of anthracnose progress on the production of secondary inoculum. A relationship between n_o and n_d and associated resistance components, such as latent period, infectious period, and spore production under more controlled and reproducible conditions needs to be established.

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