Anthracnose Development in Pure and Mixed Stands of the Pasture Legume *Stylosanthes scabra*

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ABSTRACT

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Development of anthracnose, incited by *Colletotrichum gloeosporioides,* was examined over three summer seasons in pure and mixed stands of the tropical pasture legume *Stylosanthes scabra.* Three accessions and two cultivars with different levels of resistance to four races of the pathogen were used. The susceptible cultivar Fitzroy was either excluded or included in increasing proportions in four different mixtures. Significant reduction in the area under the disease progress curve (AUDPC) in mixtures over the pure stand of the components occurred only in certain mixtures in some years. In the absence of Fitzroy, AUDPC in mixture was lower than the arithmetic mean of AUDPC of the components in pure stands in only one of the three growing seasons. In the mixture containing equal proportions of all components including Fitzroy, AUDPC was similar to the mean of pure stands in all 3 yr. In mixtures with more than 50% Fitzroy, AUDPC was significantly less in 2 of the 3 yr. There was no improvement in the survival of plants of individual accessions in mixtures compared with that in pure stands. In general, Fitzroy had consistently less AUDPC in mixtures with more than 50% Fitzroy than in its pure stand, by contrast, AUDPC of the more resistant accessions did not differ significantly from their respective pure stands in any year. Severity of individual accessions did not increase significantly with increasing Fitzroy content of a mixture except for Seca and Fitzroy in 1988 and 1989, respectively. The study emphasizes the need for long-term monitoring of host and pathogen populations in perennial pastures to ascertain the role of genotype mixtures in restricting anthracnose.

The improvement of native pastures in the wet and semiarid tropics of northern Australia depends on the successful establishment and persistence of suitably adapted legumes under minimal management inputs. Since the early 1960s, species of *Stylosanthes,* tolerant of soils with low phosphorus, have been regarded as highly suitable for this region and no fewer than 12 cultivars have been released (10; D. F. Cameron, *unpublished*). However, large-scale commercial seed production has ceased for all cultivars except *S. guianensis* (Aubl.) Sw. 'Oxley,' *S. hamata* (L.) Taub. 'Verano' and 'Amiga,' and *S. scabra* Vog. 'Seca.' This mainly is due to the susceptibility of the other cultivars to anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz.

Two types of anthracnose have been recognized in Australia based on symptomatology, histopathology, and host range (12). The type A pathogen causes discrete lesions on aerial parts of plants in most species of *Stylosanthes,* whereas type B causes blighting of aerial shoots in mainly *S. guianensis.* Type A poses a major threat to the current economically important species of *Stylosanthes* because new pathogenic races continue to arise following the release of cultivars. *S. scabra* 'Fitzroy' was discarded within 5 yr of its release in 1979 due to severe damage from highly virulent strains (8). Cultivar Seca, highly resistant to all known strains of the pathogen at the time of its release in 1976 (12), became infected by a new race (race 3) in 1982 (8). Race 3 has, however, proved to be of limited concern as Seca populations generally show a high degree of resistance in the field. Four races now have been identified within the type A group in Australia (13). These include one race virulent on *S. scabra* accessions 36260 and Q10042 (9) and one race virulent on *S. viscosa* Sw. accession 33941 (12).

Creating genetic diversity by physically mixing host genotypes, each carrying resistance to a different race, has been suggested as a possible strategy for controlling diseases caused by such variable pathogens (23). In tropical Australia, pastures increasingly are being established with a mixture of Seca and Verano, with some 500,000 ha estimated to have been sown already. Severe anthracnose epidemics have not been recorded in an interspecific mixture of *Stylosanthes* at a field site in north Queensland (R. D. Davis, R. M. Boland, and C. Howitt, *unpublished*). In South America, susceptible accessions of *S. guianensis* had lower disease severity levels and higher yields in mixtures with resistant accessions than in their pure stands in the first year (17). No information is available on whether the level of anthracnose in heterogeneous mixtures of *S. scabra* is lower than the simple mean of their components grown as pure stands.

This paper summarizes the results of a field experiment conducted with *S. scabra* over three successive years to assess the performance of mixtures of genotypes in restricting anthracnose development. Increasing proportions of the agronomically desirable Fitzroy were included in mixtures to test if this susceptible cultivar could be protected from anthracnose in mixed stands. Anthracnose progress over time among all other host accessions was studied to assess their severity levels with increasing proportions of Fitzroy in the mixture.

MATERIALS AND METHODS

Selection of host accessions and their mixtures. Four moderately resistant accessions of *S. scabra,* 36260, 55860, Q10042, and 40292 (cv. Seca), and the susceptible 40205 (cv. Fitzroy) were used. Reactions of these accessions to isolates of *C. gloeosporioides* representing four type A races were determined in a glasshouse bioassay using methods described previously (4,8). All accessions except Seca showed varying levels of disease with all isolates (Table 1), while Seca showed a very high level of resistance to all isolates except SR 24. Accession 36260 was most severely affected by isolates WRS 20 and WRS 32, and 55860 only showed slight to moderate disease with all isolates. The difference in susceptibility to WRS 20 and WRS 32 in Q10042 may be only a quantitative one as similar levels of disease severity with both

isolates have been recorded in some tests (4). Four different mixtures (Table 2) with varying percentages of the susceptible Fitzroy were selected.

Establishment and maintenance of field plots. Plots were established at the CSIRO Samford Pasture Research Station (27° 22 S; 152° 53′ E). The site has an average annual rainfall of 105 cm with a humid subtropical climate and a prairie soil. The area had been used for *Stylosanthes* evaluation in previous years. Fertilizer dressings of 50 kg/ha superphosphate and 50 kg/ha muriate of potash were given each year.

Single seedlings were raised in a 1:3 peat:sand mixture (v/v) in 4- × 4-cm peat cups 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% nitrogen, 4% phosphorus, and 18% potassium, as well as trace elements. Pure plots of each accession were established on the first week of January 1987 by transplanting 6-wk-old seedlings into 4.5- × 4.5-m areas. Between and within row spacings of 50 cm accommodated 100 plants per plot. Mixture plots were similarly established by transplanting the different accessions at predetermined randomly allocated positions within a plot. Plots were sprayed with the preemergent herbicide oryzalin (Surflan, Elanco Products Co., NSW 2114, Australia). The nine treatments (five pure stands and four mixtures) 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 in the first year by inoculating each of these spreader plants with a different isolate of *C. gloeosporioides*. Isolates SR 4 (race 1), SR 24 (race 3), and WRS 20 (race 4a, which was severe on both 36260 and Q10042, see Table 1) were grown using methods described before (8), and the spreader Fitzroy plants were inoculated in the afternoon of 23 April 1987 between 4 and 6 p.m. by spraying a conidial suspension of 10^6 conidia per milliliter to incipient runoff. Inoculated plants were covered with a reflective plastic bag (Agricultural Plastics, Sydney, Australia) for about 20 h to provide the necessary leaf wetness (6).

At the end of each growing season, all plants were cut to a height of 10 cm with a reciprocating mower. Plots in the second and third seasons consisted of plants that regrew in spring, and data on the number of plants surviving in each plot were collected in November 1987 and 1988. Plants that did not survive the winter and/or anthracnose attack were replaced with 6-wk-old glasshouse-grown seedlings in early summer (second and third weeks of January in 1987 and 1988, respectively). Both the regrowth on surviving plants and the seedlings were at the vegetative growth stage 12 according to the Winch system of legume growth stage keys (14). Infected plant residues were presumed to be the source of primary inoculum in 1988 and 1989.

Disease assessments. All 100 plants in each plot were assessed for disease and growth stage (14) on seven occasions at approximately 1–2 wk intervals in 1987 and 1988. In 1989, assessments were made on each of the 100 plants per plot in pure stands of Fitzroy and on 25 randomly selected plants per plot in pure stands of all other accessions on seven occasions. For mixtures, five randomly selected plants, where applicable, were assessed for each accession/plot. In 1987 assessment began in the first week of April. In both 1988 and 1989 assessments began in the first week of February. Disease incidence (number of infected plants) was determined for each plot. The proportion of diseased leaf area (severity) also was estimated from the top 10–15 cm length of a randomly selected branch for each plant using a 10-point visual assessment key (4) based on the Horsfall and Barratt (11) scale. In each year, assessments were made only during the growing season (January–May), with the first and last assessments made at plant growth stages 12 and 53 (14), respectively.

Statistical analysis. For each year disease progress curves were determined for each accession in both pure and mixed stands and for each of the nine treatments (five pure stands and four mixtures). The area under the disease (severity) progress curve (AUDPC) was calculated using the trapezoid rule where the area was approximated by joining the points at the observed times by line segments and calculating the area of the resultant trapezoids. The heterogeneity of error variance for AUDPC data associated with the different treatments could not be adequately corrected by transformations, including the log and square root transformations. For each accession, mean disease severity and its standard error were calculated from the three replicate plots of pure and mixed stands. Comparison between means was carried out using Student's *t* tests. A linear regression was used to analyze disease severity on individual components in a mixture with increasing proportion of the susceptible Fitzroy as the independent variable. Data on percentage survival of individual accessions for 1987 and 1988 were averaged and an analysis of variance was performed with the number of plants of each accession that constituted the various pure and mixture plots used as a weight. The SAS (20) and Genstat 5 (19) software packages were used for analysis.

RESULTS

Fitzroy spreader plants developed anthracnose symptoms within a week of inoculation with the three isolates. Anthracnose was, however, first detected on some plants 3 wk before inoculation of the spreader plants. This epidemic initiation by exogenous inoculum from a nearby field made it necessary to commence weekly disease assessments from the time anthracnose was first noted.

In 1987 anthracnose incidence, expressed as the percentage of infected plants, was initially low in all accessions grown either as pure stands or in mixtures (data not presented). Incidence increased towards the end of the growing season in most accessions. For Fitzroy in both pure and mixed stands, there was a sharp increase in disease incidence from approximately 1 wk after inoculation of the spreader plants. In both 1988 and 1989, anthracnose incidence remained high to very high (100% in many cases) in Fitzroy and 36260, moderate in Q10042 and 55860, and low to very low in Seca (data not presented).

The number of plants that survived from one year to the next varied between plots. A less severe winter in 1988 resulted in an improvement in the survival of all accessions except 36260, and data from the two seasons were averaged to remove the influence of seasonal conditions on survival. Accessions 36260 and Q10042, respectively, had the most (56.4%) and the least (23.5%) number of plants surviving over the two seasons. There was no significant effect of mixtures on the survival of individual accessions (Table 3)

TABLE 1. Reactions of a differential set of *Stylosanthes scabra* genotypes to four isolates of *Colletotrichum gloeosporioides*

Differential	Percent leaf area necrotic			
	SR4 (1)[a]	SR24 (3)	WRS32 (4)	WRS20 (4a)
Fitzroy	57.5	97.5	25.3	93.2
Seca	0.6	19.0	0.0	0.0
36260	2.0	25.3	97.5	97.5
Q10042	3.0	5.0	9.5	33.8
55860	3.0	12.6	3.0	5.0

[a]Numbers in parentheses indicate race numbers.

TABLE 2. Composition of the different mixtures of *Stylosanthes scabra* accessions

Accession	Mix 1 (%)	Mix 2 (%)	Mix 3 (%)	Mix 4 (%)
36260	25	20	12	5
55860	25	20	12	5
Q10042	25	20	12	5
Seca	25	20	12	5
Fitzroy	0	20	52	80

Anthracnose severity progress curves for the five accessions grown in pure stands or as a component of the different mixtures are presented for the 1987 season only (Fig. 1). As with disease incidence, severity of Fitzroy increased sharply after inoculation of the spreader plants. This trend was also noted for 36260, although its severity did not increase as rapidly as in Fitzroy. Trends in disease progress were similar in both 1988 and 1989; however, severity of anthracnose in most accessions was higher than in 1987. From the beginning of 1989, disease severity in pure stands of Fitzroy increased rapidly to a very high level; severity in mixtures increased slowly and reached levels similar to that in pure stands only towards the end of the season (data not presented).

Anthracnose severities in pure and mixed stands were compared using the AUDPC of individual accessions grown in pure and mixed stands (Table 4). For mix 1 (containing no Fitzroy) or mix 2 (with 20% Fitzroy), there was no significant difference in the AUDPC between pure and mixed stands for any accession in either 1987 or 1989. In 1988 AUDPC was lower for both 36260 (significant at $P < 0.005$) and Q10042 (significant at $P < 0.06$) in mix 1 than in the pure stand. Although not significant, AUDPC of 36260 in mix 1 was also lower than in pure stand in both 1987 and 1989. In mix 2, AUDPC of individual accessions did not differ significantly from their respective pure stands in any of the 3 yr of this study. In mix 3 (with 52% Fitzroy) and mix 4 (with 80% Fitzroy) AUDPC of accessions other than Fitzroy were not significantly different from their respective pure stands (Table 4). Anthracnose severity in the susceptible Fitzroy was lower than in its respective pure stands in all mixtures except mix 2 in 1988. In mix 3, there was a reduction in AUDPC of Fitzroy over its pure stand of 51.2, 8.3, and 38.2% (significant at $P < 0.04$) in 1987, 1988, and 1989, respectively, and in mix 4, the reductions were 7.8, 12.9 (significant at $P < 0.08$), and 34.7% (significant at $P < 0.04$) in 1987, 1988, and 1989, respectively (Table 4). Overall, Seca developed very little disease in either pure or mixed stands during the three seasons. In 1989 the exceptionally high AUDPC (7.2) for Seca in mix 1 was due to the inherent heterogeneity (standard error of the mean 7.13) of this cultivar as some of the seedlings transplanted in the beginning of 1989 produced very high severities.

To determine the response of accessions other than Fitzroy to increasing proportions of Fitzroy in a mixture, AUDPC of

TABLE 3. Survival of accessions of *Stylosanthes scabra* grown in pure and mixed stands

Accession/ cultivar	Percentage survival of plants[a]					Probability of a larger F
	Pure stand	Mixed stand				
		Mix 1[b]	Mix 2	Mix 3	Mix 4	
Fitzroy	42.5	...	38.3	34.7	29.7	0.355
Seca	35.5	31.3	50.8	40.0	30.0	0.718
Q10042	25.3	26.0	24.2	22.0	20.0	0.997
36260	52.8	45.3	64.2	66.3	53.3	0.837
55860	39.0	38.0	34.2	30.2	30.0	0.947

[a]Mean percentage of plants that regrew in the springs of 1987 and 1988.
[b]Details on mixture composition provided in Table 2.

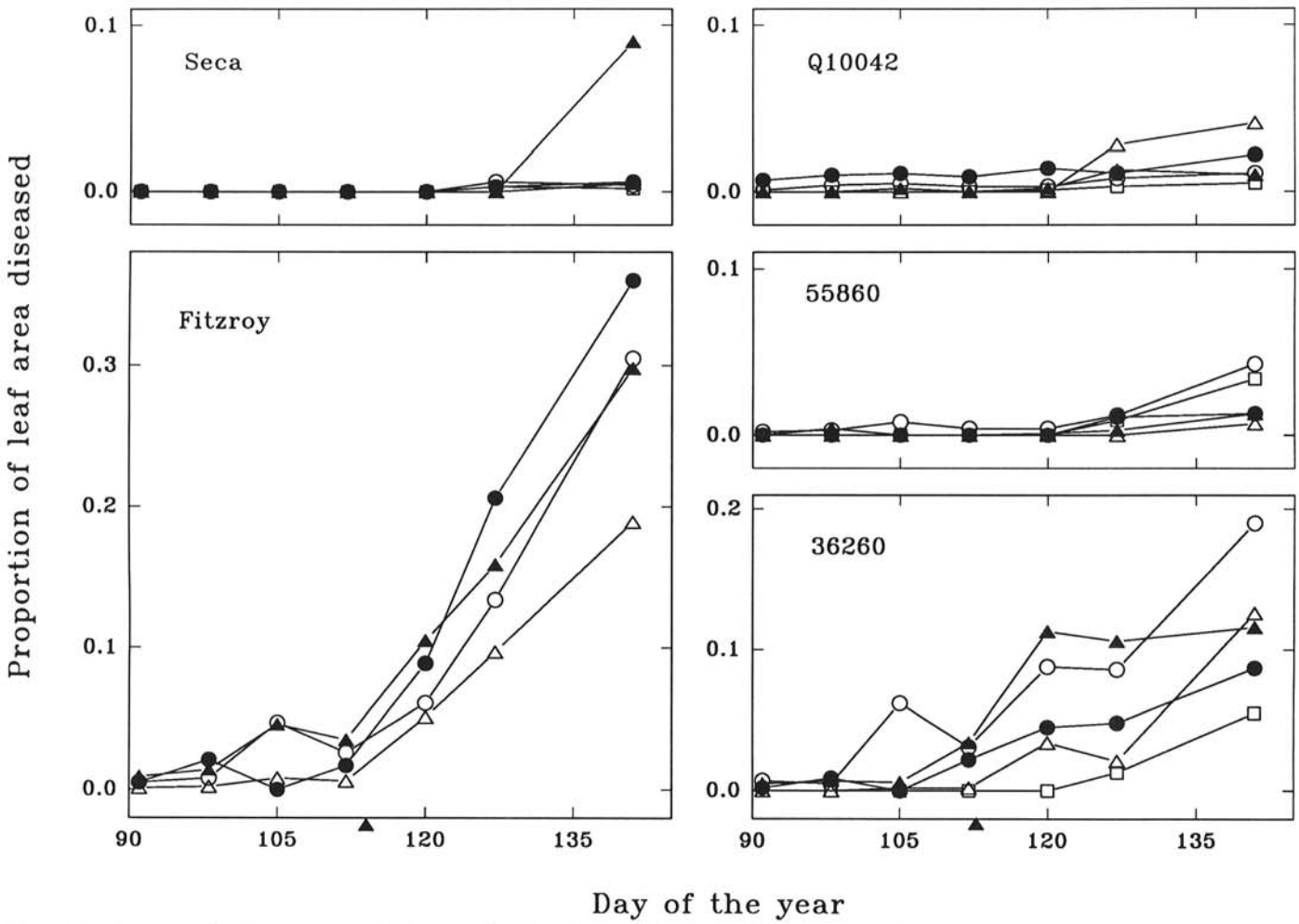

Fig. 1. Development of anthracnose severity (proportion of leaf area diseased) on five genotypes of *Stylosanthes scabra* grown either as a pure stand (●) or as a part of a mixture containing equal proportions of all genotypes except Fitzroy (□), equal proportions of all five genotypes (○), 52% Fitzroy and 12% each of the other four genotypes (△), and 80% Fitzroy and 5% each of the other four genotypes (▲). Arrowhead at bottom shows the date when plots were inoculated with isolates of *Colletotrichum gloeosporioides*.

TABLE 4. Area under disease severity progress curve (AUDPC) for individual accessions of *Stylosanthes scabra* grown as a component of a mixture or as a pure stand

Accession/ cultivar	AUDPC					
	Pure stand	Mixed stand				
		Mix 1[a]	Mix 2	Mix 3	Mix 4	$P > h_0$[b]
1987						
Fitzroy	20.5	...	15.1 (0.60)[c]	10.0 (0.26)	18.9 (0.88)	0.42
Seca	0.3	0.1 (0.65)	0.3 (0.98)	0.1 (0.52)	2.7 (0.46)	0.21
36260	5.3	2.0 (0.42)	8.9 (0.66)	4.2 (0.82)	12.1 (0.39)	0.27
Q10042	1.8	0.4 (0.17)	0.6 (0.27)	1.6 (0.94)	0.9 (0.37)	0.47
55860	0.7	1.6 (0.39)	1.3 (0.70)	0.2 (0.51)	0.6 (0.93)	0.24
1988						
Fitzroy	110.4	...	110.8 (0.98)	101.2 (0.41)	96.2 (0.08)	0.74
Seca	0.0	0.2 (0.30)	0.3 (0.30)	1.0 (0.30)	1.5 (0.19)	0.05
36260	75.1	11.4 (0.005)	57.7 (0.16)	35.1 (0.11)	61.8 (0.39)	0.07
Q10042	16.0	6.9 (0.06)	33.4 (0.21)	26.8 (0.27)	26.6 (0.14)	0.22
55860	26.8	13.6 (0.28)	21.2 (0.56)	19.8 (0.61)	30.8 (0.70)	0.10
1989						
Fitzroy	182.8	...	102.9 (0.11)	112.9 (0.04)	119.4 (0.06)	0.03
Seca	0.8	7.2 (0.47)	0.8 (0.97)	0.4 (0.63)	1.8 (0.52)	0.36
36260	57.8	39.7 (0.69)	65.0 (0.90)	28.3 (0.49)	21.9 (0.42)	0.36
Q10042	15.3	16.4 (0.93)	11.5 (0.72)	13.5 (0.85)	6.5 (0.33)	0.37
55860	13.4	15.1 (0.90)	38.3 (0.26)	14.8 (0.88)	8.5 (0.43)	0.34

[a] Details of mixture composition in Table 2.
[b] Probability of $> t$ for testing the null hypothesis that the regression parameter equals zero.
[c] Figure in parentheses is the $P > t$ for comparison of means between pure and mixed stands.

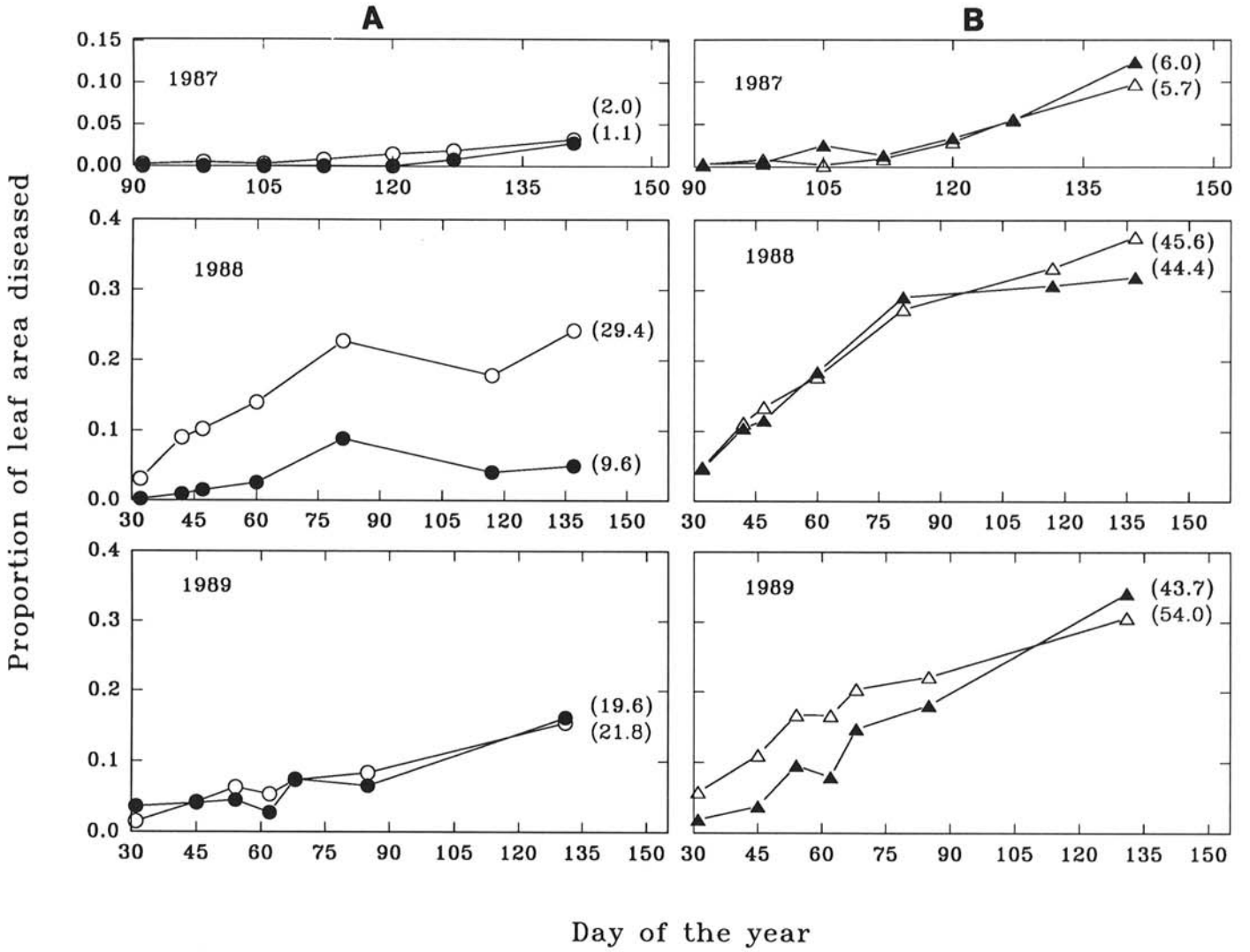

Fig. 2. Development of anthracnose severity in mixtures of *Stylosanthes scabra* containing **A,** equal proportions of Q10042, 36260, 55860, and Seca (mix 1, ●) and the arithmetic mean of severity of the components grown separately (○); **B,** equal proportions of Q10042, 36260, 55860, Seca, and Fitzroy (mix 2, ▲) and the arithmetic mean of severity of the components grown separately (△) during three summer seasons. Figure in parentheses is the area under the disease progress curve.

the respective accessions in mix 1, mix 2, mix 3, and mix 4 were regressed on the Fitzroy-content of these mixtures; for Fitzroy, AUDPC in mix 2, mix 3, mix 4, and its pure stand were used instead. A significant increase in AUDPC with increasing proportions of Fitzroy was only detected for Seca in 1988 and Fitzroy in 1989 (Table 4). Accession 36260 showed a similar trend in 1988, although this was not significant at $P < 0.05$. There was no consistent influence of increasing proportions of Fitzroy on the disease severity of Q10042 and 55860 over the 3 yr.

Progress of anthracnose severity in mix 1 and mix 2 was compared with the arithmetic mean of severity of the respective components grown in pure stands. In both 1987 and 1989, anthracnose severity increased at a similar rate in both mix 1 and the pure stands (Fig. 2). In 1988 severity increased more rapidly in the pure stands to result in a terminal severity higher than that in mix 1. For mix 2, there was no difference in disease progress between pure and mixed stands for 1987 or 1988. Severity in mix 2 during the early part of 1989 remained lower than the mean severity of its components in pure stands; this effect, however, disappeared towards the end of the season with both pure and mixed stands showing similar terminal severities (Fig. 2).

To test the efficacy of mixtures in restricting anthracnose, the AUDPC of mixtures were compared with the arithmetic mean of pure stands of the respective components. For comparisons with mix 3 and mix 4, the AUDPC of pure stands were adjusted (by multiplying Fitzroy AUDPC with 0.52 for mix 3 and with 0.8 for mix 4 and by multiplying AUDPC of all other components by 0.12 for mix 3 and 0.05 for mix 4) to match the proportion of individual components in these mixtures. A "standard normal statistic" (Z statistic) was used for comparison. This was calculated as follows:

$$Z = \frac{Mn_{ps} - Mn_{mx}}{\sqrt{SE_{ps}^2 - SE_{mx}^2}}$$

Mn_{ps} is mean severity of pure stands; Mn_{mx} is mean severity of a mixture; SE_{ps} is standard error of mean of the pure stands; SE_{mx} is the standard error of mean of the mixture.

In the mixture containing no Fitzroy, there was a significant ($P < 0.01$) reduction in the AUDPC over the mean of pure stands in 1 yr of the 3 yr (Table 5). AUDPC for mix 2 did not differ significantly from the mean AUDPC of its components in any season. In 2 yr out of the 3 yr, the AUDPC for both mix 3 and mix 4 were significantly lower than the arithmetic mean of the components in pure stands. On a plot-wise basis, disease severity in pure Fitzroy plots was higher than in any other pure or mixture plots in all 3 yr (Table 5).

DISCUSSION

This study for the first time reports the development of anthracnose epidemics in simple heterogeneous mixtures of the perennial tropical pasture legume *S. scabra*. The AUDPC in mixtures was only significantly less than that of the components in pure stands for certain mixtures in some years. In the absence of the susceptible Fitzroy, a significant reduction in the AUDPC, compared to the arithmetic mean of AUDPC of the components in pure stands, was only detected during one of the three growing seasons. In the mixture containing equal proportions of all components including Fitzroy, AUDPC was similar to the mean of pure stands in all 3 yr. In mixtures with more than 50% Fitzroy, AUDPC was significantly less in 2 yr of the 3 yr.

Among accessions other than Fitzroy, significantly less disease than in their respective pure stands developed only in Q10042 and 36260 in the mixture with no Fitzroy in 1988. In the mixtures containing Fitzroy, AUDPC of accessions other than Fitzroy did not differ significantly from their respective pure stands in any season. Fitzroy did have less severe disease in mix 3 and mix 4 than in its pure stand, although this difference was only significant in 1989. This agrees with the observation that in mixtures, reduction in disease levels below the pure stand means is mainly due to less disease on the susceptible component (16). Fitzroy had lower AUDPC in mixtures with more than 50% Fitzroy than in its pure stand in all 3 yr. In years of severe anthracnose, this reduction in AUDPC by up to 35% may make a worthwhile contribution, if reduced severity directly translates into increased dry matter yield. Quantitative relationship between anthracnose severity in *S. scabra* and dry matter yield has not been established. In a South American study, mixtures of six accessions of *S. guianensis,* ranging from susceptible to resistant, were compared with their pure stands (17). Although there was no difference in terminal anthracnose severity levels for the susceptible accessions between pure and mixed stands in the second year, some susceptible accessions in a mixture maintained improvements of

TABLE 5. Area under disease severity progress curve for accessions of *Stylosanthes scabra* grown in pure and mixed stands

Treatments	1987	1988	1989
Fitzroy Pure stand	20.5 (6.3)[a]	110.4 (4.8)	182.8 (4.8)
Seca Pure stand	0.3 (0.2)	0.0 (0.0)	0.8 (0.6)
Q10042 Pure stand	1.8 (0.7)	16.0 (2.3)	15.3 (6.7)
36260 Pure stand	5.3 (3.1)	75.1 (8.1)	57.8 (34.8)
55860 Pure stand	0.7 (0.6)	26.8 (8.1)	13.4 (5.0)
Mixture without Fitzroy (mix 1)[b]	1.1 (0.1)	9.6 (2.9)	19.6 (12.8)
Mixture with 20% Fitzroy (mix 2)	6.0 (3.5)	44.4 (1.0)	43.7 (18.7)
Mixture with 52% Fitzroy (mix 3)	5.9 (2.6)	63.9 (7.9)	26.3 (6.0)
Mixture with 80% Fitzroy (mix 4)	16.3 (6.8)	84.8 (3.3)	14.3 (0.4)
Mean of pure stands without Fitzroy (1)	2.0 (0.8)	29.4 (2.3)	21.8 (8.9)
Mixture without Fitzroy (mix 1) (2)	1.1 (0.1)	9.6 (2.9)	19.6 (12.8)
Z statistic[c] for contrast between 1 and 2	1.04 ns[d]	5.34**	0.14 ns
Mean of all pure stands (3)	5.7 (1.4)	45.6 (2.5)	54.0 (7.2)
Mixture with 20% Fitzroy (mix 2) (4)	6.0 (3.5)	44.4 (1.0)	43.7 (18.7)
Z statistic for contrast between 3 and 4	−0.08 ns	0.17 ns	0.52 ns
Mean of pure stands (5)[e]	11.6 (0.7)	71.6 (0.6)	105.5 (1.0)
Mixture with 52% Fitzroy (mix 3) (6)	5.9 (2.6)	63.9 (7.9)	26.3 (6.0)
Z statistic for contrast between 5 and 6	2.14*	0.96 ns	12.92**
Mean of pure stands (7)	16.8 (1.0)	94.2 (0.8)	150.6 (0.8)
Mixture with 80% Fitzroy (mix 4) (8)	16.3 (6.8)	84.8 (3.3)	14.3 (0.4)
Z statistic for contrast between 7 and 8	0.08 ns	2.81*	142.15**

[a]Figure in parentheses is the standard error of the mean.
[b]Details on mixture composition provided in Table 2.
[c]Standard normal statistic $Z = (Mn_{ps} - Mn_{mx})/\sqrt{SE_{ps}^2 - SE_{mx}^2}$; Mn_{ps} = Mean severity of pure stands, Mn_{mx} = Mean severity of a mixture, SE_{ps} = Standard error of mean of the pure stands, SE_{mx} = Standard error of mean of a mixture.
[d]ns = Not significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.
[e]Mean of pure stands adjusted to match the proportion of the different components in the respective mixtures.

up to 60% in yield and 14% in survival in the second year (3). Plant survival did not improve in mixtures in our study.

Because inoculum produced on one genotype will be avirulent on other genotypes, mixtures with components differing in their susceptibility operate by reducing the number of alloinfections (infection on a genotype resulting from propagules produced on a different genotype; 1). Because there is no effect on autoinfections resulting from spores produced on the same genotype, mixtures may not provide effective disease control for splash-dispersed pathogens (23) such as *C. gloeosporioides,* where autoinfections contribute more towards epidemic development. In our study, accessions other than Seca only show a quantitative difference in their susceptibility to the three isolates applied as inoculum. This partial resistance against all pathogen isolates in accessions such as 55860 has made them useful as a source of race nonspecific resistance (5). These accessions support the multiplication of inoculum of all three races. Even with a highly specialized pathogen such as the barley powdery mildew, disease in a mixture, where components did not discriminate among pathogen races, increased to the same level as the mean of the pure stands towards the end of a season (7). Similarly, for unspecialized pathogens, such as *Septoria nodorum* Berk. in wheat, although the disease was restricted during the early stages of an epidemic (16), given time disease in mixtures equaled the mean of the pure stands (15). In annual crops such as cereals, however, advantage from mixtures is generally realized due to the short period of time they are exposed to the pathogen inoculum in each growing season.

Most work on the effectiveness of mixtures in restricting epidemic development has been done with cereals. In other plants, disease restriction by genotype mixtures has not been as spectacular. For example, in celery, although mixtures were initially satisfactory in controlling *Cercospora apii* Fres., the effect was only temporary (2). In swede (*Brassica napus* L.), initial reduction in powdery mildew (*Erysiphe polygoni* DC.) due to a mixture of partially resistant and highly susceptible cultivars was lost after an initial period (21).

Apart from the epidemiological consequences of splash dispersal and the failure of most accessions to discriminate among pathogen races, other experimental factors may also influence the results. Variability in the data could be addressed by increasing the size of plots, borders between plots, and the number of replicates to improve accuracy. Other constraints, such as the heterogeneity in some accessions and the presence of plants of different age in a plot may also have influenced our results. Under field conditions, pastures almost always contain plants that differ widely in their age and the ability to provide protection against anthracnose over a prolonged period of time under these conditions is crucial to the success or failure of genotype mixtures in *S. scabra* pastures.

There is evidence of only very low levels of anthracnose severity in a pasture sown to an interspecific mixture of *Stylosanthes* in north Queensland. A detailed survey of this pasture, conducted 13 yr after establishment, has shown a diversity of *C. gloeosporioides* strains and host genotypes, with natural outcrossing playing an important role (R. D. Davis, R. M. Boland, and C. Howitt, *unpublished*). Despite susceptible plants making up a large proportion of the surviving population, serious anthracnose epidemics have not occurred at this site. In the mainly self-pollinating *S. scabra,* 1–2% outcrossing has been estimated using isozyme analysis (22). Natural outcrossing in populations of the related *S. capitata* Vog. can be as high as 20% (18). In the present study, host variation due to natural outcrossing was not allowed to develop. The results, therefore, may not predict the long-term performance of genotype mixtures under grazing in broadacre pastures. Through monitoring of host and pathogen populations as well as the physical environment and the use of modified experimental designs, current studies are aimed at determining the long-term success of genotype mixtures in controlling the severity of anthracnose.

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