Section B

Anthracnose resistant *Stylosanthes*

B1: Germplasm evaluation & cultivar development

Chapter 10

Regional evaluation of *Stylosanthes* germplasm in Brazil

Celso Dornelas Fernandes[1], Sukumar Chakraborty[2], Bela Grof[3], Hortência Maria Abranches Purcino[4], Maria do Socorro Bona Nascimento[5], Maria José D' Ávila Charchar[6], Jaqueline Ros emeire Verzignassi[7] and José Marcelino Sobrinho[8]

Summary

Accessions and cultivars of *Stylosanthes capitata, S. macrocephala, S. guianensis, S. scabra* and *S. seabrana* were evaluated for agronomic performance and anthracnose resistance at five field sites in Brazil spanning the states of Chapadão do Sul-MS, Goiânia-GO, Planaltina-DF, Sete Lagoas-MG and Teresina-PI. The field trials were laid out as a complete randomised block design replicated four times and planted at the beginning of the rainy season. Each replicate 5x2 m plot consisted of four rows of each accession/cultivar. Data on dry matter, seed yield and anthracnose severity were collected during two years of this study and analysed separately for each site. Results showed considerable genetic variability among the germplasm of *Stylosanthes* studied for each character. The most promising accessions yielded 11–16 t dry matter/ha annually and seed yields reached up to 600 kg/ha. Anthracnose was a serious problem on susceptible accessions at some sites with high rainfall. The study forms a basis for the targeting of specific accessions to suit regional climatic conditions.

[1] Embrapa Gado de Corte/Uniderp, C.P. 154, 79002-970, Campo Grande-MS, Brazil
[2] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia
[3] Amiga Court, Palmwoods, Queensland 4555, Australia
[4] EPAMIG/CTCO, Rod. MG 424 Km 64, 35715- 000, Sete Lagoas-MG, Brazil
[5] Embrapa Meio Norte, Av. Duque de Caxias, 5660, Teresina-PI, Brazil
[6] Embrapa Cerrados, Rod. BR 020, Km 18, 73-301-970, Planaltina-DF, Brazil
[7] Universidade Estadual de Maringá, Maringá-PR
[8] Universidade Católica de Goiás, Goiânia-GO
Correspondence should be addressed to: Celso Dornelas Fernandes
celsof@cnpgc.embrapa.br

Introduction

Species of the genus *Stylosanthes* are widely distributed in tropical and subtropical regions of the Americas, Africa and Asia. The main centre of origin and diversity is Brazil, the native habitat of 25 of the 45 described species (Stace & Edye 1984). The genus is adapted to a variety of edaphic and climatic conditions and has good potential as cultivated forage in Brazil. Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., the anamorphic form of *Glomerella cingulata* (Stonem.) Spauld & Scherenk, has been the main limitation to the commercial utilisation of *Stylosanthes*. This fungus has an extensive genetic diversity in Brazil (Weeds et al 2003) and resistant cultivars of the legume can potentially become susceptible to the disease only a few years after their commercial release.

The savanna soils are often low in available nitrogen, and low forage availability and quality of native or cultivated grass pastures in the dry season is a major constraint to livestock production. Forage legume species adapted to these soils and weather conditions can have an important impact on pasture-based animal production and soil fertility when used as a pasture crop with grasses or as a cover crop to improve fertility through biological nitrogen fixation (Andrade et al, this volume; Cameron & Chakraborty, this volume).
The introduction of legumes in grass-based pastures improves the quality of forage due to increased protein availability, and animal performance improves as a consequence (Hall & Glatzle, this volume). The genus *Stylosanthes* contains many multi-purpose species that have been used to integrate animal and crop production, and aid in the revegetation of fragile environments in Asia (Phaikaew et al, this volume), Africa (Pengelly et al, this volume) and Australia (Hall & Glatzle, this volume).

There is a long history of research and development on *Stylosanthes* germplasm in Brazil but the commercial success with released cultivars has been short lived in most countries in South America including Brazil (Andrade et al, this volume). No fewer than ten cultivars have been released in Brazil; early cultivars were imported from Australia but many have been developed through evaluation and selection by EMBRAPA and its international collaborators such as the Centro Internacional Agriculture Tropical (CIAT) (Andrade et al, this volume). The main reasons for failure include poor seed yield, susceptibility to anthracnose, poor persistence under grazing, a lack of consideration of alternative usage of a pasture species, and a reliance of farmers on N fertilised grass pastures (Andrade et al, this volume; Miles & Lascano 1997). Extensive evaluations of germplasm through a network of regional sites had been carried out for a number of years. However, this activity has been greatly reduced in recent years and, as a consequence, identification of promising *Stylosanthes* germplasm through national and international programs has almost come to a standstill. To increase the range of materials available to Brazil, and to select well-adapted, productive and anthracnose-resistant *Stylosanthes*, new germplasm and bred lines from EMBRAPA, Australia and CIAT were evaluated in a collaborative project funded by the Australian Centre for International Agricultural Research (ACIAR) during 1996–2003. This paper reports on the productivity and anthracnose resistance of these new materials from a regional evaluation across selected edaphic and climatic conditions in Brazil.

Materials and Methods

A total of 26 accessions and cultivars of *S. guianensis, S. scabra, S. capitata, S. macrocephala* and *S. seabrana* from EMBRAPA, CSIRO (Australia) and CIAT were evaluated during a two-year period at five separate field sites in Brazil, representing different agroclimatic zones. Sites were selected to represent areas where *Stylosanthes* species have either shown promise or have the potential to be used as a pasture and forage legume. Replicated plots were established at Chapadão do Sul in Mato Grosso do Sul, Teresina in Piauí, Sete Lagoas in Minas Gerais, Goiânia in Goiás and Planaltina in the Federal District (DF). The number of accessions of the various species and the geographical location of these sites are given in Table 10.1.

At each site a field trial was laid out in a complete randomised block design with four replicate plots established at the beginning of the rainy season. Annual dry matter (DMY) and seed yield (SY) were recorded from replicate plots of each accession from an area of at least 2x3 m in each established plot. Anthracnose severity was assessed during the growing season on a randomly selected branch on each plant within each plot at approximately 15-day intervals. Plants were visually assessed for disease severity using a 10-point rating scale, where: 0 = disease free; 1 = 1–3% leaf area diseased and defoliated; 2 = 4–6%; 3 = 7–12%; 4 = 13–25%; 5 = 26–50%; 6 = 51–75%; 7 = 76–87%; 8 = 88–94%; 9 = 95–100% (Chakraborty 1990).

Data on DMY, SY and anthracnose severity were separately analysed for each site. Results were compared by analysis of variance and clustering and means were separated at the 5% probability level (Scott & Knott 1974). Data on anthracnose severity (sev.) were √(sev. +0.01) transformed before analysis to stabilise variance.

Results and Discussion

The number of accessions/cultivars evaluated at each site varied from 21 to 26 (Table 10.1). Accessions of the five *Stylosanthes* species were initially selected for the regional sites according to their expected performance at these sites, and not all species or accessions were grown at every site. Therefore, results for each site are given separately. However, many accessions were common to all sites and their relative performance at each of the five sites was also compared; findings are given for a small subset of accessions. This work forms the basis for the evaluation, selection and release of a new *S. macrocephala – S. capitata* composite cultivar for use in central Brazil (Verzignassi et al 2002). This has come seven years after the release of Mineirão in 1993 and has the potential to start a renewed interest in *Stylosanthes* in Brazil, following

Table 10.1 *Stylosanthes* species accessions and cultivars evaluated at field sites in Brazil.

Site and state	Latitude and Longitude	*S. capitata*	*S. macrocephala*	*S. guianensis*	*S. scabra*	*S. seabrana*	Total
Chapadão do Sul, MS	20º 26' S, 54º 42' W	5	6	5	7	0	23
Goiânia, GO	16º 36' S, 49º 16' 50" W	5	6	5	5	0	21
Planaltina, DF	15º 36' S, 47º 12' W	5	5	2	12	2	26
Sete Lagoas, MG	19º 28' S, 45º 15' W	5	5	2	8	2	22
Teresina, PI	5º 14' 35" S, 42º 11' 38" W	5	6	5	5	0	21

disappointments with many of the earlier released cultivars. Unrelated to this work, a second new *S. capitata* cultivar is at a pre-release stage (Andrade et al, this volume). In the final section of this study we record and review the performance of cultivar Campo Grande.

Performance of accessions at each site

Chapadão do Sul Overall, accessions of *S. capitata* performed the best at this site, with all accessions reaching over 11 t/ha. This species was followed by *S. guianensis* and *S. macrocephala*, and *S. scabra* was the least productive (Table 10.2). However, the *S. guianensis* cv. Mineirão (accession GC 984) produced the highest DMY yield at 13.8 t/ha, with the *S. capitata* – *S. macrocephala* cultivar Campo Grande (GC 2260) a close second at a DMY reaching up to 13.4 t/ha.

Stylosanthes macrocephala produced the highest quantity of seeds, generally close to 300 kg/ha, and *S. capitata* produced over 200 kg/ha. Both *S. guianensis* and *S. scabra* were poor seed producers, although some *S. guianensis* produced nearly 100 kg/ha of seeds. The poor herbage and seed yield of all *S. scabra* accessions indicate that this species was not suited to the local agroclimatic conditions. The *S. guianensis* cv. Mineirão had the lowest seed yield of only 5.6 kg/ha despite its high forage availability and DMY. Poor seed yield of Mineirão in the first few years of its establishment has been the most significant impediment to its large-scale commercial utilisation in Brazil (Andrade et al, this volume).

The data on anthracnose severity indicate considerable variation in resistance among the various accessions tested. Overall, *S. macrocephala* and *S. guianensis* showed higher resistance than *S. capitata* or *S. scabra*. Among the accessions, GC 1496 (*S. scabra* cv. Fitzroy) was the most susceptible and GC 1507, GC 1508 (both *S. macrocephala*) and GC 1585 (*S. guianensis*) were the most resistant.

Based on their overall performance, accessions GC 1466, GC 1585 and GC 2260 were selected for further studies.

Goiânia The overall DMY of *S. guianensis* was the best at Goiânia, although accessions did not produce the same level of DMY at this site as in Chapadão do Sul (Table 10.2). No other species produced over

Table 10.2 Dry matter, seed yield and anthracnose severity rating of accessions of *Stylosanthes* species evaluated at Chapadão do Sul in Mato Grosso do Sul and Goiânia in Goias over a two-year period.

		Chapadão do Sul			Goiânia		
Accession No. (GC)	Species	Dry matter yield (t/ha)	Seed yield (kg/ha)	Anthracnose severity	Dry matter yield (t/ha)	Seed yield (kg/ha)	Anthracnose severity
348	*S. guianensis*	9.1 b[1]	47.5 d	1.2 e	5.6 c	29.8 d	2.4 a
984	*S. guianensis*	13.8 a	5.6 d	1.4 d	11.5 a	8.5 e	1.7 c
1082	*S. capitata*	12.8 a	287.8 a	2.2 b	8.0 c	625.0 a	2.0 a
1173	*S. capitata*	12.8 a	273.5 a	2.2 b	7.1 c	500.3 b	1.9 b
1466	*S. capitata*	11.4 a	277.5 a	2.0 c	6.2 c	335.8 b	1.6 c
1468	*S. guianensis*	9.3 b	100.4 c	1.5 d	12.9 a	157.5 c	1.2 d
1469	*S. capitata*	12.3 a	223.3 b	2.3 b	7.0 c	287.3 c	1.6 c
1490	*S. scabra*	5.8 c	40.8 d	2.3 b	5.4 c	110.3 d	2.1 a
1493	*S. scabra*	5.1 c	84.0 c	2.0 c	4.8 c	200.9 c	1.2 d
1496	*S. scabra*	–	–	2.8 a	–	–	–
1498	*S. scabra*	6.3 c	79.6 c	2.2 b	6.1 c	483.3 b	1.4 d
1500	*S. scabra*	–	–	1.6 d	–	–	–
1507	*S. macrocephala*	7.2 c	316.2 a	1.1 e	5.1 c	124.5 d	2.2 a
1508	*S. macrocephala*	8.5 b	309.0 a	1.1 e	5.1 c	76.3 d	2.2 a
1511	*S. macrocephala*	9.2 b	214.6 b	1.3 d	6.0 c	157.0 c	2.2 a
1536	*S. scabra*	6.3 c	47.7 d	2.1 b	4.8 c	259.5 c	1.9 b
1538	*S. scabra*	4.6 c	99.0 c	2.2 b	6.2 c	222.3 c	1.9 b
1557	*S. guianensis*	8.7 b	40.1 d	1.7 c	8.8 b	78.8 d	0.8 e
1582	*S. macrocephala*	9.1 b	328.2 a	1.3 d	6.0 c	206.3 c	2.2 a
1585	*S. guianensis*	10.9 b	106.5 c	1.1 e	12.5 a	138.3 d	1.4 d
1586	*S. guianensis*	9.7 b	161.0 c	1.4 d	10.1 b	164.5 c	1.9 b
1587	*S. macrocephala*	9.1 b	298.5 a	1.4 d	6.7 c	105.5 d	2.2 a
2260	*S. capitata* – *S. macrocephala*	13.4 a	314.6 a	1.8 c	7.0 c	432.8 b	1.6 c

[1] Means followed by a different letter are not significantly different according to the Scott-Knott test (P≥0.05)

10 t/ha and there was very little difference in DMY between *S. capitata, S. macrocephala* and *S. scabra,* or between accessions among these species. Seed yield of all species and accessions was generally higher than in Chapadão do Sul and *S. capitata* GC 1082 produced over 600 kg/ha of seeds. In contrast to Chapadão do Sul, some *S. scabra* accessions produced >400 kg/ha seed; however, the relative ranking of most accessions remained the same as at the Chapadão do Sul site and, despite improvements in seed yield for nearly all accessions, Mineirão produced only 8.5 kg/ha seed. Anthracnose severity ratings of species and accessions had a narrower range than at Chapadão do Sul and both the least and most resistant accessions were from *S. guianensis.* GC 1468 and GC 1585 were selected for further study.

Germplasm evaluation and co-workers at the Goiânia site.

Planaltina and Sete Lagoas Overall, DMY of most accessions was lower at Planaltina than at Sete Lagoas. Only one accession at Planaltina produced over 10 t/ha, whereas ten accessions produced over 10 t/ha at Sete Lagoas (Table 10.3). The DMY of *S. guianensis* GC 348 at Goiânia and Planaltina was <7 t/ha (Table 10.2), but over 15 t/ha, as high as the cultivar Mineirão, at Sete Lagoas.

Although *S. guianensis* accessions generally had the highest DMY at both sites, the same accession did not produce the highest seed yield at both sites. Seed production at the two sites largely followed trends in DMY; however, at Sete Lagoas the cultivar Mineirão did not produce any seed and data on seed yield were unavailable for a large number of other accessions. The most prolific seed producers were GC 1517 (*S. macrocephala*), GC 1579 (*S. guianensis*), GC 1588 (*S. seabrana*) and GC 2256 (*S. scabra*) at Planaltina; and GC 1093, GC 1463, GC 1511, GC 1582 and GC 2260 at Sete Lagoas.

Table 10.3 Dry matter, seed yield and anthracnose severity rating of accessions of *Stylosanthes* species evaluated at Planaltina in Brasilia-DF and Sete Lagoas in Minas Gerais over a two-year period.

		Planaltina			Sete Lagoas		
Accession No. (GC)	Species	Dry matter yield (t/ha)	Seed yield (kg/ha)	Anthracnose severity	Dry matter yield (t/ha)	Seed yield (kg/ha)	Anthracnose severity
348	*S. guianensis*	6.4 b1	161.1 f	1.8 b	15.2 a	19.5 c	0.3 a
984	*S. guianensis*	8.2 a	3.0 f	0.7 d	15.2 a	0.0 c	0.3 a
1059	*S. capitata*	5.0 c	101.3 f	1.6 b	7.3 d	562.3 b	0.3 a
1084	*S. capitata*	4.5 c	20.2 f	2.2 a	9.6 c	413.0 b	0.1 a
1093	*S. capitata*	2.1 d	48.6 f	1.8 b	5.8 d	759.0 a	0.3 a
1179	*S. capitata*	5.3 c	45.6 f	1.3 c	11.0 b	408.8 b	0.1 a
1463	*S. guianensis*	9.4 a	617.0 b	2.2 a	14.3 a	500.8 a	0.3 a
1496	*S. scabra*	4.7 c	579.2 b	0.8 d	–	–	–
1498	*S. scabra*	6.6 b	116.4 f	1.3 c	–	–	–
1500	*S. scabra*	5.1 c	36.0 f	1.0 d	7.3 d	–	0.1 a
1511	*S. macrocephala*	7.5 b	467.2 c	1.7 b	12.1 b	1066.3 a	0.4 a
1517	*S. guianensis*	10.5 a	776.4 a	2.1 a	12.7 b	401.8 b	0.1 a
1540	*S. scabra*	6.8 b	116.3 f	1.1 d	7.9 c	–	0.3 a
1579	*S. guianensis*	8.8 a	797.6 a	2.1 a	11.5 b	180.0 b	0.1 a
1582	*S. macrocephala*	7.4 b	630.0 b	1.0 d	14.2 a	948.0 a	0.1 a
1588	*S. seabrana*	4.5 c	810.7 a	1.4 c	9.0 c	–	0.3 a
1589	*S. seabrana*	4.9 c	573.2 b	1.3 c	6.9 d	–	0.1 a
2252	*S. scabra*	5.9 b	121.6 f	1.0 d	–	–	–
2253	*S. scabra*	4.9 c	198.5 e	1.2 c	7.2 d	–	0.3 a
2254	*S. scabra*	4.9 c	92.3 f	2.2 a	10.6 b	–	0.9 a
2255	*S. scabra*	6.1 b	321.0 d	2.2 a	4.2 d	–	0.1 a
2256	*S. scabra*	3.7 c	686.5 a	1.6 b	4.5 d	–	0.3 a
2257	*S. scabra*	6.0 b	361.9 d	1.4 c	9.5 c	-	0.6 a
2258	*S. scabra*	6.4 b	92.6 f	1.8 b	6.8 d	–	0.1 a
2259	*S. scabra*	7.0 b	337.7 d	1.1 d	–	–	–
2260	*S. capitata* – *S. macrocephala*	4.3 c	187.4 e	1.9 b	12.8 b	1675.0 a	0.1 a

[1] Means followed by a different letter are not significantly different according to the Scott-Knott test (P≥0.05)

There was a range of anthracnose resistance among accessions of *S. guianensis, S. capitata* and *S. scabra* at Planaltina, but anthracnose was not a problem at Sete Lagoas and most accessions developed very low anthracnose severity. GC 1463 and GC 1517 were selected for further study at Planaltina and GC 1463, GC 1582 and GC 2260 at Sete Lagoas.

Eight bred *S. scabra* lines and two *S. seabrana* selections from Australia were also evaluated at these two sites. At Planaltina their DMY were in the range 3.7–6.4 t/ha, which was lower than many other accessions, but the seed yield of one *S. seabrana* line (810 kg/ha) was comparable to the best seed producers at this site. There was a similar trend in DMY of the Australian selections at Sete Lagoas, but seed production data were not available for this site.

Germplasm evaluation at the Planaltina site.

Teresina Overall, many accessions of *S. guianensis, S. capitata, S scabra* and *S. macrocephala* produced their highest DMY at Teresina, and seed yield was moderate to high (Table 10.4). *Stylosanthes capitata* GC 1082, *S. scabra* GC 1498, *S. macrocephala* GC 1511 and *S. guianensis* GC 1557 were among the best, with DMY in the range 15–17 t/ha. The seed production of some ten accessions reached above 300 kg/ha, although Mineirão produced only 0.4 kg/ha. The semi-arid conditions at this site meant that weather was not conducive to severe anthracnose development. Most accessions developed only a few minor lesions to allow any discrimination between accessions or species. The most promising accessions according to DMY and seed yield were GC 1082, GC 1173, GC 1469, GC 1498 and GC 2260.

Table 10.4 Dry matter, seed yield and anthracnose severity rating of accessions of *Stylosanthes* species evaluated at Teresina in Piau over a two-year period.

Accession No. (GC)	Species	Dry matter yield (t/ha)	Seed yield (kg/ha)	Anthracnose severity
348	*S. guianensis*	11.2 b[1]	17.5 c	0.1 a
984	*S. guianensis*	13.8 b	0.4 c	0.1 a
1082	*S. capitata*	16.1 a	613.2 a	0.1 a
1173	*S. capitata*	16.0 a	605.8 a	0.1 a
1466	*S. capitata*	16.2 a	613.7 a	0.1 a
1468	*S. guianensis*	13.5 b	121.8 c	0.1 a
1469	*S. capitata*	15.9 a	617.3 a	0.1 a
1490	*S. scabra*	13.7 b	336.8 b	0.1 a
1493	*S. scabra*	12.6 b	339.5 b	0.1 a
1498	*S. scabra*	17.0 a	—	0.1 a
1507	*S. macrocephala*	8.8 c	486.3 b	0.1 a
1508	*S. macrocephala*	10.8 c	400.0 b	0.1 a
1511	*S. macrocephala*	14.0 a	491.2 b	0.1 a
1536	*S. scabra*	8.6 c	119.5 c	0.1 a
1538	*S. scabra*	14.8 a	—	0.1 a
1557	*S. guianensis*	15.0 a	155.9 c	0.1 a
1582	*S. macrocephala*	13.5 b	648.5 a	0.1 a
1585	*S. guianensis*	13.3 b	144.8 c	0.1 a
1586	*S. guianensis*	13.2 b	20.8 c	0.1 a
1587	*S. macrocephala*	11.6 b	342.2 b	0.1 a
2260	*S. capitata- S. macrocephala*	12.8 b	608.9 a	0.1 a

[1]Means followed by a different letter are not significantly different according to the Scott-Knott test (P≥0.05)

Relative performance at the five sites

Although the performance of the five accessions varied across the five sites, *S. guianensis* cv. Mineirão produced the highest DMY at all sites and, except at Goiânia and Planaltina, *S. capitata – S. macrocephala* cv. Campo Grande also performed well (Figure 10.1). Overall, Sete Lagoas and Teresina had the highest DMY of most accessions. In contrast, seed production was very patchy across all sites and accessions and no single accession consistently produced high seed yield at all five sites (Figure 10.1). *Stylosanthes guianensis* accession 348 and Mineirão consistently produced very poor seed yield at all five sites. Based on these results, Sete Lagoas appears to be the best site for seed production of *S. macrocephala* and needs to be considered as a site for commercial seed production of the newly released cv. Campo Grande. The five accessions were very well separated according to their anthracnose resistance at Planaltina, which has long been recognised as a site for severe anthracnose development due to conducive weather and inoculum availability (Chakraborty et al 1997). Mineirão had the lowest severity of all five accessions at this site, indicating its superior anthracnose resistance.

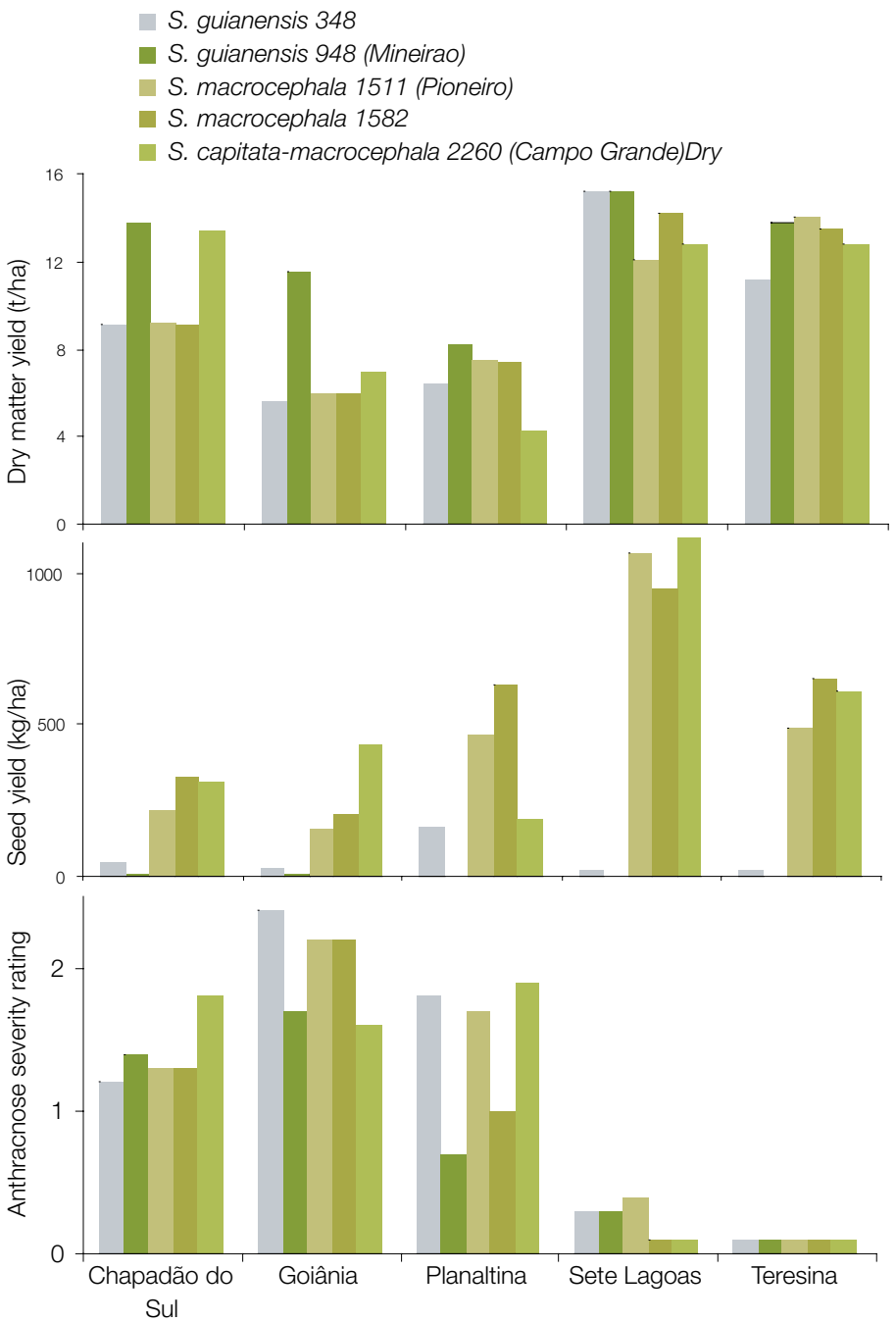


Figure 10.1 Dry matter, seed yield and anthracnose severity of five selected *Stylosanthes* spp. accessions at five different field sites in Brazil.

There was very little anthracnose at either Sete Lagoas or Teresina, and all five accessions had very high DMY at these two sites in the absence of a severe anthracnose challenge.

Commercial release of cultivars

These regional evaluations form the basis for the release of the *S. capitata* – *S. macrocephala* multiline cultivar 'Estilosantes Campo Grande' (accession GC 2260 in this paper). It was released commercially by the EMBRAPA Beef Cattle centre in 2000 for use in association with *Brachiaria decumbens* mainly on sandy soils of central Brazil (Embrapa Gado de Corte 2000). The variety is recommended for reclamation of mixed grass–legume pastures, where the legume is either sown with fertilisers in rows using zero tillage seeders, following a herbicide application to suppress grass growth; or established after burning the grass-dominated pasture. Seeds were initially multiplied in Chapadão do Sul and then later produced by a large consortium from Mato Grosso do Sul, Minas Gerais and São Paulo states, under EMBRAPA´s supervision. Seeds were commercially available to cattle farmers in 2001. Currently, an area >640 ha is under commercial seed production and the estimated area under grazing in Brazil is up to 100,000 ha. The strong interest in this variety among producers is driven by: a yield of 12–13 t of dry matter/ha/year, leading to an 18–20% increase in liveweight gain in mixed pastures with *Brachiaria decumbens* compared to the grass alone; good seed production (200–700 kg/ha) with further prospects of reducing seed cost from mechanical seed harvesting; over five years' persistence under grazing in well-managed mixed *B. decumbens* pastures due to acceptable levels of anthracnose resistance and high natural reseeding in the pasture; and adding nearly 180 kg N/ha through biological nitrogen fixation in three months compared to 95 kg by *S. guianensis* Mineirão and 88 kg by *S. macrocephala* Pioneiro.

Acknowledgments

The Australian Centre for International Agricultural Research and the Cooperative Research Centre for Tropical Plant Protection, among others, funded much of the research reported in this review. We thank the staff and management of Ribeirão Agropecuária in Chapadão do Sul for their enthusiasm, encouragement and assistance with the field trial at this site. We are grateful to Segenet Kelemu, Ademir Hugo Zimmer, Cesar Heraclides Behling Miranda, José Marques da Silva, José Raul Valério, Josias de Carvalho, Leônidas da Costa Schalcher Valle, Manuel Cláudio Motta Macedo, Roza Maria Schunke and many other individuals who have contributed invaluable ideas and offered technical and other assistance throughout the course of this work.

***Stylosanthes capitata*-*S. macrocephala* cultivar 'Estilosantes Campo Grande' in a commercial farm that is increasingly used for cattle in feedlots (photo: S. Chakraborty).**

References

Chakraborty S. 1990. Expression of quantitative resistance to *Colletotrichum gloeosporioides* in *Stylosanthes scabra* at different inoculum concentrations and day-night temperatures. Australian Journal of Agricultural Research 41, 89–100.

Chakraborty S., Fernandes C.D., Charchar M.J.d'A., Kelemu S. & Cameron D. 1997. Epidemiology of anthracnose at the centre of diversity. Tropical Grasslands 31, 408–416.

Embrapa Gado de Corte 2000. *Estilosantes* Campo Grande: estabelecimento, manejo e produção animal. Campo Grande. Embrapa Gado de Corte. Comunicado Técnico, 618 pp.

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I. *Stylosanthes* development and utilization in South America. Tropical Grasslands 31, 454–459.

Scott, A.J. & Knott, M.A. 1974. Cluster analysis method for grouping means in the analysis of variance. Biometrics 30, 507–512.

Stace, H.M. & Edye, L.A. (eds.). The biology and agronomy of *Stylosanthes*. Academic Press: Sydney, Australia.

Verzignassi, J.R., Fernandes, C.D. & Miranda, C.H.B. 2002. *Estilosantes* Campo Grande**:** current situation and perspectives. *Stylosanthes* international, newsletter issue 7, September 2002

Weeds, P.L., Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A., Ramesh, C.R., Kexian, Y. & Kelemu, S. 2003. Genetic diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centres of origin and utilization. Phytopathology 93, 176–185.

Chapter 11

Regional evaluation of *Stylosanthes* germplasm in India

C.R. Ramesh[1], N.P. Melkania[2], J.S. Desale[3], A.H. Sonone[3], M.R. Manjare[3], J.R. Patel[4], J.P. Yadavendra[4], C.C. Patel[4], M.A. Nair[5], S. Lakshmi[5], J. Pillai[5], A. Gopalan[6], G. Vijayakumar[6], F. Khan[6], K. Jhansi[7] and M.A. Sultan[7]

Summary

This chapter deals with the evaluation of *Stylosanthes* germplasm for dry matter yield (DMY), green fodder yield and anthracnose resistance at eight regional sites in India to select promising accessions for potential release as commercial cultivars. In a preliminary evaluation 18 germplasm of *S. guianensis, S. hamata, S. scabra, S. seabrana* and *S. viscosa* were grown at eight sites over a two-year period. All except two *S. guianensis* cultivars performed reasonably well. Based on superior DMY, 11 accessions were included in an advanced evaluation along with nine other accessions at five of these sites. An extended range of 27 accessions was evaluated at the master site at Dharwad in Karnataka. Overall, Trivandrum was the most productive site with an average DMY of 13.7 t/ha, followed by Rahuri (11.7 t/ha), Dharwad (7.8 t/ha), Anand (1.8 t/ha) and Coimbatore. One or more *S. seabrana* accessions were among the most productive entries at each site, and *S. scabra* 36260 produced the least DMY at most sites. Anthracnose severity was low to very low at all sites and the overall mean severity for Trivandrum was 2.03, Anand 0.9, Rahuri 0.8, Coimbatore 0.45 and Dharwad 0.32. Despite the overall low severity, the damage to *S. seabrana* is a cause for concern since the anthracnose pathogen has adapted to this species within a few years of its use in India. The work has demonstrated a high level of productivity of *S. seabrana* accessions in India and its suitability to a broad range of soil and climatic conditions at the regional sites. Further work should result in the release of one or more cultivars to expand the limited range currently available in India.

[1] Indian Grassland and Fodder Research Institute, Regional Station, Dharwad, Karnataka, 580 005
[2] Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh 284 003
[3] All India Coordinated Research Project on Forage Crops (AICRPFC), Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra 413 722
[4] AICRPFC, Gujarat Agricultural University, Anand, Gujarat 388 110, India
[5] AICRPFC, College of Agriculture, Kerala Agricultural Unversity Vellayani, Kerala 695 522, India
[6] AICRPFC, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India
[7] AICRPFC, Acharya NG Ranga Agricultural University (ANGRAU), Hyderabad, 500030, India

Introduction

Ever since the first introduction of *Stylosanthes* (stylo) to India, nearly 60 years ago (Whyte 1957), interest in this tropical legume genus has continued unabated. Introductions from Australia, South America, Africa and the USA of species and accessions have continued to the present day (Ramesh et al 1997). Along with the introduced species, the native perennial *Stylosanthes fruticosa* Alston is widely distributed throughout the southern peninsular regions (Hooker 1872–1897). There is a significant history of research on various aspects of this legume in India and a substantial body of information has been published, predominantly in Indian research journals and organisational annual reports (eg annual reports of the Indian Grassland and Fodder Research Institute (IGFRI) and the various agricultural universities). Much of this has been reviewed in this volume (Phaikaew et al; Rao et al; Ramesh et al) and in other publications (Ramesh et al 1997).

India boasts the world's largest *Stylosanthes* seed industry, producing nearly a thousand tonnes of seed each year (Rao et al, this volume). Most of this is used domestically in the revegetation of wasteland and problem soils through government-sponsored development programs. However, the legume is very versatile, and research in India has shown its usefulness in a number of production systems (Jayan 1995) including agro forestry and plantation horticulture. A small fraction of the seed is used by the private sector as a fresh forage crop to feed animals, as mixed legume–grass pastures, as a nitrogen fixer in mixed legume–cereal crops, as a legume in rangelands, as a legume for short fallows, as a cover crop to control weeds, and to add nitrogen and produce fodder in plantations of horticultural and forestry species.

The usefulness of *Stylosanthes* as hay when fresh or processed, and as a component of feed concentrate when dried and ground, has been tried in India (Gupta et al 1992). Recent research on broiler liveweight gain and meat and egg yoke quality (Guodao et al, this volume) has generated renewed interest in the potential of *Stylosanthes* leaf meal as a component of commercial feed formulations. Currently, there is not enough information on the suitability of the different species and cultivars, or a large enough area under *Stylosanthes* production that is suitable for making high quality leaf meal. This is the case for other utilisation schemes as well, since the information necessary to target a specific species or cultivar to a production system for a given agroecological condition is largely unknown.

The reasons for this lack of relevant knowledge, and hence a poor adoption rate, are many and varied. There have been many regional evaluations of introduced germplasm in India. In the 1970s *S. humilis, S. scabra* (CPI 34925, 40205, 40289, 40292), *S. hamata* (CPI 38842) and *S. viscosa* (CPI 34904, 40264) were evaluated for green and dry fodder yield under a Drought Prone Area Program at the Central Arid Zone Research Institute in Jodhpur. During 1988, 11 *S. hamata* accessions from Florida consisting of nine erect types (EC168634, 168633, 168641, 168640, 168635, 168629, 168630, 168639, 168628), one semi-erect (EC168638) and one prostrate type with narrow leaves (EC 168631), along with the cultivar Verano, were evaluated at the IGFRI Regional Research Station at Dharwad (P.K. Jayan, pers. comm.). A wide range of variability was noticed in plant height (36 to 68.8 cm), number of main branches (4.3 to 6.5) and days to flowering (66 to 110). In addition, one or more accessions of *Stylosanthes* have been included in several regional trials by the All India Coordinated Research Project on Forage Crops and other IGFRI initiatives (C.R. Hazra, pers. comm.). Despite such a wealth of useful information, no cultivar has been formally released in India, including the two Australian cultivars *S. scabra* cv. Fitzroy and *S. scabra* cv. Verano, which are widely grown in India.

In common with other countries where attempts have been made to use *Stylosanthes,* anthracnose is a serious threat to most cultivars; Fitzroy is highly susceptible to severe damage from most races of the pathogen and Verano is only partially resistant to anthracnose (Chakraborty, this volume). Commercial seed production of *S. scabra* suffered serious damage from anthracnose during the 1997 growing season at Ananthapur in Andhra Pradesh and many farmers did not grow this species in 1998.

Extensive regional evaluation of germplasm in India has been greatly reduced in recent years and, as a consequence, new promising *Stylosanthes* germplasm identified through international programs has not been made available or tested in India. To increase the range of materials available and to select well-adapted, productive and anthracnose-resistant *Stylosanthes*, new germplasm and bred lines from EMBRAPA, Australia and CIAT were evaluated in a collaborative project funded by the Australian Centre for International Agricultural Research (ACIAR) during 1997–2003. The germplasm were evaluated over a 5-6-year period at a number of regional sites selected to represent the range of edaphic and climatic conditions in India. This paper reports on their productivity and anthracnose resistance.

Materials and Methods

Selected accessions, cultivars and breeding lines of *S. guianensis, S. hamata, S. scabra, S. seabrana* and *S. viscosa* from Australia, Colombia and Brazil were imported under quarantine permit through the National Bureau of Plant Genetic Resources. A total of 89 different accessions, cultivars and breeding lines were available. These were initially grown in pots or small 2x2 m plots at the master site of the IGFRI regional research station in Dharwad, Karnataka during 1997–98. From these, 18 accessions (Table 11.1) were selected for a preliminary evaluation during 1998–2000; seeds were multiplied at Dharwad and distributed to eight regional sites (Table 11.2).

Preliminary evaluation

The preliminary evaluation was carried out at Anand (Gujarat Agricultural University), Coimbatore (Tamil Nadu Agricultural University), Hyderabad (Acharya NG Ranga Agricultural University in Andhra Pradesh), Kalyani (Bidhan Chandra Krishi Vidyalaya in West Bengal), Palampur (IGFRI regional research station in Himachal Pradesh), Rahuri (Mahatma Phule Krishi Vidyapeeth in Maharashtra), Uruli Kanchan (Bharatiya Agro Industries Foundation in Maharashtra) and Trivandrum (Kerala Agricultural University). These sites represent the agroecological and climatic conditions of regions where *Stylosanthes* may potentially have a major role in silvipasture and dryland agriculture. Using a randomised complete block design, three to five replicate plots of each accession were established at each site with 50 cm spacing between rows, and seeds were directly sown after a mild scarification. Plot sizes varied at the different sites, with 2.4x5 m at Rahuri, 4x3 m in Dharwad and 4x2.5 m at the other sites.

Observations were recorded on germination, establishment, days to flowering, green forage yield (GFY), dry matter yield (DMY), anthracnose severity (SEV) and persistence at each site. Other assessments including seed yield and leaf:stem ratio were only recorded at the Dharwad site. Anthracnose severity was assessed from ten randomly selected plants in each replicate during June to December each year to coincide with the rainy season. A 0–9 severity rating score (Chakraborty 1990) was used, where 0 = no visible disease symptom and 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–94%, and 9 = 95–100% tissue necrotic.

Table 11.1 List of accessions and bred lines of *Stylosanthes* species used in primary and advanced evaluations at regional sites in India.

Species	Cultivar/ Accession number	Collector/ Breeder	Collection site	Preliminary evaluation	Advanced evaluation
S. guianensis	cv. Oxley			•	
S. guianensis	cv. Cook			•	
S. hamata	CPI 61670	R. Smith	Venezuela	•	•
S. scabra	CPI 93116 (CIAT 1053)	V.M. Patino	Brazil		•
S. scabra	Q10042	J.P. Ebersohn	Monteiro	•	•
S. scabra	CPI 40205 (Fitzroy)	R.J. Williams	Cuy des Almas, Brazil	•	•
S. scabra	CPI 40292 (Seca)	R.J. Williams	70 km W Recife, Brazil	•	•
S. scabra	CPI 36260 (IRI 1593)		Brazil	•	•
S. scabra	RRR 94-96	D.F. Cameron	Bred lines		•
S. scabra	RRR 94-96-1-8	D.F. Cameron	Bred lines		•
S. scabra	RRR 94-56	D.F. Cameron	Bred lines		•
S. scabra	RRR 94-97	D.F. Cameron	Bred lines		•
S. seabrana	EC 408401				•
S. seabrana	EC 408403			•	
S. seabrana	EC 408404			•	
S. seabrana	EC 408405			•	•
S. seabrana	EC 408406			•	•
S. seabrana	CPI 2523	L.A. Edye	Brazil	•	•
S. seabrana	CPI 2534	L.A. Edye	Brazil	•	•
S. seabrana	CPI 2539	L.A. Edye	Brazil	•	•
S. seabrana	CPI 110372 (CIAT 10517)	CENARGEN	6 km W Juayeiro, Brazil	•	•
S. seabrana	CPI 110370C (CIAT 10119)	R. Schultze-Kraft	7 km N Palmeiras, Colombia	•	•
S. seabrana	CPI 104710 (CIAT 10026)	R. Schultze-Kraft	75 km W Seabra, Brazil	•	•
S. seabrana	CPI 105546B	N. Sousa Costa	5 km E Cactite, Brazil	•	
S. viscosa	CPI 33941				•
Total				**18**	**20**

Table 11.2 Location and characteristics of the master site at Dharwad and the eight regional sites used in preliminary and/or advanced evaluation of *Stylosanthes* germplasm.

Site	Latitude, longitude & elevation	Annual average rainfall (mm)	Soil type	pH
Anand, Gujarat	22°35'N, 72°55'E, 49 m	850	Sandy loam	7.9
Coimbatore, Tamil Nadu	11°N, 76°58'E, 426 m	690	Clay loam	8.2
Dharwad, Karnataka	15°28'N, 75°E, 700 m	1200	Black	6.0
Hyderabad, Andhra Pradesh	17°22'N, 78°28'E, 489 m	750	Sandy loam	8.2
Kalyani, West Bengal	23°5'N, 88°E, 10 m	1500	Sandy loam	6.6
Palampur, Himachal Pradesh	32°7'N, 76°31'E, 1472 m	900	Alluvial clay	6.0
Rahuri, Maharashtra	19° 47'N 74° 18'E	550	Black	8.0
Uruli Kanchan, Maharashtra	18° 5'N 73° 8'E	400	Black	7.5
Trivandrum, Kerala	8° 28'N 76° 55'E, sea level	2000	Red lateritic	4.5

Advanced evaluation

Eleven accessions that performed well in the preliminary evaluation and nine new accessions of *S. hamata, S. scabra* and *S. seabrana* were further tested during 2000–03 in advanced evaluations at the master site and four (Anand, Coimbatore, Rahuri and Trivandrum) of the regional sites used in the preliminary evaluation. Uniform plots of 4x3 m, each accommodating ten 4 m-long rows with 30 cm between rows, were established with four replications using a randomised block design at each site.

Data analysis

Data on green forage and dry matter yield were separately analysed for each site using an analysis of variance for a randomised complete block design using Genstat (Payne et al 1987). Data on anthracnose severity rating were analysed using a restricted maximum likelihood (REML) approach. For the advanced evaluation a combined stability analysis based on 12 genotypes which are common across all five locations was used to compare performance among sites. On the basis of Genotype*Environment interaction, separate dendrograms and biplots were developed for GFY, DMY and SEV.

Results and Discussion

Accessions of most species, including *S. seabrana,* established well at most sites when scarified seeds were sown, despite initial difficulties at some sites due to inadequate scarification and heavy rain affecting germination of seeds. Two cultivars of *S. seabrana* have been recently released in Australia; these require inoculation with specific *Bradyrhizobium* strains, whereas inoculation is not required in Brazil where the native strains occur widely. When nodulation, plant performance and nitrogen fixation were compared using a *Bradyrhizobium* strain selected in Australia, an Indian strain of the cowpea miscellany and naturally occurring strains in Indian soils, both glasshouse (at Dharwad) and field (at Rahuri) results consistently showed that *S. seabrana* can nodulate well with the native *Bradyrhizobium* strains present in Indian soils (Ramesh et al, this volume). Hence, seeds of *S. seabrana* were sown without artificial inoculation with any specific *Bradyrhizobium* strains.

Preliminary evaluation

Of the four species used in the preliminary evaluation, *S. seabrana* accessions had the best overall DMY, GFY and seed yield at most sites (data not shown). Many *S. seabrana* accessions produced nearly 5 t DMY/ha and over 350 kg seed/ha. Some *S. scabra* accessions and the *S. hamata* 61670 had moderate DMY, but both *S. guianensis* cultivars had poor DMY at most sites and this species was excluded from further evaluations at all sites except the wet coastal site at Trivandrum.

Among the sites, most accessions established well at the master site Dharwad as scarified seeds were sown. Accessions of *S. seabrana* were the best performers in terms of both DMY and seed yield. Plant growth was excellent for *S. seabrana* 105546 and good for most entries except *S. guianensis* at Anand. Over 50% flowering was recorded for several *S. seabrana* accessions including 105546, 408414, 408416, 408417 and 408418, indicating a potential for good seed yield. Two cuts of forage were taken during the year; at the first cut *S. scabra* cv. Seca had the highest GFY of 13 t/ha, followed closely by *S. seabrana* 408414 and 105546 B (>7 t/ha), but the DMY was highest (3 t/ha) for *S. seabrana* 408414. At Coimbatore the germination was erratic and *S. viscosa* and some accessions of *S. scabra* failed to emerge, with consequent sparse plant population per plot. At Hyderabad *S. seabrana* 408413 established well but some *S. scabra* accessions had poor establishment. *Stylosanthes seabrana* 408417 had the highest DMY followed by *S. scabra* cv. Fitzroy. At Rahuri *S. seabrana* 115995 had the highest GFY (9 t/ha) followed by *S. seabrana* 408414 and 104710. The crude protein content was also highest in *S. seabrana* 115995. At Urulikanchan establishment was 50% for *S. seabrana* 408416 and *S. scabra* Q10042 but <20% for the others, and Q10042 produced the highest DMY (1.4 t/ha). Establishment was poor to very poor at both Kalyani and Palampur and plants at Kalyani suffered from a phyllody-type symptom most likely caused by the legume little leaf phytoplasma. No data on DMY, GFY or SEV were available from these two sites.

Anthracnose was not recorded on most accessions at Anand during initial evaluation, while only low severity was recorded on some accessions at the other sites except Dharwad, where moderate to severe anthracnose was recorded on Fitzroy and Q10042.

Overall, the crude protein percentages of *S. seabrana* accessions were comparable to those of *S. hamata* and *S. scabra* at most sites, with obvious difference between sites. Data are shown for Anand and Coimbatore only (Figure 11.1). Given their high DMY, total crude protein availability from *S. seabrana* crops would be considerably higher than that of other species.

Advanced evaluation

Eleven accessions were selected based on the preliminary evaluation, and nine new accessions of *S. hamata, S. scabra* and *S. seabrana* were included in a further evaluation during 2000–03 at Dharwad, Anand, Coimbatore, Rahuri and Trivandrum. In addition, seven other accessions, mostly of *S. seabrana,* were also evaluated at the master site Dharwad. Overall, there was significant (P<0.001) difference in DMY and GFY for accessions at each site, and the effect of year and the accession*year interaction were significant (P<0.02) for all sites except for DMY at Dharwad (output of analysis not shown), the interaction largely indicating the variability in weather and other growing conditions during the three years. Trivandrum was the most productive site with an average DMY of 13.7 t/ha, followed by Rahuri (11.7 t/ha), Dharwad (7.8 t/ha), Anand (1.8 t/ha) and Coimbatore. One or more *S. seabrana* accessions were among the most productive entries at each site and *S. scabra* 36260 produced the least DMY at most sites.

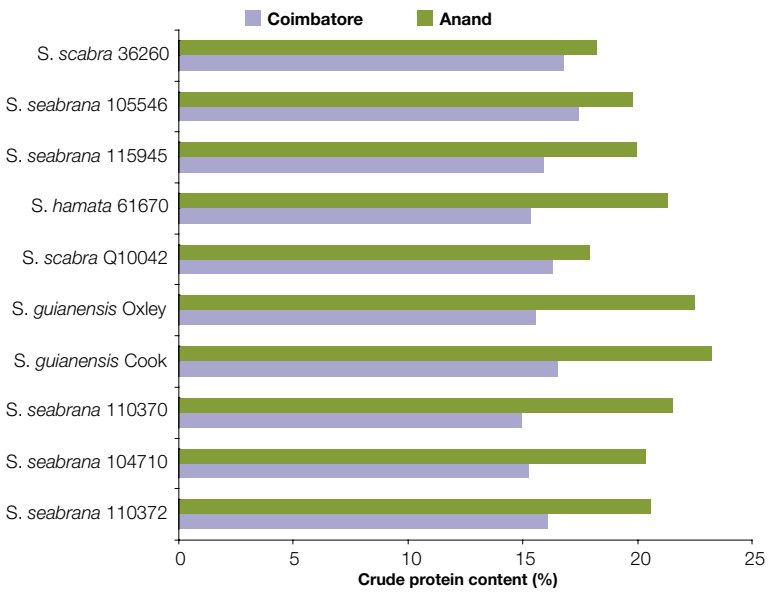


Figure 11.1 Crude protein content of *Stylosanthes* germplasm at the preliminary evaluation during 1998–99 at Anand and Coimbatore sites.

At the master site at Dharwad, six of the top ten performers in DMY were *S. seabrana,* and the top three accessions, ATF 3350, 408409 and 408406, yielded significantly higher than all others (Figure 11.2). The trend was similar for GFY. However, *S. seabrana* accessions were not among the highest seed producers and only one accession, 408406, was among the top ten, along with several *S. scabra* and *S. hamata* accessions (data not shown).

At Trivandrum, which had the highest overall DMY, only three of the top ten productive accessions were from *S. seabrana,* while several *S. scabra*, including Fitzroy, Q10042 and 93116, were among accessions with the highest DMY at this high rainfall site (data not shown). In contrast, of the top ten accessions for DMY, seven at Rahuri and six at Anand were *S. seabrana* (Figure 11.3). At both Anand and Rahuri *S. scabra* Fitzroy and Q10042 were also among the top ten. Overall, *S. seabrana* accessions were by far the best performers at Coimbatore (data not shown).

Anthracnose severity was low to very low at all sites and the overall mean severity for Trivandrum was 2.03, Anand 0.9, Rahuri 0.8, Coimbatore 0.45 and Dharwad 0.32 (data not shown). Despite the low severity ratings, the difference in anthracnose resistance between accessions was significant *(P*<0.001) at all sites except Anand. The overall mean SEV exceeded 2.00 only for *S. scabra* 36260, Fitzroy and Q10042 and *S. seabrana* 104710, and at least four accessions did not develop any disease at any location. A lack of severe damage to the most susceptible entries such as Fitzroy indicates that the range of pathogenic variants in India may be relatively small, and existing strains may not be highly aggressive. This is supported by recent analysis of pathogen races in

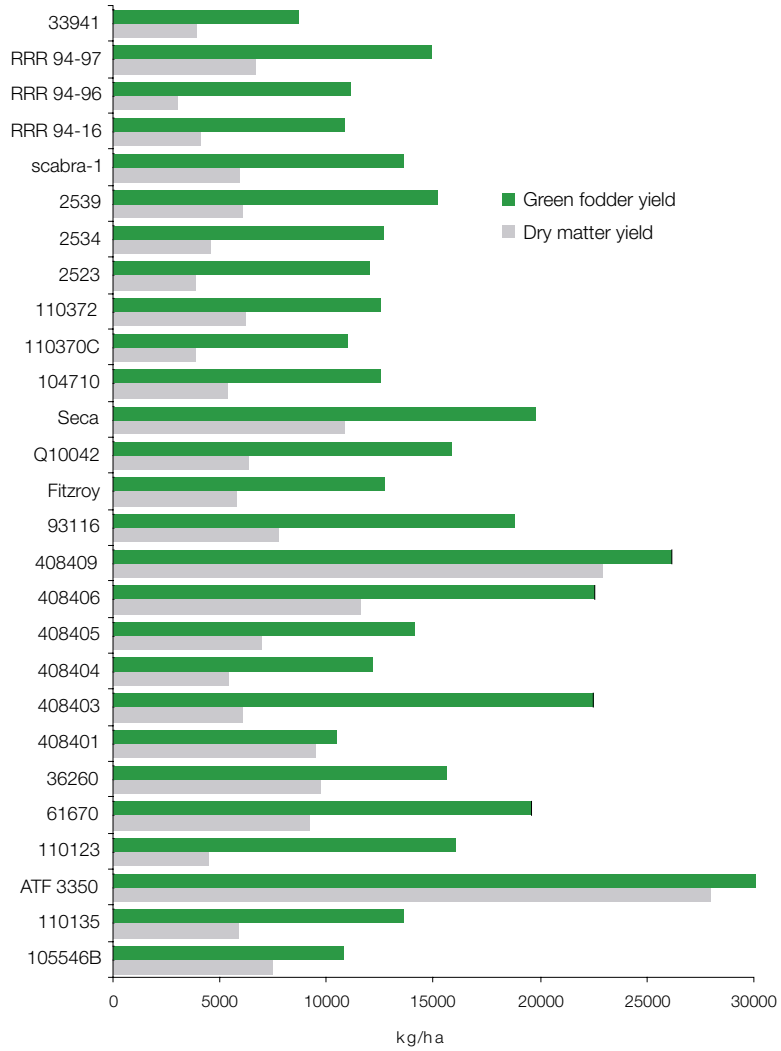


Figure 11.2 Dry matter and green fodder yield of ***Stylosanthes scabra*** **(36260, 93116, Fitzroy, Q10042, RRR 94-16, RRR 94-96, RRR 94-97, scabra-1, Seca)*, S. seabrana* (2523, 2534, 2539, 104710, 105546B, 110370C, 110372, 408401, 408403, 408404, 408405, 408406, 408409, ATF 3350), *S. hamata* (61670, 110123, 110135) and *S. viscosa* (33941) germplasm at the master site Dharwad.

India that shows eight race clusters, of which six are weakly pathogenic on many accessions and cultivars (Chakraborty et al, this volume). The fact that Fitzroy is still the dominant cultivar in use for commercial seed production (Rao et al, this volume) also indicates a weakly aggressive pathogen population. Despite the overall low SEV, the damage to *S. seabrana* 104710 is a cause for concern. In Australia *S. seabrana* cultivars Primar and Unica have become susceptible only two years after their commercial release (Trevorrow et al 1998). This species is a new introduction to India, having only been used in these regional evaluations since 1998, and a similar rapid adaptation by the pathogen population towards this species is evident from these studies.

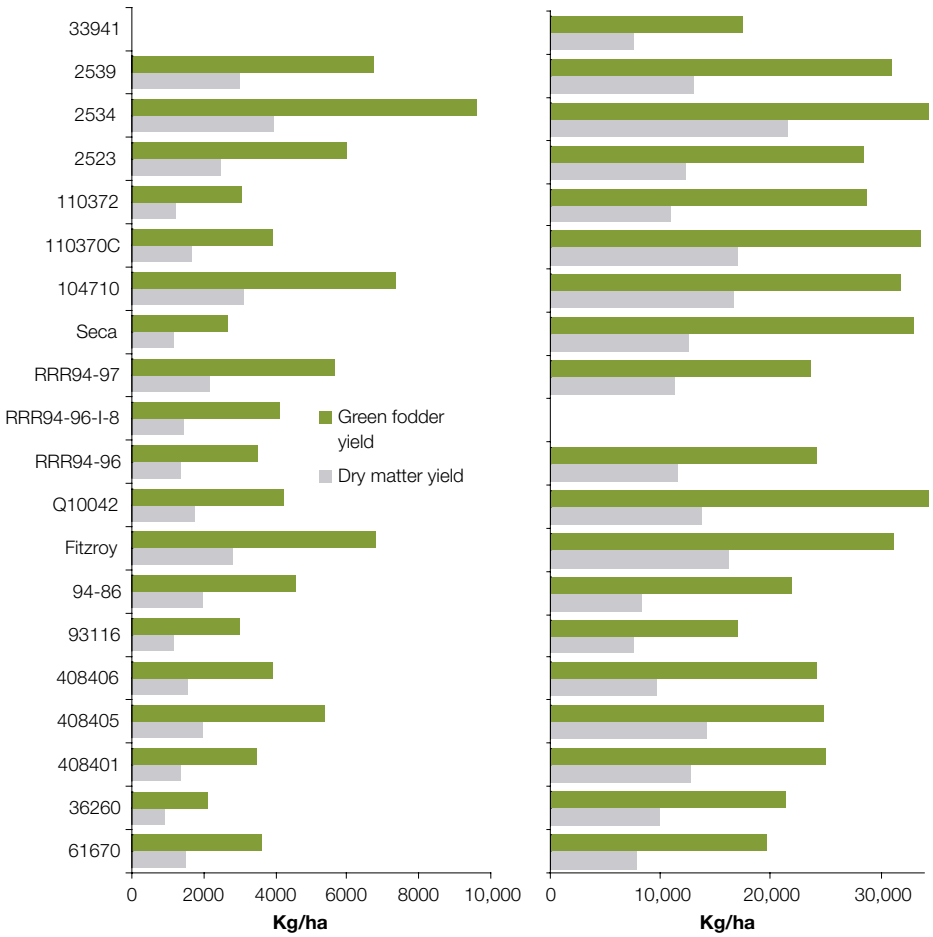


Figure 11.3 Dry matter and green fodder yield of *Stylosanthes scabra* (36260, 93116, Fitzroy, Q10042, RRR 96-1-8, 94-86, RRR 94-96, RRR 94-97, Seca), *S. seabrana* (2523, 2534, 2539, 104710, 110370C, 110372, 408401, 408405, 408406), *S. hamata* (61670) and *S. viscosa* (33941) germplasm at Anand (left) and Rahuri (right).

Consistency in performance for DMY, GFY and SEV among the five sites was analysed using data on 12 accessions that were common to all sites: *S. hamata* 61670; *S. scabra* 36260, 93116, Fitzroy, Q10042, Seca; and *S. seabrana* 2523, 2534, 2539, 104710, 408405 and 408406. Of these, with 56.8% coefficient of variation for DMY, *S. hamata* 61670 was the most consistent across all sites, whereas *S. scabra* 36260 and 93116 and *S. seabrana* 2523 all had coefficients of variation >90% (Figure 11.4). The low overall DMY of *S. scabra* 36260 is also evident from its lowest coefficient of determination of only 8.7%.

Among the sites, Anand and Rahuri offered similar environments for DMY of accessions, while Dharwad and Trivandrum represented very different environments (Figure 11.5). This indicates that either Anand or Rahuri could be used in future studies without losing vital information, and resources saved could be directed towards a new regional site with very different environmental conditions for a more comprehensive evaluation across a broad range of environments. Although some accessions could be grouped together according to their DMY, these were not from the same species, and accessions within a species were as diverse as those from other species (Figure 11.5).

This study has increased the range of available germplasm for India and has demonstrated the superiority of many accessions in DMY, GFY and SEV at one or more of the regional sites used. Of particular significance are *S. seabrana* accessions that have performed better than the existing cultivars of *S. scabra* and *S. hamata*. The stage is now set for the release of one or more new *S. seabrana* cultivars using these and/or additional data on regional evaluation. Given the long and involved process of cultivar release at the national level, it may be better to proceed with releases at a state level. Initially, one to two *S. seabrana* cultivars are expected for Maharashtra, where the superior performance of some *S. seabrana* accessions has been confirmed from three other sites within the state.

Apart from the role of *Stylosanthes* as a cut-and-carry fodder for domestic and farm animals, stylo is increasingly utilised in a number of other production systems. These include revegetation of wasteland, use of intercropping and legume leys for soil fertility enrichment, conservation of degraded soils and weed management in plantation horticulture (Phaikaew et al, this volume). Recent research has demonstrated a potential role for dried leaf meal in commercial poultry feed (Guodao et al, this volume), and leaf meal made from *S. guianensis, S. scabra* or

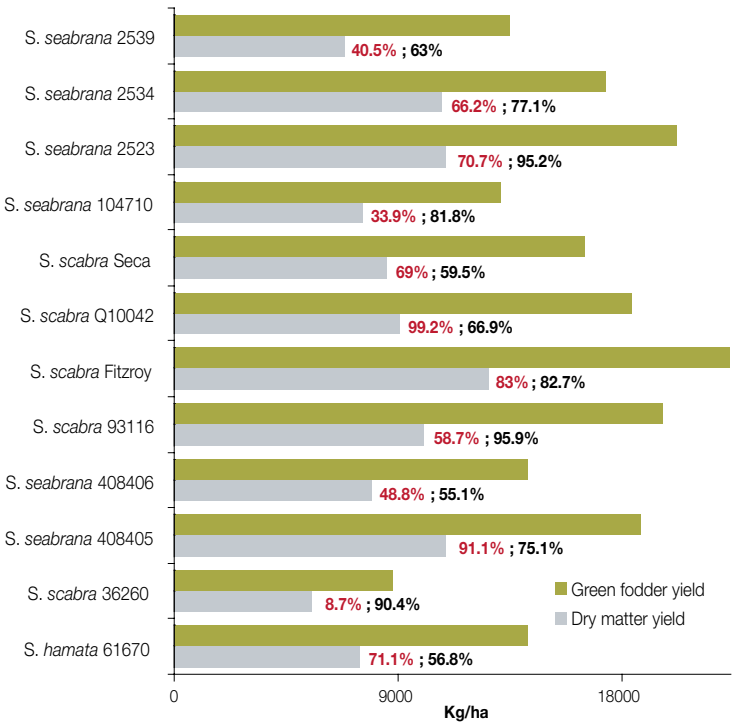


Figure 11.4 Stability of dry matter yield of selected accessions grown at five sites determined from the coefficient of determination (red numerals) and the coefficient of variation (black numerals).

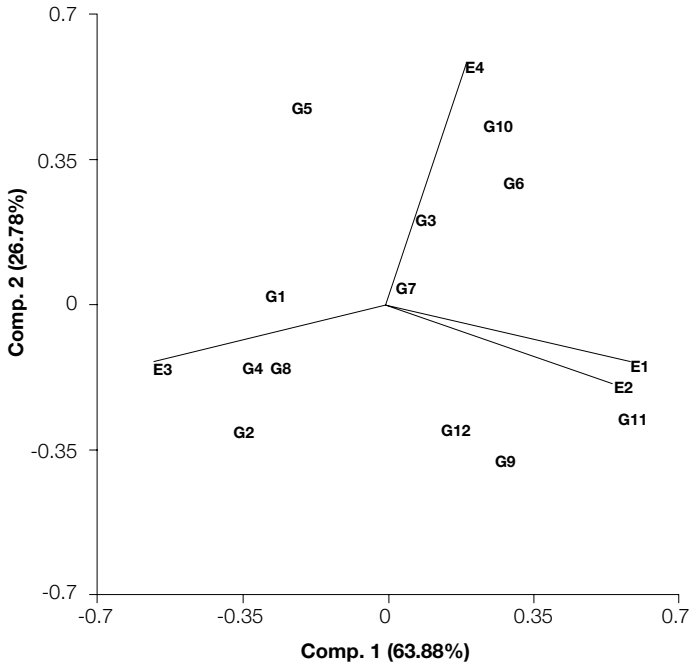


Figure 11.5 Biplot of 12 *Stylosanthes* accessions: G1, *S. hamata* 61670; G2, *S. scabra* 36260; G3, *S. seabrana* 408405; G4, *S. seabrana* 408406; G5, *S. scabra* 93116; G6, *S. scabra* Fitzroy; G7, *S. scabra* Q10042; G8, *S. scabra* Seca; G9, *S. seabrana* 104710; G10, *S. seabrana* 2523; G11, *S. seabrana* 2534; G12, *S. seabrana* 2539 evaluated at 4 field sites: E1, Anand; E2, Rahuri; E3, Dharwad; and E4, Trivandrum.

S. seabrana are equally useful in achieving liveweight gain among broilers. *Stylosanthes* leaf meal is already a success in southern China where it is used to feed poultry, pigs, fish, cattle and ducks among others. Given their high DMY, *S. seabrana* cultivars would be an ideal choice to start large-scale production facilities supplying leaf meal to poultry feed manufacturers.

Acknowledgments

We are grateful to Drs C.R. Hazra, P.S. Pathak, Bhagmal, R.P. Singh, P.S. Tomer, Amresh Chandra, B. Gangaiah, A.N. Mukherjee, Hassan, V.B. Aher, D.Y. Khandale and many others for their input and assistance with this work. We are grateful to Mr Dharmendra Kar of ICRISAT for the statistical analysis of the data. We gratefully acknowledge Dr Kuhu Chatterjee, Ms Natalie Daldar, Mary Lupul and Vicki Poole of the Australian Centre for International Agricultural Research (ACIAR), New Delhi office, for providing assistance and advice, and ACIAR and the Australian Department of Industry, Science and Technology for funding.

References

Chakraborty, S. 1990. Expression of quantitative resistance to *Colletotrichum gloeosporioides* in *Stylosanthes scabra* at different inoculum concentrations and day-night temperatures. Australian Journal of Agricultural Research 41, 89–100.

Gupta, J.J., Yadav, B.P.S. & Gupta, H.K. 1992. *Stylosanthes* protein in broiler rations. Indian Journal of Poultry Science 27, 87–90.

Hooker, J.D. 1872–1897. The Flora of British India. Vol 2. L. Reeve and Co. Ltd.: Kent, England.

Jayan, P.K. 1995. Fodder resources and their development in Peninsular India. Indian Grassland and Fodder Research Institute, Regional Research Station: Dharwad, Karnataka.

Payne, R.W., Lane, P.W., Ainsley, A.E., Bicknell, K.E., Digby, P.G.N., Harding, S.A., Leech, P.K., Simpson, H.R., Todd, A.D., Verrier, P.J., White, R.P., Gower, J.C. & Wilson, G.T. 1987. Genstat 5 reference manual. Clarendon Press: Oxford, 749 pp.

Ramesh, C.R., Bhag Mal, Hazra, C.R., Sukanya, D.H., Rammamurthy, V. & Chakraborty, S. 1997. Status of *Stylosanthes* development in other countries: III. *Stylosanthes* development and utilisation in India. Tropical Grasslands 31, 467–475.

Trevorrow, R.M., Chakraborty, S. & Jansen, P.I. 1998. Anthracnose resistance in *Stylosanthes seabrana.* In Michalk, D.L. & Pratley, J.E. 'Agronomy – Growing Greener Future: Proceedings of the 9th Australian Society of Agronomy Conference', pp. 211–212. Wagga Wagga, NSW, 20–23 July 1998.

Whyte, R.O. 1957. The grassland and fodder resources in India. Indian Council of Agricultural Research: New Delhi, India, 312 pp.

Chapter 12

Selecting high-yielding anthracnose-resistant *Stylosanthes* in Hainan

Bai Changjun[1], Liu Guodao[1] and Wang Dongjin[1]

Summary

Stylosanthes accessions were evaluated to select for high yield and anthracnose resistance in three separate experiments using the Grey Systematic Analysis system during 1996–2001 at the Chinese Academy of Tropical Agricultural Sciences farm in southern China. In experiment I, 34 accessions were tested in rows and mini-plots; in experiment II, a selection of 12 from the 34 accessions and 2 cultivars were compared in multi-row 10 m^2 plots; and in experiment III, 16 other accessions were evaluated in 10 m^2 plots. The preliminary results show that *S. guianensis* Mineirão and GC 1581 have the highest forage dry matter yield (DMY) of 11.4 t/ha/year and 11.6 t/ha/year, respectively, among the 34 accessions, and that these exceeded the DMY of *S. guianensis* Reyan 5 by 16.2% and 18.5%, respectively. The superior performance of these two promising germplasm was due to their high winter survival, and DMY during the dry cool season accounted for over 29% of their total DMY. Both have high anthracnose resistance and drought and cold tolerance, and the survival rate between seasons was over 30% for Mineirão and nearly 42% for GC 1581, with stem:leaf ratio of 1.74 and 1.67, respectively. These results show that both Mineirão and GC 1581 have higher DMY, anthracnose resistance, drought and cold tolerance, and fertility than those of the recently released Chinese cultivars *S. guianensis* Reyan 2 and Reyan 5. Among the other 16 germplasm tested in experiment III, GC 1480 and GC 1517 had superior DMY, stem: leaf ratio and winter survival than most of the current Chinese cultivars, but the level of anthracnose was similar or only slightly better. A number of promising new materials were detected among the germplasm evaluated in experiment III, and multi-site evaluations over two to three years are necessary to fully test their potential.

[1] Tropical Pasture Research Center, CATAS, Danzhou 571737, Hainan, PR China

Introduction

The value of *Stylosanthes* species (stylo) for improving tropical grassland was first recorded for *S. humilis* in Australia in 1914 and for *S. guianensis* in Brazil in 1933. Introductions of *Stylosanthes* species to South-East Asia started in the 1940s with the main species introduced from Australia (Edye 1997). Introduction of *Stylosanthes* species to China commenced with *S. humilis* in 1962 for use as a green manure crop under rubber plantations (Guodao et al 1997). A wide range of introductions from Australia and Colombia were evaluated in the early 1980s, included *S. hamata*, *S. guianensis* Cook, *S. guianensis* Graham, *S. scabra* Seca, *S. guianensis* CIAT 184 and *S. guianensis* CIAT 136, among others (Guodao et al 1997; Hwang et al 1986; Michalk et al 1993). From a total of 45 species and accessions, *S. guianensis* CIAT 184 was selected for release as Reyan No. 2 in southern China in 1992 for its high adaptability to environmental conditions, including acid soils and arid conditions, good anthracnose resistance and high dry matter yield (DMY), approximately 36% more than that of *S. guianensis* Graham. At the same time *S. hamata* Verano and *S. scabra* Seca were also released for use in arid and semi-arid areas. Also released was *S. guianensis* Reyan No. 5, originally selected as a single plant from CIAT 184 in 1987 with early flowering, higher seed yield and nearly the same DMY as CIAT 184. Reyan No. 5 has become an important cultivar in tropical and subtropical regions in southern China (Guodao et al 2001).

Stylosanthes cultivars are now grown on more than 150,000 ha in southern China. They are a very important feed source for livestock, and as a forage legume they are used to improve natural pasture or as a component of sown pastures. They are often used as a cover crop by intercropping under fruit trees such as mango, lychee and longan, and in rubber plantations in Hainan (Guodao et al 1997). More recently, they

have been used on sloping lands and as roadside plantings on highways to control water and soil erosion. One major utilisation scheme has been the production of *Stylosanthes* leaf meal and hay for animal production.

In common with most countries where *Stylosanthes* is commercially grown, anthracnose caused by the fungus *Colletotrichum gloeosporioides* has been the single most important determinant driving germplasm evaluation and cultivar development in China (Chakraborty, this volume). While still showing reasonable levels of field resistance, Reyan 2 and Reyan 5 are known to be susceptible to some strains of the anthracnose pathogen (Yi Kexian, pers. comm.). There is a danger that highly virulent strains of the pathogen may evolve in the future to severely damage cultivars that currently have an acceptable level of resistance. Selecting new materials with high anthracnose resistance and DMY is therefore a critical research objective. To increase the range of materials available to China, and to select well-adapted, productive and anthracnose-resistant materials, improved *Stylosanthes* germplasm and bred lines from Australia and the Centro Internacional Agriculture Tropical (CIAT) were evaluated in a collaborative project funded by the Australian Centre for International Agricultural Research (ACIAR) during 1996–2003. This paper describes their evaluation and selection.

Materials and Methods

Location, soil and climate

Starting in August 1996, the new *Stylosanthes* germplasm were evaluated at the Tropical Pasture Research Center of the Chinese Academy of Tropical Agricultural Sciences (CATAS). The site (19°30'N, 109°30'E, elevation 149 m) consists of a moderately acidic (pH 5.1) lateritic soil with 0.08% total N, 48.8 mg/kg available K and low available P (20 mg/kg). The climate is classified as 'season transitional tropical' with predominance of summer rainfall (over 82% of the 2136 mm annual total) but with a significant spring–winter component (Figure 12.1). Summer storms are particularly intense from May to October, and may produce as much as 350 mm rainfall in a 24-hour period. The mean temperature during the period of the experiment was 23.9°C. In the coldest month, December, 1.8°C was the lowest temperature recorded during the experiment and temperatures below 5°C occurred for about five consecutive days. The dry season occurs during the cooler months, extending from November to April, and the highest maximum temperature recorded was 40°C in April 2001.

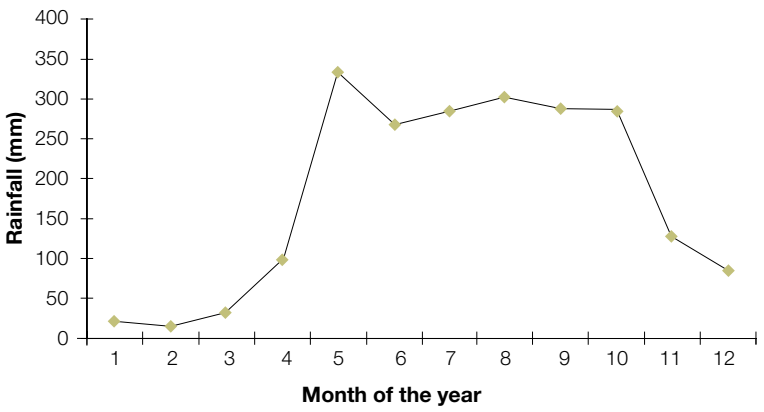


Figure 12.1 Mean monthly rainfall during the study period at the evaluation site near the Chinese Academy of Tropical Agricultural Sciences in Hainan, PR China.

Field plots and germplasm

Accessions and cultivars of *S. guianensis, S. scabra, S. capitata, S. macrocephala* and *S. hamata* were introduced from collections held at CSIRO in Australia, EMBRAPA in Brazil and CIAT in Colombia and the Philippines. Selected germplasm was evaluated in three separate experiments: in experiment I, a preliminary evaluation was made of 34 accessions and cultivars of *Stylosanthes* in rows or mini-plots (5.0 m long and 1.5 m apart) with three replications in 1996–97 (Table 12.1); in experiment II, 12 accessions that performed well in experiment I and two existing cultivars were further evaluated using an expanded range of characteristics including anthracnose resistance, agronomic performance and plant phonological attributes in 10 m2 plots with four replications during 1998–2000 (Tables 12.2, 12.3, 12.4); in experiment III, 16 other accessions were evaluated in 10 m2 plots with four replications over three years spanning October 1998 to December 2001, and included the Brazilian accession GC 1579 and the two CIAT 184 selections, Reyan 2 and Reyan 5 released in China as new cultivars (Table 12.5). Reyan 2 and Reyan 5 were common to all three experiments and GC 1579 was included in experiment I. Seeds of some accessions used in experiment III were obtained from both EMBRAPA and CIAT through its regional office at the International Rice Research Institute (IRRI) and these were treated as separate entries.

The single row plots used in experiment I to evaluate 34 accessions of *Stylosanthes* species at the Chinese Academy of Tropical Agricultural Sciences in Hainan, PR China.

Table 12.1 Yield performance and a systemic analysis of the 34 accessions of *Stylosanthes* species used in a preliminary evaluation in experiment I.

No.	Accession/cultivar	Fresh wt (kg/plot)	Height (cm)	Mean rating	Mean vigour	Xa	Xb	Xc	Systemic value	Rank
1	*S. guianensis* FM9405P5	3.4 hij	65.5	1.3 bc	1.90 ijklm	0.068	0.939	0.396	0.468	25
2	*S. guianensis* CIAT 2312	6.6 ghij	22.0	3.9 g	1.77 jklm	0.133	0.622	0.369	0.375	31
3	*S. guianensis* GC 1581	47 a	108.0	2.1 f	4.80 a	0.946	0.841	1.000	0.929	3
4	*S. guianensis* FM9405P6	9.5 fghij	56.0	1.4 bc	2.00 hijklm	0.191	0.939	0.417	0.516	22
5	*S. guianensis* FM9405P3	14.6 defghij	68.0	1.4 bc	3.33 cdefg	0.294	0.927	0.694	0.638	12
6	*S. guianensis* CIAT 184	19.7 cdefg	68.7	1 ab	3.33 cdefg	0.396	0.976	0.694	0.689	8
7	*S. guianensis* cv. Mineirão	32.3 bc	63.0	0.8 a	4.23 abcde	0.651	1.000	0.881	0.844	4
8	*S. guianensis* GC 1578	26 bcde	62.3	1.1 ab	3.57 bcdef	0.523	0.963	0.744	0.743	7
9	*S. guianensis* GC 1579	7.2 fghij	66.0	4.2 h	1.67 klm	0.145	0.585	0.348	0.359	32
10	*S. capitata/macrocephala* GC 1580	0 j	49.0	1 ab	1.00 m	0	0.976	0.208	0.395	28
11	*S. guianensis* CIAT 11844	11.7 fghij	67.7	1.2 b	2.90 fghijk	0.235	0.951	0.604	0.597	16
12	*S. guianensis* CIAT 11833	10.3 fghij	64.7	1.3 bc	2.00 hijklm	0.207	0.939	0.417	0.521	21
13	*S. capitata* Multiline 5	0.98 hij	44.7	1.8 d	1.43 lm	0.020	0.878	0.298	0.399	27
14	*S. guianensis* FM05-1	13.4 defghij	66.7	1.3 bc	3.00 efghij	0.270	0.939	0.625	0.611	14
15	*S. guianensis* FM05-2	0.53 ij	67.0	1.3 bc	1.00 m	0.011	0.939	0.208	0.386	30
16	*S. guianensis* FM05-3	15.3 defgh	65.0	1.3 bc	2.90 fghijk	0.308	0.939	0.604	0.617	13
17	*S. guianensis* CIAT 10417	0.73 hij	40.0	1 ab	1.57 lm	0.015	0.976	0.327	0.439	26
18	*S. guianensis* FM03-2	12.1 efghij	68.3	1.3 bc	2.87 fghijk	0.264	0.939	0.598	0.600	15
19	*S. guianensis* CPI 55848	5.33 ghij	29.3	1.2 b	1.77 jklm	0.107	0.951	0.369	0.476	24
20	*S. scabra* cv. Siran (L3-93)	9.9 fghij	88.0	1.2 b	2.57 fghijkl	0.199	0.951	0.535	0.562	19
21	*S. guianensis* CPI 58719	3.9 hij	33.3	0.9 a	2.20 fghijklm	0.078	0.988	0.458	0.508	23
22	*S. guianensis* CPI 87830	10.7 fghij	35.0	4.5 i	1.90 ijklm	0.215	0.549	0.396	0.387	29
23	*S. guianensis* cv. Graham (L7-84)	10.8 fghij	56.7	1.2 b	2.53 fghijkl	0.217	0.951	0.527	0.565	18
24	*S. guianensis* CPI 67652	15 defghi	59.3	2.4 f	3.20 defgh	0.302	0.805	0.667	0.591	17
25	*S. guianensis* cv. Cook (L1-82)	4.8 hij	39.3	7.8 j	1.43 lm	0.097	0.146	0.298	0.180	34
26	*S. guianensis* CIAT 136	37 ab	80.3	2 ef	4.33 abcd	0.744	0.854	0.902	0.833	5
27	*S. scabra* cv. Seca	18.5 cdefg	95.0	1 ab	3.13 defghi	0.372	0.976	0.652	0.667	11
28	*S. guianensis* cv. Graham	21.7 cdef	61.3	1.5 c	3.43 cdefg	0.437	0.915	0.715	0.689	9
29	*S. guianensis* cv. Reyan 5	49.7 a	77.3	1.9 ef	4.47 abc	1.000	0.866	0.931	0.932	2
30	*S. guianensis* cv. Cook	3.6 hij	50.5	4.7 l	1.67 klm	0.072	0.524	0.348	0.315	33
31	*S. guianensis* cv. Reyan 2	49.3 a	90.7	2.1 f	4.77 ab	0.992	0.841	0.994	0.942	1
32	*S. hamata* cv. Verano	4.7 hij	32.0	1 ab	2.67 fghijkl	0.095	0.976	0.556	0.542	20
33	*S. guianensis* L8	26.3 bcd	59.7	1.2 b	3.77 abcde	0.53	0.951	0.785	0.755	6

Xa, Xb and Xc are weighted means of dry matter yield, anthracnose severity rating and plant vigour, respectively, used in calculating the systemic value. Means followed by different letters within a column do not differ significantly ($P<0.05$) according to Duncan's multiple range test.

Table 12.2 Anthracnose severity rating, winter survival, stem:leaf ratio, seed yield, 1000 seed weight and dry matter yield (DMY) of 12 selected accessions and the two Chinese cultivars Reyan 2 and Reyan 5 of *Stylosanthes guianensis* used in an advanced evaluation in experiment II.

Accession/cultivar	Disease rating	Winter survival	Stem:leaf ratio	Date of flowering	Seed yield (g/plot)	1000 seed weight (g)	DMY (t/ha)
S. guianensis GC 1581	1.74 (3) ab	41.68 ab	1.03 bc	29 Nov	6.0	2.5	11.4 bc
S. guianensis FM9405P6	2.09(5) abc	17.50 d	0.96 bc	9 Dec	14.0	3.9	6.5 fg
S. guianensis FM9405P3	2.71 (7) e	16.68 d	0.79 c	7 Oct	160.6	2.8	6.9 fg
S. guianensis CIAT 184	2.16 (7) bcd	36.68 b	0.99 bc	28 Oct	125.4	2.2	9.5 d
S. guianensis cv. Mineirão	1.67 (4) a	30.83 bcd	1.07 bc	9 Dec	5.25	2.7	11.6 ab
S. guianensis GC 1578	2.75 (9) e	0.83 e	1.07 bc	7 Oct	164.2	2.0	7.1 fg
S. guianensis FM05-1	3.82 (9) g	0 e	1.05 bc	28 Oct	95.0	2.0	5.5 g
S. guianensis FM05-3	3.25 (9) f	0 e	1.11 bc	28 Oct	148.9	2.2	7.5 ef
S. guianensis FM03-2	2.41 (6) cde	55.83 a	1.07 bc	7 Oct	138.5	2.2	9.2 de
S. guianensis CIAT 136	2.31 (6) cde	36.68 b	1.42 ab	9 Dec	80.9	2.0	9.1 de
S. guianensis cv. Reyan 5	2.56 (6) de	19.18 cd	1.36 abc	28 Oct	152.5	2.0	9.8 cd
S. guianensis cv. Reyan 2	2.06 (5) abc	40.83 ab	1.73 a	28 Oct	126.4	2.2	13.2 a
S. guianensis L8	2.01 (5) abc	27.50 bcd	1.20 bc	28 Oct	83.8	2.4	10.8 bcd
S. guianensis E3	2.32 (5) cde	33.33 bc	0.98 bc	7 Oct	101.0	2.0	7.5 ef

Table 12.3 Dry matter percentage (DM%), crude protein (CP), crude fat (CE), crude fibre (CF), ash, non-N extract (NFE), Ca, P, Mg and K content of 12 selected accessions and the two Chinese cultivars Reyan 2 and Reyan 5 of *Stylosanthes guianensis* used in an advanced evaluation in experiment II.

Accession/cultivar	DM%	CP	CE	CF	Ash	NFE	Ca	P	Mg	K
S. guianensis GC 1581	33.66	15.36	2.85	32.23	5.45	44.11	1.21	0.35	0.35	1.02
S. guianensis FM9405P6	36.72	14.46	1.92	36.49	5.26	41.87	1.55	0.22	0.14	0.95
S. guianensis FM9405P3	35.41	12.52	1.74	28.15	8.28	49.31	1.89	0.29	0.19	0.78
S. guianensis CIAT 184	29.64	13.00	2.23	30.65	8.91	45.21	1.65	0.29	0.26	1.59
S. guianensis cv. Mineirão	33.94	11.55	3.20	37.36	8.58	39.31	1.29	0.21	0.19	1.07
S. guianensis GC 1578	36.93	10.86	1.77	36.71	6.89	43.77	1.71	0.15	0.28	0.57
S. guianensis FM05-1	36.08	12.50	2.12	41.30	7.07	37.01	1.45	0.19	0.35	0.87
S. guianensis FM05-3	30.59	11.24	2.40	30.75	8.02	47.59	1.93	0.26	0.12	1.21
S. guianensis FM03-2	31.92	14.23	2.22	30.06	7.51	45.98	1.73	0.22	0.10	1.15
S. guianensis CIAT 136	32.34	11.99	1.72	38.96	5.95	41.37	1.39	0.16	0.34	0.91
S. guianensis cv. Reyan 5	35.21	12.71	1.69	36.37	8.31	45.14	1.36	0.29	0.31	1.19
S. guianensis cv. Reyan 2	29.92	14.80	1.59	38.10	6.39	39.12	1.76	0.30	0.12	0.87
S. guianensis L8	29.89	15.12	1.76	34.01	8.03	41.08	1.58	0.29	0.13	1.56
S. guianensis E3	35.71	12.66	1.93	41.79	8.89	34.73	1.87	0.34	0.41	0.68

Table 12.4 The overall performance of 12 selected accessions and the two Chinese cultivars Reyan 2 and Reyan 5 of *Stylosanthes guianensis* used in an advanced evaluation in experiment II using a systemic analysis of biomass (X_1), dry matter yield during the dry season (X_2), anthracnose severity rating (X_3), plant survival (X_4), crude protein content (X_5), ratio of stems to leaves (X_6) and seed production (X_7).

Accession/cultivar	X1	X2	X3	X4	X5	X6	X7	Systemic value	Rank
S. guianensis GC 1581	0.256	0.239	0.198	0.088	0.200	0.064	0.021	1.066	3
S. guianensis FM9405P6	0.185	0.123	0.190	0.042	0.188	0.062	0.021	0.811	10
S. guianensis FM9405P3	0.180	0.104	0.177	0.042	0.167	0.057	0.048	0.755	13
S. guianensis CIAT 184	0.220	0.118	0.188	0.059	0.170	0.063	0.037	0.855	8
S. guianensis cv. Mineirão	0.262	0.250	0.200	0.053	0.157	0.165	0.020	1.107	1
S. guianensis GC 1578	0.184	0.100	0.177	0.034	0.152	0.165	0.050	0.862	7
S. guianensis FM05-1	0.169	0.096	0.159	0.033	0.167	0.065	0.031	0.720	14
S. guianensis FM05-3	0.191	0.109	0.168	0.033	0.155	0.067	0.044	0.767	12
S. guianensis FM03-2	0.215	0.120	0.183	0.100	0.185	0.065	0.040	0.908	6
S. guianensis CIAT 136	0.214	0.105	0.185	0.059	0.162	0.080	0.028	0.833	9
S. guianensis cv. Reyan 5	0.224	0.169	0.182	0.043	0.168	0.077	0.045	0.908	5
S. guianensis cv. Reyan 2	0.300	0.218	0.191	0.065	0.191	0.100	0.037	1.102	2
S. guianensis L8	0.242	0.137	0.191	0.050	0.196	0.070	0.029	0.915	4
S. guianensis E3	0.191	0.111	0.185	0.055	0.168	0.062	0.032	0.804	11

Table 12.5 Anthracnose severity rating, winter survival, stem:leaf ratio, date of flowering, seed yield, systemic value and ranking according to systemic value of 14 selected accessions and the two Chinese cultivars Reyan 2 and Reyan 5 of *Stylosanthes guianensis* used in an advanced evaluation in experiment III.

Accession /cultivar	Source of seed	DM (t/ha)	Disease rating	Winter survival	Stem: leaf ratio	Date of flowering	Seed yield (kg/ha)	Systemic value	Rank
FM07-2	IRRI	6.5 fg	2.6 (7) bcd	37.5 bc	1.14 ab	9 Apr	43.5	0.57 bc	12
FM07-3	IRRI	4.6 i	2.8 (8) d	17.5 cd	0.85 abcd	9 Apr	39	0.49 c	16
GC 1463	IRRI	9.4 abc	2.3 (6) abcd	50 b	0.89 abcd	28 Oct	268.5	0.70 ab	2
GC 1480	IRRI	11.4 a	2.1 (6) abc	77.5 a	0.69 bcd	18 Nov	223.5	0.82 a	1
GC 1517	EMBRAPA	9.3 bc	2.0 (5) ab	49.2 b	0.54 d	28 Oct	244.5	0.68 abc	5
GC 1517	IRRI	8.7 ab	2.0 (5) ab	32.5 bcd	0.82 abcd	18 Nov	360	0.68 abc	6
GC 1524	EMBRAPA	6.3 fg	2.4 (6) abcd	26.7 bcd	1.20 a	10 Jul	150	0.56 bc	13
GC 1524	IRRI	4.7 ghi	2.6 (8) bcd	18.3 cd	0.75 abcd	16 Sep	216	0.60 bc	9
GC 1528	IRRI	5.9 fgh	2.4 (7) abcd	22.5 cd	1.03 abc	28 Oct	262.5	0.58 bc	10
GC 1557	IRRI	4.5 i	2.7 (8) cd	22.5 cd	1.2 a	16 Sep	106.5	0.57 bc	11
GC 1576	IRRI	7.9 cd	2.2 (6) abcd	48.3 b	0.95 abcd	26 Aug	229.5	0.69 ab	4
GC 1576	EMBRAPA	6.9 de	2.3 (7) abcd	28.3 bcd	0.89 abcd	16 Sep	403.5	0.69 ab	3
GC 1579	EMBPAPA	8.7 ab	2.2 (6) abc	36.7 bc	0.59 cd	28 Oct	130.5	0.67 abc	7
GC 348	EMBRAPA	2.9 hi	4.5 (9) e	8.3 d	1.08 ab	28 Nov	49.5	0.52 bc	15
Reyan 2	CATAS	8.9 abc	1.9 (5) a	26.7 bcd	0.78 abcd	28 Oct	90	0.63 bc	8
Reyan 5	CATAS	6.6 ef	2.6 (7) bcd	10.8 d	0.75 abcd	10 Jul	145.5	0.54 bc	14

More than one source of seeds was available for some accessions and these have been treated as separate entries. Means followed by different letters within a column do not differ significantly ($P<0.05$) according to Duncan's multiple range test. Figure in parenthesis is the maximum disease severity rating.

Experiment I was laid out as a randomised complete block design consisting of 5 m-long single row mini-plots, 1.5 m apart, with 16 plants in each row. All observations were made on each replicate. The ten middle plants of every row were cut for fresh forage and dry matter yield (DMY). In experiment II, 5x2 m plots were established 2 m from one another using a randomised complete block design and 40 seedlings were transplanted in each plot. Anthracnose severity and survival rate were recorded on three replicates, which were cut at regular intervals to measure DMY from all plants in a plot, while observations on vigour, height and harvesting seed yield were recorded from the fourth replicate. For experiment III, the design, number of plants/plot and processing of replicates were similar to those in experiment II, except that plots were 1.5 m apart and all 40 plants in the three replicates were cut every three months for DMY.

Recording of observations

Anthracnose resistance was assessed using a 0–9 severity rating score (Chakraborty 1990) where 0 = no visible disease symptom and 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–94%, and 9 = 95–100% tissue necrotic. The number of surviving plants was counted before cutting and at the beginning of each season for survival and persistence, respectively. A 1–5 visual rating score, where 5 = excellent, 4 = very good, 3 = good, 2 = poor and 1 = very poor was used to assess vigour. Dried vegetative plant materials were used to estimate crude protein (CP), crude fat (CE), crude fibre (CF), ash, non-N extract (NFE), Ca, P, Mg and K using standard techniques (Hood & Jones 1996). Other assessments included date of flowering, seed yield and leaf:stem ratio for some of the experiments.

Statistical analysis

Data were analysed using an analysis of variance, and means were compared using standard algorithms for multiple means comparison in SAS version 6.12 (SAS Institute, Cary, North Carolina). A synthetic evaluation was adopted using the Grey Systematic Analysis (Qingci & Xilai 2003; Zhon et al 2002) which standardises scores for different characters such as biomass, disease rating and plant vigour scores; and calculates absolute maximum and minimum values for each character to compute systematic coefficients and a putative ranking by using the weighed means. The original data are standardised using xi/max xi, where xi = original value. The standardised values for the seven characters (k = 7) biomass (x1), DMY of dry season (x2), disease rating score (x3), survival rate (x4), crude protein (x5), ratio of stems to leaves (x6) and potential seed production (x7) are used to calculated the absolute (Δij (k)) maximum (Δmax) and minimum (Δmin) values of each character. The systematic coefficient is calculated as

$\xi ij(k) = (\Delta min+\rho\Delta max)/(\Delta ij(k) +\rho\Delta max)$ with $\rho = 0.05$,

and the systematic value as the weighed mean

$ri = W(k)\xi i(k)$.

Results

Experiment I

Biomass Three cuttings were taken during the 1996–97 season and the preliminary results (Table 12.1) show that there was significant difference in fresh weight among the 34 accessions or cultivars. Several accessions had significantly higher biomass, eg GC 1581, Mineirão, GC 1578 and L8, among others, with fresh weights of 47, 32.3, 26 and 26.3 kg/plot, respectively. The Chinese cultivars Reyan 2 and Reyan 5 and CIAT 136 also had high biomass.

Anthracnose resistance The first severity assessment was made five weeks after sowing and observations were continued at three-weekly intervals, giving a total of 22 observations during the period 1996–97. The preliminary results on mean severity show that, although all accessions were affected by anthracnose to a greater or lesser extent, there were significant differences in anthracnose resistance among the 34 accessions and cultivars (Table 12.1). Mineirão and CPI 58719 had the highest anthracnose resistance, with mean disease ratings of 0.8 and 0.9 respectively. CIAT 184, GC 1580, CIAT 10417, GC 1578 and E3 had moderate to high anthracnose resistance with disease rating scores around 1.0. With a rating of 2.1, these were followed by GC 1581 and Reyan 2. Others, including both selections of Cook (L1-82 and old), CPI 87830, GC 1579 and CIAT 2312, had little or no resistance, with rating scores >4.0, and developed very serious anthracnose. Cook (L1-82) was nearly destroyed by the disease.

Plant vigour During 1996–97 vigour was assessed three times before cutting, and the preliminary results showed that there were significant differences among accessions (Table 12.1), with GC 1581 showing the highest rating for vigour with a mean score of 4.8. There were no significant differences amongst Reyan 2, Reyan 5, CIAT 136 or Mineirão, but all were significantly higher than GC 1580, FM05-2, Cook and Multiline 5, which all showed generally poor vigour.

Synthetic evaluation The systematic coefficients and degree were calculated from weighed means of biomass, disease rating and plant vigour scores. The systematic coefficients were significant for accessions according to a t test ($P<0.0005$). The two recently released cultivars, Reyan 2 (0.942) and Reyan 5 (0.932) had the highest systematic coefficients of all materials tested, indicating their superiority in overall performance among all the materials evaluated; these were followed by GC 1581 (0.929) and Mineirão (0.844). Cultivar Cook had the lowest coefficient, as did some of the non-guianensis accessions. A high systematic coefficient was one of the important criteria used to select a subset of 12 accessions and cultivars for further detailed evaluation. Reyan 2 and Reyan 5 were included as controls.

Experiment II

DMY The first cut was taken three months after transplanting and this continued every three months, giving a total of 12 cuts during the experimental period. The mean DMY was significantly different for accessions ($P<0.0001$). Among the evaluated germplasm Mineirão and GC 1581 had the highest DMY with 11.6 and 11.4 t/ha/year, respectively. These yields were significantly higher (16–18%) than that of Reyan 5 but lower than the DMY of Reyan 2 (Table 12.2). L8 was among other accessions with high DMY, while FM05-1, FM9405P3, GC 1578 and FM9405P6 had low DMY. There was a close positive correlation between mean DMY and rainfall ($r = 0.57$) and a negative correlation with mean minimum temperature ($r = -0.47$).

Separate DMY for the dry season was determined as an estimate of drought tolerance of the accessions. This showed that the overall DMY in the dry and cold season was about 17.5% of the total DMY, and this was significantly ($P<0.0001$) different for the accessions. Mineirão and GC 1581 had the highest dry season DMY of 3.4 t/ha/year and 3.3 t/ha/year, respectively, which were significantly higher than that of Reyan 2 (3.1 t/ha/year). Interestingly, these two accessions continued to grow during the dry season and the DMY during this period contributed to over 29% of their total DMY (data not shown). Some accessions with high DMY had high leaf:stem ratio, making them potentially less palatable to animals (Table 12.2). These included Reyan 2, CIAT 136, Reyan 5 and L8, while FM9405P3, with a relatively low ratio, would potentially be more palatable.

Anthracnose resistance Starting four weeks after transplanting, visual assessments of anthracnose severity were made every three weeks, giving a total of 45 observations during the 1998–2000 period. Although there were significant differences in mean anthracnose score ($P<0.0001$), all accessions were affected by anthracnose to a greater or lesser extent. The preliminary results show that Mineirão and GC 1581 had the highest anthracnose resistance; their mean disease rating scores were 1.67 and 1.74 and their maximum disease rating scores were 4.0 and 3.0 respectively (Table 12.2). Accessions L8, Reyan 2, FM9405P6 and CIAT 184 had moderate anthracnose resistance and their mean disease rating scores, between 2.01 and 2.16, were not significantly different. FM05-1, FM05-3 and GC 1578 had the least resistance with mean ratings ranging from 2.75 to 3.82 and a maximum rating reaching the highest score of 9.0. These accessions were severely affected by anthracnose and many plants died during the three-year period. As expected, there was a significant negative correlation between anthracnose rating score and mean minimum temperature ($r = –0.56$).

Plant survival The survival of accessions declined at different rates during the experiment, and the rate differed significantly among accessions ($P<0.01$). FM03-2, GC 1581 and Reyan 2 persisted well with 55.8%, 41.6% and 40.8% of plants surviving, respectively, at the end of the experiment. In contrast, all plants of GC 1578, FM05-1 and FM05-3 were dead by the end of the three-year period. Two of these accessions, GC 1578 and FM05-3, were particularly poor, their survival declining rapidly. One reason for the rapid loss in survival was the extremely low minimum temperature of 1.8°C in December 1999 that continued for five days. This, combined with their high anthracnose susceptibility, killed off accessions FM05-1 and FM05-3.

Seed production potential As the commercial success of any cultivar depends on its high seed production ability, the seed production potential of the accessions was assessed from harvested plant tops that were dried to determine seed yield. FM9405 parcela 3 (FM9405P3) produced the highest seed yield, but GC 1578 and Mineirão did not produce much seed under conditions at the CATAS farm (Table 12.2). As expected, high seed yield was generally associated with early flowering. Accessions such as FM03-2 and E3 commenced flowering in early October, but Mineirão and CIAT 136 did not flower until early December.

Nutrient content Although crude protein (CP), crude fat (CE) and crude fibre (CF) content differed significantly between accessions, differences were generally small. GC 1581 had relatively high crude protein (15.36%) and Mineirão had high crude fat (3.20%), while FM05-1 and E3 had high CF (Table 12.3).

Synthetic evaluation The weighted means for DMY, DMY in dry season, disease rating, survival rate, crude protein, ratio of stems to leaves and seed production potential, and the ranking of accessions according to systematic coefficient, are given in Table 12.4. With high systematic coefficient, Mineirão (1.107), Reyan 2 (1.102) and GC 1581 (1.066) were the overall best performing accessions; L8, Reyan 5 and FM05-2 followed these; and FM05-1, FM9405P3, FM05-3 and E3 had low systematic coefficients.

Experiment III

DMY There was an overall significant ($P<0.0001$) difference between accessions in DMY assessed from a total of 17 cuts. With 11.37 t/ha/year, GC 1480 had the highest DMY of any accession and this was >26% and >72% more than the DMY of Reyan 2 and Reyan 5, respectively (Table 12.5). GC 1463 and GC 1517 (IRRI) were the other high-yielding accessions, and their yield was similar to that of Reyan 2 but significantly higher than that of Reyan 5. GC 1557, FM07-3 and GC 348 yielded poorly. Overall, the DMY for the dry season accounted for over 33% of the total DMY in these accessions. GC 1480 had the highest dry season DMY, followed by GC 1517 (EMBRAPA) and GC 1579. These DMY were similar to that of Reyan 2 but higher than that of Reyan 5.

The ratio of stems to leaves varied significantly among accessions ($P<0.02$); GC 1524 (IRRI) had the highest ratio, followed by GC 1557, while GC 1517 (EMBRAPA) had the lowest ratio of stems to leaves, which indicates a high quality of forage (Table 12.5). The ratio of stems to leaves changed significantly with time; the mean ratio was highest in August, average during December and lowest in June (data not shown).

Anthracnose resistance There were 69 disease assessments made on these accessions during the period 1998–2001 and all accessions developed anthracnose. Although there was significant difference in mean anthracnose rating between accessions ($P<0.0001$), most were moderate to highly resistant with severity ratings between 1.90 and 2.79, and only GC 348 was highly susceptible with a severity rating of 4.47 (Table 12.5). The maximum severity rating of GC 1517 from both EMBRAPA and IRRI, and Reyan 2 was 5, while that of some accessions with similar mean severity ratings ranged between 6 and 8. Maximum severity rating of the most susceptible GC 348 was 9. The weather dependence of anthracnose development was clearly evident from its significant fluctuation between months of the year; the rating was highest in September and lowest in July–August.

There was a significant negative correlation between anthracnose rating and rainfall ($r = –0.60$), possibly due to rain washing pathogen spores off the surface of stylo leaves and stems; and a significant negative correlation with mean temperature ($r = –0.91$) and monthly minimum temperature ($r = –0.43$) (data not shown).

Winter survival GC 1480 (IRRI) persisted best with more than 77% of the plants surviving. Nearly 50% of plants survived for GC 1463, GC 1517 (IRRI) and GC 1576 (IRRI), but only just over 8% of plants of GC 348 survived (Table 12.5). There was a negative correlation between winter survival and rainfall ($r = –0.275$), and a significant negative correlation with monthly minimum temperature ($r = –0.542$). Survival declined rapidly during winter and the dry season in 1999–2000, with extreme minimum temperature of 1.8°C in December 1999 that continued for five days. The high survival of GC 1480 (IRRI) may indicate its cold tolerance.

Nutrient content As before, accessions varied in their crude protein, crude fat and crude fibre content, but the differences were not large (data not shown). GC 1576 (EMBRAPA) had relatively high crude protein (16%), GC 1517 (IRRI) had high crude fibre (41.7%), and GC 1528 had high crude fat (2%).

Potential seed production GC 1576 (EMBRAPA) and GC 1517 (IRRI) had high potential seed production and FM07-3, GC 348 and FM07-2 were poor seed producers (Table 12.5).

Synthetic evaluation There was significant difference in systemic value among accessions ($P<0.01$); GC 1480, GC 1463, GC 1576 (EMBRAPA) and GC 1576 (IRRI) had the highest values, indicating their superior overall performance on the nine characteristics used. GC 348 and FM07-3 had the lowest values, indicating their poor performance.

Discussion

In this work we have evaluated a comprehensive range of *S. guianensis* germplasm from a number of sources in three major field experiments at a CATAS experiment station in southern China, and compared the performance of selected germplasm with two of the recently released Chinese cultivars of *S. guianensis*, Reyan 2 and Reyan 5. Many of the accessions are new to this region. Overall, a number of promising new materials were detected in the germplasm evaluated in the three trials.

Using the systemic value as a measure of overall performance in the two experiments where detailed measurements of their performance were taken, Mineirão, GC 1581, L8, Reyan 2 and Reyan 5 were the top five performers in experiment II. Of these, Mineirão, released in 1993, has largely failed to establish itself as a significant commercial pasture legume in Brazil, due mainly to poor seed production (Miles & Lascano 1997), and it did not produce adequate seeds under the conditions at CATAS (Table 12.2). Similarly, despite its strong winter survival, GC 1581 also flowered late, and poor seed production will limit its use to more targeted areas such as the northern latitudes of southern China. In experiment III Reyan 2 and Reyan 5 only ranked 8th and 14th among the 16 accessions tested. Whether most of the germplasm used in experiment III are truly superior to the two Chinese cultivars cannot be easily judged from these results, as the performance of the two cultivars differed in the two experiments. In experiment II Reyan 2 produced 13.2 t/ha DMY and Reyan 5 yielded 9.8 t/ha; in contrast, these two cultivars yielded 8.9 and 6.6 t/ha, respectively, in experiment III, although the relative performance of the two cultivars remained the same. A combined trial using selected accessions from both experiments II and III is necessary to further select a small number of the most promising materials.

There were moderate to high levels of anthracnose resistance in most of the germplasm tested in experiments II and III, with the exception of three accessions. FM05-1 and FM05-3 had severity ratings >3 and GC 349 was highly susceptible, with a >4 severity rating. Reyan 2 and Reyan 5, with moderate levels of resistance, were comparable to most except Mineirão and GC 1581 (experiment II). However, given the intrinsic variability associated with anthracnose severity assessments, such minor differences in severity may not be consistently obtained under field conditions and hence would be difficult to select for. The two current cultivars appear adequate in their anthracnose resistance levels.

Lack of low temperature tolerance is one of the main limitations of the current commercial cultivars in China, and Reyan 2 and Reyan 5 had less than 30% survival in experiment III under severe winter conditions. Two of the new introductions, GC 1480 and GC 1463, had excellent winter survival of 77% and 50%, respectively, and both accumulated over 30% of their DMY in the dry season. These two also ranked first and second according to their systemic value, indicating their overall superior performance.

Further evaluation of selected germplasm must be carried out at more than one site to cover the range of agroclimatic conditions where stylo can potentially be utilised in China. Accessions including GC 1480, GC 1463, GC 1581, L8 and GC 1576 (EMBRAPA) must be included in these evaluations. New potential cultivars developed from such evaluations would have high DMY, winter survival and anthracnose resistance. In the meantime, the current cultivar Reyan 2, with at least good DMY, seed yield and anthracnose resistance, should perform adequately.

Acknowledgments

The Australian Centre for International Agricultural Research and the Cooperative Research Centre for Tropical Plant Protection funded much of the research reported in this paper. We are indebted to Drs Segenet Kelemu, John Miles and Peter Kerridge of CIAT; Drs Celso Fernandes and Maria Jose Charchar of EMBRAPA; Drs Don Cameron and Sukumar Chakraborty of CSIRO; Drs C.R. Ramesh, P.S. Pathak and N.P. Melkania of IGFRI; and other colleagues from CATAS for access to germplasm and input into ideas and discussions. Mr He Huaxuan, Mr Chen Zhiquan, Mr Yu Daogen, Tang Jun and Ms Cai Biyuan provided technical assistance.

References

Chakraborty, S. 1990. Expression of quantitative resistance to *Colletotrichum gloeosporioides in Stylosanthes scabra* at different inoculum concentrations and day-night temperatures. Australian Journal of Agricultural Research 41, 89–100.

Edye, L.A. 1997. Commercial development of *Stylosanthes* pastures in northern Australia. I: Cultivar development within *Stylosanthe*s in Australia. Tropical Grasslands 31, 503–508.

Guodao, L., Changjun, B. & Huaxuan, H. 2001. The selection and utilization of *Stylosanthes guianensis* cv. Reyan 5. Acta Agrestia Sinica 9, 1–7.

Guodao, L., Phaikaew, C. & Stur, W.W. 1997. Status of *Stylosanthes* development in other countries: II. *Stylosanthes* development and utilisation in China and south-east Asia. Tropical Grasslands 31, 460–467.

Hood, T. & Jones, J.B.Jr. (eds). 1996. Soil and plant analysis in sustainable agriculture and environment. Marcel Dekker: New York.

Hwang, M.Y., Wun, L.X. & Zhang, C.Z. 1986. The main varieties of forages and their evaluation in Southern China. Proceedings of an international workshop, Cisarua, Indonesia, 19–23 August, 1985, p. 202. (ACIAR proceedings series No. 12).

Michalk, D.L., Fund, N.P. & Zhu, C.M. 1993. Improvement of dry tropical rangelands in Hainan island. Society for Range Management 46, 331–339.

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I. *Stylosanthes* development and utilisation in South America. Tropical Grasslands 31, 454–459.

Qingci, F. & Xilai, L. 2003. The relation between alpine meadow productivity and climate factors in Qinghai region. Pratacultural Science 20, 8–12.

Zhon, Z., Xilai, W. & Haisheng, M. 2002. Qinghao-Tibet Plateau: an analysis of relation between herbage, water and heat factors. Pratacultural Science 19, 11–15.

Chapter 13

Stylosanthes cultivars in China: Their development and performance

Liu Guodao[1], Bai Changjun[1], Wang Dongjin[1] and He Huaxuan[1]

Summary

This chapter documents the history of development of *Stylosanthes* cultivars in China and provides a summary of their performance in multi-location trials. Reyan 2–Zhuhuacao was the first *S. guianensis* cultivar to be developed and released in China and it replaced many of the Australian cultivars such as *S. hamata* cv. Verano and *S. guianensis* cv. Graham that had been used previously. Since then, several new cultivars have been released in China following difficulties with seed production in some CIAT 184 derived cultivars such as Reyan 2 and the gradual erosion of anthracnose resistance. Many of these *S. guianensis* cultivars have improved seed yield and winter survival and all are equal or better than Reyan 2 in dry matter yield: Reyan 5 is a CIAT 184 selection; Reyan 7 is a selection from CIAT 136; Reyan 10 originates from CIAT 1283; Reyan 13 is from CIAT 184; and cultivar 907 has been developed through Cr64-radiation technology. Other new germplasm of *S guianensis* and *S. seabrana*, recently introduced and evaluated through international collaboration, also show promise and may soon become new cultivars for China.

[1] Tropical Pasture Research Center Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, Hainan, PR China

Introduction

China has over 22% of the world's population supported on only 7% of the farming land, with a growing percentage of the population migrating to urban areas. This has led to a greater demand for food from the rural sector and a consequent intensive exploitation of resources, especially land. At the same time, a rapid increase in the consumption of livestock products has occurred that is predicted to continue, with beef consumption expected to double in developing countries between 1993 and 2020. In China the consumption of livestock products has increased by a staggering 27.8% every year. South China consists of Hainan, Guangdong, Guangxi, Fujian and Yunnan provinces and makes up 10% of China's land area, where some 11% of the country's population lives (Goudao et al 1994). This area contains 32 million ha of grasslands supporting an estimated 35 million head of ruminants (cattle, buffaloes and goats), 74 million pigs and 830 million head of poultry raised on state and smallholder farms, which constitute the livestock industry in the region. Consequently, the area suffers from a severe shortage of fodder and feed and the less than 10% increase in annual feed production is not enough to keep pace. According to statistics, the annual shortage of feed in China at present is about 50 million t and of these 15 million t is protein feed (Kexian 2002).

A key agricultural problem has been the declining soil fertility and degradation of marginal crop lands leading to a reduced ability to support sustainable production systems. The problem is magnified by increases in human population. As food production intensifies and expands onto more marginal agricultural lands, there is an urgent need to develop more sustainable agricultural systems to maintain and improve the fertility of both the marginal and other crop lands. In addition to food production, marginal lands are also major

contributors to livestock production and to forest products, which are in high demand for fuel, fibre and construction materials. Crop/livestock and silvipastoral production systems are degrading due to depletion of soil nutrients, loss of vegetation cover, soil erosion and build-up of diseases and pests.

Species of the tropical legume *Stylosanthes* (stylo) can improve fertility in marginal lands because they are well adapted to infertile soils and climatic conditions ranging from humid to semi-arid. They have the potential to be more widely grown in the subtropics and tropics than any other genus. This applies to Asia as well as Africa, Australia and South America. Since 1950 over 500 forage accessions have been introduced to tropical China. Of these, cultivars of *S. guianensis, S. hamata* and *S. scabra* have delivered the most significant benefits to increasing crop and animal production. Effective international collaboration has resulted in the introduction of many promising accessions including *S. guianensis* CIAT 184 and CIAT 136 that have resulted in highly successful cultivars such as Reyan 2–Zhuhuacao. Intercropping of CIAT 184 derived cultivars and Graham with perennial crops such as rubber, coconut, cashew and other fruit trees is already considered a success. *Stylosanthes hamata* is used in grazing lands. The use of stylo in a range of production systems including commercial use as leaf meal in animal feed has been detailed elsewhere (Phaikaew et al, this volume).

The disease anthracnose is a major constraint in these production systems in China, with damaging strains of the pathogen limiting the utilisation of *S. guianensis* cultivars Cook and Graham. Tolerance to anthracnose and high fodder production of more than 12 t/ha of Reyan 2–Zhuhuacao has encouraged its acceptance among small farmers. It covers a significant portion of the 200,000 ha improved with tropical forages in southern China. However, low seed yield and increased susceptibility to anthracnose in recent years has led to the evaluation and selection of germplasm to develop new high-yielding and anthracnose-resistant cultivars to suit the multi-purpose use of stylo in China. Several new cultivars have been released recently and this chapter documents the history of their origin and performance. Details on the performance of promising new germplasm, yet to be released as new cultivars, appear in Changjun et al (this volume).

Origin and Performance of Chinese Cultivars

Introduction of *Stylosanthes* species to China commenced with *S. humilis* in 1962, which was used as a green manure crop under rubber trees (Edye 1997). More widespread introduction and evaluation occurred in the 1980s with *S. guianensis* cultivars Schofield, Endeavour, Graham and Cook; *S. hamata* Verano; and *S. scabra* Seca from Australia and *S. guianensis* CIAT 136 and CIAT 184 from Colombia (Guodao et al 1997). Of these, Graham was among the most widely used cultivar in China (Devendra & Sere 1992) and continued to be so for nearly 20 years. As the evaluation of newly introduced germplasm progressed, it was soon realised that there were superior materials than Graham with higher dry matter yield (DMY) and anthracnose resistance. A selection of these promising accessions have since been evaluated at a number of regional sites and released as new cultivars.

Stylosanthes guianensis cultivar Reyan 2

Twenty-five accessions of *S. guianensis* were introduced from CIAT (Colombia) in 1982 and these were extensively tested at field sites in southern China. Among a total of 45 species and accessions, *S. guianensis* CIAT 184 was highly adapted to acid soils and arid conditions, had good anthracnose resistance and high DMY, approximately 36% more than that of Graham. It recorded a DMY of 15–22.5 t/ha/year, producing the highest crude protein in its vegetative stage (Tables 13.1 and 13.2). It has produced significant gains in animal weight gain/day/head, and improved calf birth and calf survival rate to reduce the cattle raising period from birth to market from 3–4 years to 1.5–2 years (China National Agriculture Museum 1991). It is a vigorously growing plant with a low stem to leaf ratio and high palatability. Increased availability of feed and high N availability in pastures improved with CIAT 184 has increased carrying capacity from 0.25 for native rangelands to 1.0 animal units/ha.

Table 13.1 Fresh fodder yield of *Stylosanthes guianensis* cultivar Reyan 2 compared with standard cultivars Cook and Graham at field sites in South China.

Site, province	Fresh weight (t/ha)	Cultivar for comparison	Change (%)
Danzhou, Hainan	30.1	Cook	235.6
Danzhou, Hainan	47.0	Graham	46.9
Jiexi, Guangdong	96.2	Cook	111.7
Baisha, Hainan	57.0	Graham	40.7
Shaya, Hainan	36.0	Graham	44.0
Ledong, Hainan	49.0	Graham	28.9
Meizhou, Guangdong	41.6	Graham	22.6
Mean	46.1	Graham	36.1

A stand of *Stylosanthes guianensis* cultivar Reyan 2 in Hainan.

Table 13.2 Dry matter percentage (DM%), crude protein (CP), crude fibre (CF), crude fat (CE), ash, non-N extract (NFE), Ca and P content of *Stylosanthes guianensis* cultivar Reyan 2 compared with standard cultivars grown in South China.

Cultivar	DM%	CP	CF	CE	Ash	NFE	Ca	P
Graham (flowering)	22.5	15.5	27.2	1.5	6.4	49.8	1.47	0.23
Reyan 2 (flowering)	18.5	15.2	39.3	1.9	6.9	36.2	1.13	0.19
Reyan 2 (vegetative)	17.0	22.7	21.7	3.2	9.2	43.2	1.54	0.31
Cook (vegetative)	17.2	20.1	22.9	2.2	9.9	44.9	1.4	0.28
Verano (flowering)	20.1	14.4	35.1	2.2	6.7	40.9	–	–
Reyan 7	21.4	16.1	32.5	2.6	6.3	41.7	1.18	0.10
Seca	24.8	14.7	39.2	2.9	5.8	37.4	1.15	0.18
Reyan 10 (vegetative)	22.5	15.6	27.1	2.9	6.7	37.6	1.08	0.43
Reyan 10 (flowering)	20.4	17.5	32.4	2.1	6.42	35.6	1.33	0.41

A stand of *Stylosanthes guianensis* cultivar Reyan 5 for seed production.

It was released in southern China in 1992 as Reyan 2–Zhuhuacao and by 1993 it had replaced large areas that had been previously under Graham. It is the most widely grown *Stylosanthes* cultivar in China and is now grown in many provinces in southern and southwestern China such as Hainan, Fujian, Guangdong, Guangxi, Yunnan, Sichuan, Guizhou and Hunan. Reyan 2 produces flowers from the middle of October to the end of November and seeds mature by the middle of January. It has good winter survival in areas that may have a short frost season and retains green fodder during the dry and cold season to produce around 225–300 kg/ha seeds each year.

Stylosanthes guianensis cultivar Reyan 5

In 1987 an early-flowering single plant was found amongst CIAT 184 plants grown in a large area for seed production. Seed was collected separately from this plant and, as the colour of the seeds was black, it was commonly referred to as 'black seed stylo'. A systematic evaluation of this selection began in 1990 using Reyan 2 as the check cultivar. In regional trials spanning five provinces, its DMY was nearly the same as that of Reyan 2, with three sites recording lower yield than that of Reyan 2 (Table 13.3). It is slightly more resistant to anthracnose, with lower severity (66% leaf area necrotic in Reyan 5 compared with 93% for Reyan 2 under artificial inoculation) and a lower anthracnose rating score of 2.3, compared with 3.2 for Reyan 2 under natural inoculum in a field environment.

The main advantage of Reyan 5 over Reyan 2 is its superior seed yield. Starting in the middle of September, Reyan 5 flowers one month earlier than Reyan 2 and matures at the beginning of December. This results in an average seed yield of over 350 kg/ha in Hainan (Table 13.4), which is more than 40% higher than the seed yield of Reyan 2. It survives well at sites that have short frosty periods and grows slowly to maintain some green fodder during the dry and cold season. Its cold tolerance is higher than that of Reyan 2 and, provided the early flowers escape frost, it can successfully set seeds when planted at high latitude and high altitude

Table 13.3 Fresh fodder yield of *Stylosanthes guianensis* cultivar Reyan 5 compared with the standard cultivar Reyan 2 at field sites in South China.

Site, province	Year	Fresh weight (t/ha)	Change %	Fresh weight of Reyan 2 (t/ha)
Lingshui, Hainan	1995	14.7	–2.1	15.1
Lingshui, Hainan	1996	78.4	4.5	75.1
Lingshui, Hainan	1997	72.1	6.4	67.7
Danzhou, Hainan	1996	9.7	–5.4	10.3
Danzhou, Hainan	1997	54.1	5.6	51.2
Danzhou, Hainan	1998	46.5	3.9	44.7
Shaoguan, Guangdong	1998	3.9	6.4	4.3
Simao, Yunnan	1998	5.9	18.0	5.0
Simao, Yunnan	1999	27.9	–38.8	33.8
Wuchang, Hubei	1999	1.4	18.8	9.6
Yibing, Sichuan	1999	19.1	3.6	18.1

***Stylosanthes guianesis* cr. Reyan 5 as a cover crop in a young commercial mango plantation in Hainan.**

Table 13.4 Seed yield of *Stylosanthes guianensis* cultivar Reyan 5 compared with the standard cultivar Reyan 2 at field sites in South China.

Site, province	Year	Seed yield (kg/ha)	Change (%)	Seed yield of Reyan 2 (kg/ha)
Lingshui, Hainan	1995	505.5	65.2	306.0
Lingshui, Hainan	1996	457.5	41.9	322.5
Danzhou, Hainan	1996	408.0	62.9	250.5
Danzhou, Hainan	1997	312.0	30.8	238.5
Danzhou, Hainan	1998	186.0	45.9	127.5
Dongfang, Hainan	1999	306.0	35.1	226.5
Ledong, Hainan	1999	417.0	33.6	312.0
Simao, Yunnan	1998	249.0	49.6	166.5
Average for Hainan		359.25	45.24	247.35

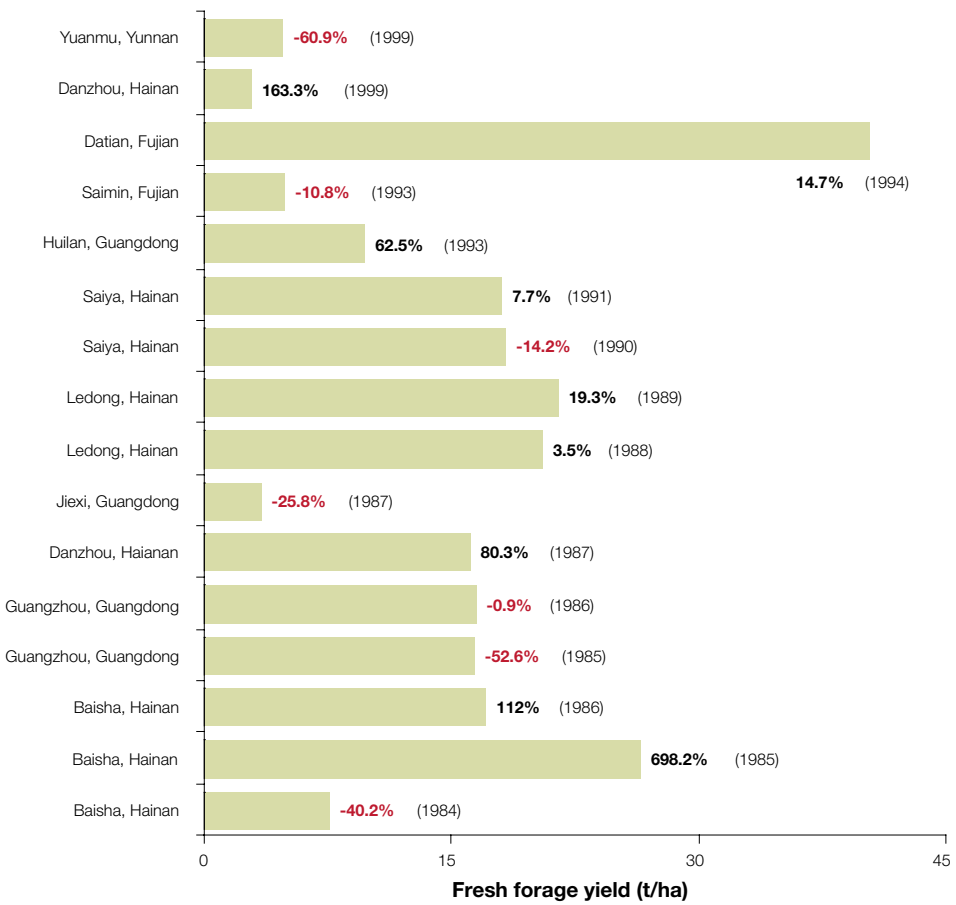


Figure 13.1 Fresh fodder yield of *Stylosanthes scabra* cultivar Seca compared with the standard *S. hamata* cultivar Verano at field sites in South China. Figure in bold represents percentage change in yield compared with Verano and figure in parenthesis is the year of evaluation.

sites in southeastern and southwestern China. For instance, at Simao in the Yunnan province it flowers at least 18 days earlier than Reyan 2, allowing seeds to be harvested; whereas at Shaoguan in northern Guangdong province, although it flowers 15–20 days earlier than Reyan 2 and can survive the cold, there is no seed set at this site. In contrast, it can flower but cannot survive in sufficient numbers at Wuchang beside the Yangtse River in Hubei province. Reyan 5 was released in 1998 for its superior seed yield and anthracnose resistance and, together with Reyan 2, has become one of the dominant varieties in southern China.

Stylosanthes scabra cultivar Seca

Seca was introduced from Australia in the 1980s and is a commonly grown variety in Hainan and parts of southern China. Seca is well adapted to arid and semi-arid environments with its high drought tolerance, and is particularly suitable for grazed pastures due to its high tolerance to trampling by grazing animals. It shows a high level of anthracnose resistance in Hainan where biotype A is less widespread than the biotype B pathogen. It produces reasonable green fodder under prevailing soil and environmental conditions in Hainan where, on average, it yields 35% higher than the comparable *S. hamata* cultivar Verano, but it has not performed well in Fujian, Guangdong or Yunnan provinces (Figure 13.1).

In Baisha, Hainan province (19ºN), Seca germinates within four to six days, and after an initial period of slow growth in the first two months it grows rapidly to produce good biomass. One drawback is its low seed yield due to late flowering, the seed yield rarely exceeding 120 kg/ha. In Hainan Seca starts to flower in early to mid November and seeds do not mature till January or February. Flowering, seed set and seed yield vary considerably according to the field site. At higher latitudes such as in Jiexi, Guangdong province (23º5N), flowering does not start until the end of November and a sufficient number of seeds do not mature because of the cold conditions that follow flowering (Table 13.5). The herbage quality of Seca is also generally poor compared to that of *S. guianensis* cultivars, with comparatively low essential amino acids (Figure 13.2). This impacts on the quality of fresh and dried feed used as hay or as leaf meal in feed rations; further details on leaf meal quality and use are given in Guodao et al (this volume).

Stylosanthes guianensis cultivar Reyan 7

CIAT 136 (released in southern China as cultivar Reyan 7 in 2000) was among several accessions of *S. guianensis* introduced from CIAT (Colombia) in the 1980s. This accession was among the top performers

Table 13.5 Beginning and end of flowering, seed maturation and seed yield of *Stylosanthes scabra* cultivar Seca at selected field sites in South China.

Year	Site, province	Start of flowering	End of flowering	Maturity	Seed yield (kg/ha)
1987–89	Saiya, Hainan	15 Aug	6 Sep	End of Dec – 5 Jan	277
1987–89	Danzhou, Hainan	16 Sep	14 Oct	End of Dec – Jan	177
1984–85	Baisha, Hainan	28 Sep	4 Nov	End of Dec – 5 Jan	150
1987–89	Jiexi, Guangdong	10 Nov	25 Nov	Did not mature	–
1985–86	Guangzhou, Guangdong	12 Sep	30 Oct	26 Dec	113
1999	Yuanmu, Yunnan	15 Oct	25 Oct	End of Dec	Not harvested

in regional evaluations for DMY and anthracnose resistance that adapted to a range of environments. With 13–20 t/ha/year DMY, its yield is similar to that of Reyan 2 and Reyan 5 and is over 45% higher than that of Graham. Because of its good winter survival (survival rate is 90%) and the ability to offer green fodder during the dry, cold season, its usage has been extended to Hainan, Fujian, Guangdong, Guangxi, Yunnan, Sichuan, Guizhou and Hunan provinces.

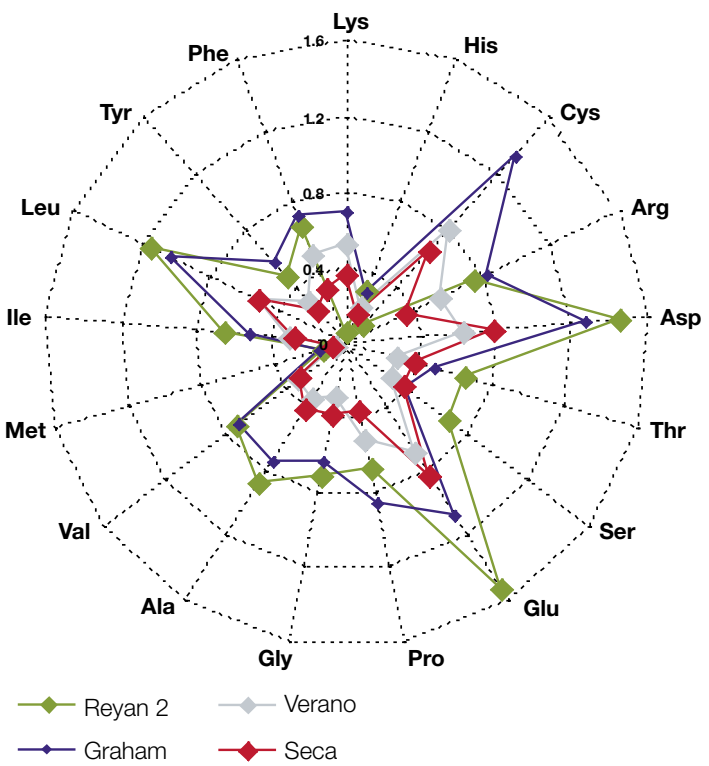


Figure 13.2 Essential amino acid composition of *Stylosanthes guianensis* cultivars Reyan 2 and Graham, *S. hamata cultivar* Verano and *S. scabra* cultivar Seca.

Stylosanthes guianensis cultivar Reyan 10

Accession 1283 (released as Reyan 10 in China) was introduced from CIAT in the 1980s and evaluated for environmental adaptation and dry matter and seed yield. Results from comparative evaluation during the period 1993–95 indicated that CIAT 1283 was well adapted to a range of environments; it grew well on sandy and dry land, had a high tolerance to drought and acid soil, and grew well under 500 mm rainfall conditions and at high altitudes. With a fresh yield of over 120 t/ha, it was slightly better that Reyan 2 (Figure 13.3) but considerably better than Reyan 5 by about 16%. It has better anthracnose resistance and nutritive value with high crude protein content than either Reyan 2 or Reyan 5, and had high cold tolerance. Reyan 10 was released in southwestern China, especially for use as a cut-and-carry fodder to supply green feed for dairy and cattle farms. In some areas it is used as a green manure crop and as an intercrop in mango, rubber and other fruit orchards.

Other cultivars and promising germplasm

Two other cultivars have been released in recent years, *S. guianensis* cv. Reyan No. 13, which originated from CIAT 184, and *S. guianensis* cv. 907, which was selected using Cr64-γ radiation breeding technology and released in Guangxi province. It is early days to make any realistic assessment of their performance or impact.

Other accessions have been introduced in the last five to six years from Australia, CIAT and EMBRAPA (Brazil), through an Australian Centre for International Agricultural Research funded project. Some accessions of *S. guianensis* including GC 1581, GC 1463, GC 1480 and cultivar Mineirão from Brazil and new accessions of *S. seabrana* have shown early promise (Changjun et al, this volume). Some of these have been compared with cvv. Reyan 2 and Reyan 5 in regional evaluations at Hainan, Yunnan, Fujian and Guangdong provinces, where the Tropical Pasture Research Center of CATAS has set up field trials. Some of these promising materials may end up as new cultivars in the near future.

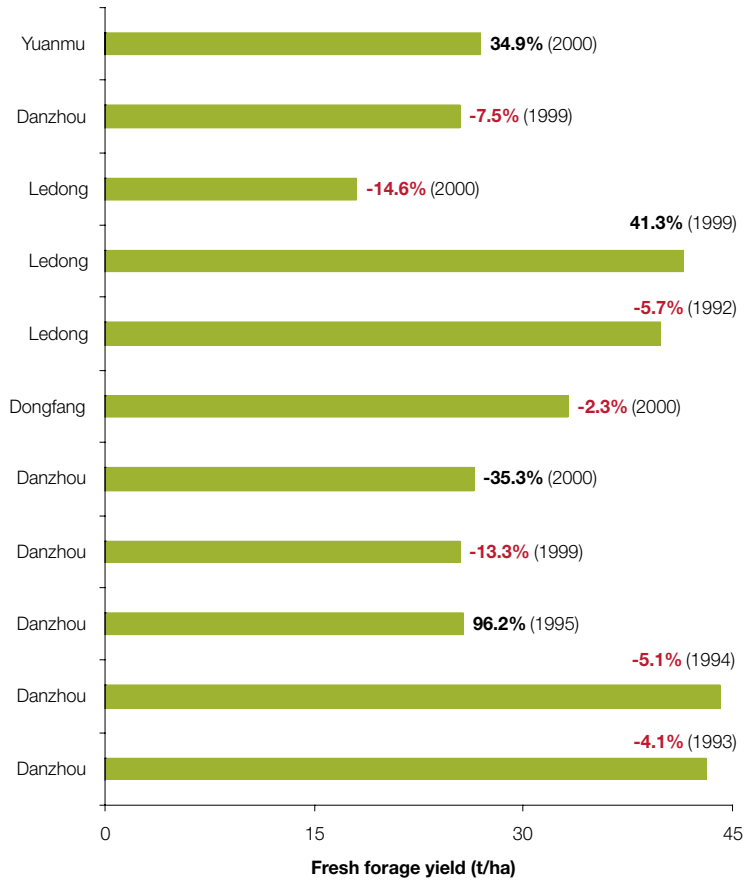


Figure 13.3 Fresh fodder yield of *Stylosanthes guianensis* cultivar Reyan 10 compared with the standard *Stylosanthes guianensis* cultivar Reyan 2 at field sites in South China. Figures in bold represent percentage change in yield compared with Verano and figure in parenthesis is the year of evaluation.

In China stylo has repeatedly proven its value as a legume crop in a number of production systems that have been successful over a number of years. In 1985 a cattle-grazing farm in Dongfang, Hainan province, established a nearly 600 ha area with a mixture of *S. hamata* cv. Verano, Reyan 2, Seca and other grasses by aerial sowing. Similarly, nearly 2400 ha has been improved by mixed stylo and *Brachiaria* pastures in the Xishui cattle farm, Baisha, Hainan province. Forage yield and grazing performance in these farms are up to four times higher than those in native pastures, with concomitant doubling of crude protein, almost a 30% increase in the birth rate of calves, increased cattle survival rate and a doubling of liveweight gain per day. Stylo leaf meal and hay have been a good source of quality protein in feed rations for many domestic and farm animals (Guodao et al, this volume). More than 200 ha of cropland and wasteland in Changjiang country, Hainan province, has been converted to pastures producing stylo and *Leucana* leaf meal and hay as feed for the dairy and other animal industries. Because of its use as an intercrop in horticultural plantations, as a cut-and-carry fodder, and as a soil conditioner fixing nitrogen and increasing soil organic matter, stylo has been a versatile legume for southern China. The new suite of cultivars will add to the flexibility and sustainability offered by stylo in farming systems.

Acknowledgments

The Australian Centre for International Agricultural Research, the Cooperative Research Centre for Tropical Plant Protection and the Chinese Academy of Tropical Agricultural Sciences funded much of the research reported in this paper. We are indebted to Drs Segenet Kelemu, John Miles and Peter Kerridge of CIAT; Drs Celso Fernandes and Maria Jose Charchar of EMBRAPA; Drs Don Cameron and Sukumar Chakraborty of CSIRO; Drs C.R. Ramesh, P.S. Pathak and N.P. Melkania of IGFRI; and other colleagues from CATAS for access to germplasm and input into ideas and discussions. Mr He Huaxuan, Mr Chen Zhiquan, Mr Yu Daogen, Tang Jun and Ms Cai Biyuan provided technical assistance.

References

China National Agriculture Museum (edited). 2000. Achievements in agricultural science and technologies (in Chinese). Agricultural Press 1991, 872 pp.

Devendra, C. & Sere, C. 1992. Assessment of the use and impact of *Stylosanthes guianensis* CIAT 184 in China. IDRC Report: Ottawa, Canada, 11 pp.

Edye, L.A. 1997. Commercial development of *Stylosanthes* pastures in northern Australia. I: Cultivar development within *Stylosanthes* in Australia. Tropical Grasslands 31, 503–508.

Guodao, L., He, H. & Zhang, Y. 1994. The current status and future needs of forage R&D in tropical China. In Stur, W.W. et al (eds) 'Forages for smallholders'. Working document No. 143, CIAT.

Guodao, L., Phaikaew, C. & Stur, W.W. 1997. Status of *Stylosanthes* development in other countries: II. *Stylosanthes* development and utilisation in China and south-east Asia. Tropical Grasslands 31, 460–467.

Kexian, Yi. 2002. Development and potentialities of stylo production and utilization in China. SEAFRAD Newsletter no. 4

Chapter 14

Response of *Stylosanthes seabrana* to *Bradyrhizobium* inoculation in India

C.R. Ramesh[1] J.H. Kulkarni[2] and J.S. Desale[3]

Summary

Stylosanthes seabrana has been recently introduced into India, where several of its accessions have produced the highest dry matter and seed yield in regional evaluations at several field sites. Two cultivars of this species, recently released in Australia, require inoculation with specific *Bradyrhizobium* for their establishment and growth, but inoculation is not required in Brazil where the native strains occur widely. In this work we have compared nodulation, plant performance and nitrogen fixation using different strains: the strain selected in Australia, an Indian *Bradyrhizobium* strain of the cowpea miscellany, and resident strains that occur naturally in Indian soils. Results from glasshouse and field studies have consistently shown that *S. seabrana* can nodulate well with the native *Bradyrhizobium* strains present in Indian soils and hence do not require inoculation with any specific strains.

[1] Principal Scientist, Indian Grassland and Fodder Research Institute, UAS Campus Dharwad 580005, India
[2] Professor of Agricultural Microbiology, University of Agricultural Sciences, Dharwad 580005, India
[3] Head, Forage Crops, Mahatma Phule Krishi Viswa Vidyapeeth, Rahuri, Maharashtra, India

Introduction

Grasslands and grazing lands are the major source of forage for livestock in India. Compared to temperate regions, grasslands in the tropics experience a significant shortage of the legume component that contributes to palatability and quality of forage and improves the soil fertility. Some studies have shown that legumes such as *Stylosanthes* fix up to 250 kg N /ha/year (Tuley 1968). During the last two decades tropical grasslands have experienced massive biotic pressure from both cattle and humans for life-supplying needs, resulting in an alarming degradation. *Stylosanthes* has gained importance as a tropical fodder legume for its adaptability to a wide range of bio-edaphic conditions (Stace & Edye 1984), and significant commercial utilisation of the species has occurred in Australia, China, Thailand, Vietnam and the Philippines, among other countries.

In India species of *Stylosanthes* have been introduced from Australia, South America, Africa and the USA since 1945 (Ramesh et al 1997). Of the five species *S. hamata, S. scabra, S. guianensis, S. humilis* and *S. viscosa* that have been evaluated, the first two are more suitable for Indian conditions. *Stylosanthes* is regarded as the most important tropical legume for semi-arid and arid regions and is used in wasteland reclamation, as cut-and-carry forage in horticultural systems, and as a component of dryland mixed cropping, with a large seed industry in the Anantpur district of Andhra Pradesh servicing the various utilisation schemes. However, most seed produced in India is predominantly of the *S. scabra* cultivar Fitzroy, which has shown extreme susceptibility to anthracnose caused by the fungus *Colletotrichum gloeosporioides.* This disease has caused the demise of many cultivars worldwide (Chakraborty, this volume; Miles & Lascano 1997) and inflicts over 50% loss in forage and seed yield in Fitzroy under Indian conditions (Ramesh et al, this volume). To increase the range

of materials available to India and to select well-adapted, productive and anthracnose-resistant materials, improved *Stylosanthes* germplasm and bred lines from Australia and CIAT were evaluated at designated regional sites in a project funded by the Australian Centre for International Agricultural Research (ACIAR). These included the newly described *S. seabrana* (Maass & Mannetje 2002), which was originally discovered in 1988 as a contaminant in a field trial of *S. scabra*. An evaluation at CSIRO field stations found that 17 lines of *S. seabrana* outperformed *S. scabra* to a considerable degree on the heavy soils of central and southern Queensland (Jansen & Edye 1996). *Stylosanthes seabrana* has unique morphological and agronomic features and also an ability to perform well on heavy soils.

Stylosanthes species nodulate with *Bradyrhizobium* species of the cowpea miscellany group that nodulate groundnut and pulse legumes. They are generally widely distributed in many soils and nodulate normally to meet the nitrogen requirement of these crops. However, there is variation in the effectiveness of *Rhizobium* species and strains for different accessions of *Stylosanthes* (Date & Norris, 1979; Mannetje 1969, 1977). In Australia *S. seabrana* requires a specific strain of *Bradyrhizobium* inoculant for its establishment and growth, and pelleted seeds of the two recently released varieties Primar and Unica containing the specific strain are commercially sold due to the absence of this strain in Australian soils (Date et al 1996). *Stylosanthes seabrana* has performed extremely well at several field sites in India, and with its high herbage and seed yield this species may prove to be a commercial success for India. Specific information on the inoculation response of *S. seabrana* to local or introduced strains of *Bradyrhizobium* is lacking in India except for early reports on the usefulness of *Rhizobium* inoculation to *Stylosanthes* species (Kale & Konde 1985), which showed that the maximum nitrogen content resulted from multi-strain inoculations rather than single individual strains. In this paper we have evaluated the performance of a *Bradyrhizobium* strain specific to *S. seabrana* in Australia following inoculation under both pot and field conditions.

Materials and Methods

Pot culture

Response of *S. seabrana* accession 105546B to *Bradyrhizobium* inoculation was studied in a pot culture experiment in a glasshouse at the Indian Grassland and Fodder Research Institute Regional Research Station at Dharwad, Karnataka. Light red sandy loam soil, not previously cropped to *Stylosanthes*, was sieved and filled into 10 kg capacity earthen pots. Seeds of *S. seabrana* were scarified and sown. *Bradyrhizobium* strain CB 3481 was obtained from CSIRO, Australia, as a peat-based inoculant under a quarantine permit. A native cowpea group miscellany strain NC 92 recommended for groundnut was obtained from the University of Agricultural Sciences, Dharwad, as a lignite-based inoculant. Individual strains were separately inoculated at the rate of 1 g per pot and placed adjoining the seed. Two un-inoculated controls were maintained: one with nitrogen fertiliser (urea) at 20 kg N/ha and one without nitrogen fertiliser. For each treatment five pots were maintained as replication. The pots were sown on 23 March 1998 and observations were taken eight weeks after sowing. Dry matter yield of shoot was taken after 21 weeks of planting. Roots were carefully separated from the soil by spraying a jet of water, and nodules per plant were counted.

Field experiment

The usefulness of the *Bradyrhizobium* strain CB 3481 as a field inoculant was evaluated for six *S. seabrana* accessions at an experimental station of the Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra. The black soil was thoroughly ploughed and harrowed to prepare a good seedbed and 2x4 m plots were laid out using a randomised block design with three replications. The seeds of each accession were scarified and 5 kg/ha was sown on 14 September 1998 with 50 cm spacing between rows. Each plot was sown with one of the six accessions either without any fertiliser or with one of the following treatments:

(a) Prior to sowing, the peat inoculant of *Bradyrhizobium* strain CB 3481 was applied to the soil at 5 g/plot.

(b) Prior to sowing, 40 kg N/ha was applied.

(c) Prior to sowing, the peat inoculant of *Bradyrhizobium* strain CB 3481 at 5 g/plot, 40 kg N/ha and 40 kg P2O5/ha were applied.

The crop was grown without irrigation and harvested in December 1999. Green and dry fodder yields were determined and crude protein yield was estimated by multiplying the nitrogen content of the dry matter by 6.25 (Parkinson & Allen 1975).

Results and Discussion

Pot culture

There were significantly more nodules in plants inoculated with the *S. seabrana*-specific *Bradyrhizobium* strain CB 3481, followed by plants inoculated with the cowpea strain from India NC 92 (Table 14.1). Uninoculated plants supplemented with N had the lowest number of nodules, significantly lower than plants that did not receive any *Bradyrhizobium* or N. However, the increased nodulation did not translate directly into increased plant biomass or N uptake (Table 14.1), and N supplementation resulted in the least biomass and N uptake. These results indicate that *Bradyrhizobium* strain CB 3481 did improve nodulation but it was not effective in fixing adequate N to enhance plant biomass production through increased N uptake.

Date (2000) summarised a number of important factors that influence both nodulation and nitrogen fixation in rhizobial associations of tropical legumes. These include inoculant quality, acid soils and acidity factors such as aluminium and manganese toxicity, calcium and phosphorus deficiency, salinity and osmotic stress, and soil and root temperatures. In addition, the competitive ability of an introduced strain in the rhizosphere

Table 14.1 Response of *Stylosanthes seabrana* accession to *Bradyrhizobium* inoculation in a pot experiment.

Treatment	No. of nodules	Plant biomass (g)	Nitrogen uptake (mg/plant)
Bradyrhizobium strain CB 3481	89.80	4.32	0.044
Bradyrhizobium strain NC 92	70.07	4.04	0.041
Uninoculated control	64.27	5.62	0.057
Uninoculated control + 20 kg N/ha	36.93	3.26	0.030
Critical difference (P<0.055)	14.19	0.496	

is a major determinant of success. With unsterilised soil used in the pot experiment, it is likely that highly competitive and well-adapted resident native strains outcompeted the introduced strain, and many of the nodules present may not have been formed by the introduced strain. Similarly, optimum temperatures for nodulation and N fixation vary between species and genera (Date 2000) and this may have been responsible for a lack of direct correlation between nodulation and N fixation.

Overall, despite poor nodulation in the uninoculated treatment, both plant biomass and N uptake are comparable to the inoculated treatments. From a practical perspective, this experiment has clearly demonstrated that accessions of *S. seabrana* can establish, grow and produce high biomass without the need to inoculate seeds with any specific *Bradyrhizobium* strains.

Field experiment

Overall, there was no clear-cut benefit from *Bradyrhizobium* inoculation on green forage yield for any of the six *S. seabrana* accessions as uninoculated controls performed more or less at similar levels (Figure 14.1). All six accessions maintained their relative rankings for green forage yield at each of the four treatments and 1155995 performed well under most treatments. However, the overall means for the four treatments showed a trend: the combined N, P and *Bradyrhizobium* treatment produced the highest green forage (20.7 t/ha), followed by the *Bradyrhizobium* treatment (20.3 t/ha) and the uninoculated treatment (17.7 t/ha); and the 40 kg N/ha treatment produced the least amount of green forage (15.8 t/ha). Although significant, these differences were relatively minor and none of the accessions showed any signs of N or other nutrient deficiency.

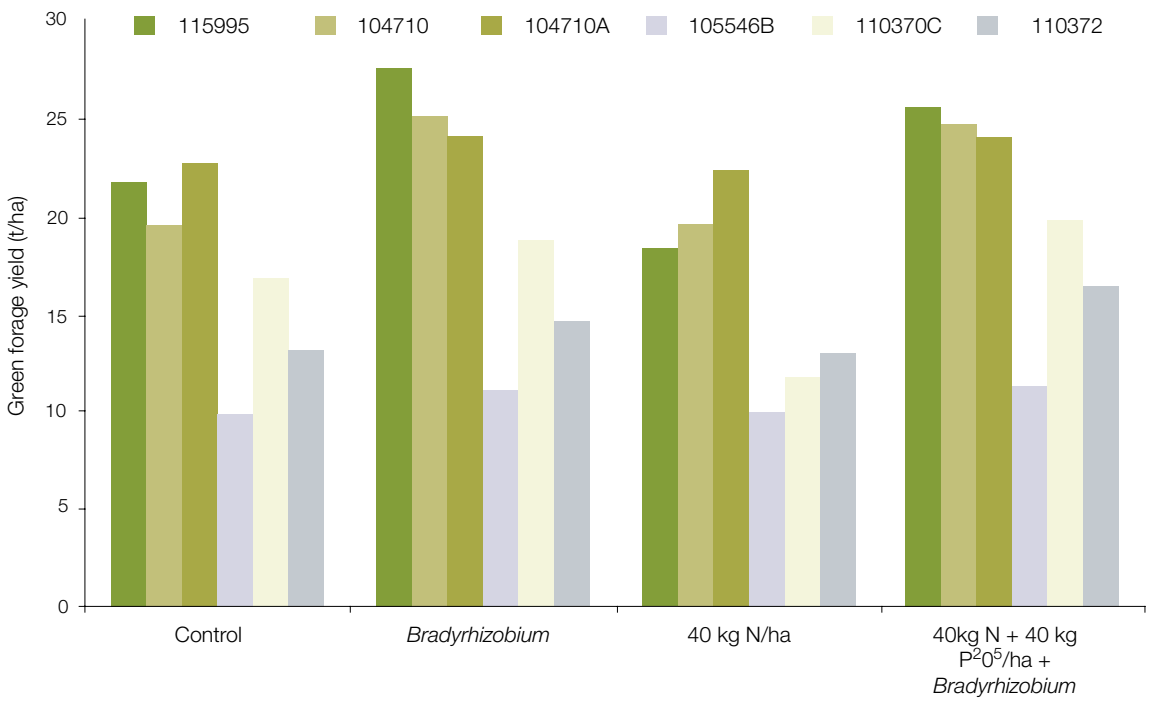


Figure 14.1 Mean green forage yield of six *Stylosanthes seabrana* accessions grown at a field site in Rahuri, Maharashtra: without any fertiliser or *Bradyrhizobium* inoculant; with peat inoculant of *Bradyrhizobium* strain CB 3481; with 40 kg N/ha; and with peat inoculant of *Bradyrhizobium* strain CB 3481 at 5 g/plot, 40 kg N/ha and 40 kg P_2O_5/ha.

These results confirm earlier findings for *S. seabrana* which demonstrated a requirement for specific *Bradyrhizobium* inoculation for establishment at sites within Australia but not in Brazil (Date 2000). There are other examples where a particular legume may not need to be inoculated at every location. For example, soybean in Thailand did not respond to inoculation (Thomson et al 1991) but requires inoculation for effective N fixation in Australia (Bushby et al 1983) or Brazil (Vargas & Suhet 1980). These results point to the different composition and effectiveness of resident soil populations of root nodule bacteria in the different countries/sites and the ability of native Indian strains of root nodule bacteria to form symbiotic associations with *S. seabrana*, allowing effective N fixation.

S. seabrana accessions are among the best performers for dry matter and seed yield at most of the ten or so sites in India where a range of germplasm from three to four *Stylosanthes* species have been under evaluation for the last three years (Ramesh et al, this volume). Results from the targeted field study at Rahuri indicating that *S. seabrana* accessions are able to perform well with native strains of the root nodule bacteria in India bode well for the future of this legume. Promising materials of this species can be used in wide geographical regions employing simple technology without the need for inoculation with specific *Bradyrhizobium* strains.

Acknowledgments

We acknowledge the Australian Centre for International Agricultural Research, the Indian Council for Agricultural Research and the Cooperative Research Centre for Tropical Plant Protection, which funded much of the research reported in this work. We are indebted to Drs P.S. Pathak, Gangaiah, Sridhar and N. Birader from IGFRI and staff at the Rahuri field site for input into ideas and discussions and technical assistance.

References

Alexander, M. 1961. Introduction to Soil Microbiology. John Wiley Publishers: New York.

Bushby, H.V.A., Date, R.A. & Butler, K.L. 1983. *Rhizobium* strain evaluation of *Gycine max* cv. Davis, *Vigna mungo* cv. Regur and *V. unguiculata* cv. Caloona for three soils in glasshouse and field experiments. Australian Journal of Experimental Agriculture and Animal Husbandry 23, 43–53.

Date, R.A. 2000. Inoculated legumes in cropping systems of the tropics. Field Crops Research 65, 123–136.

Date, R.A., Edye, L.A. & Liu, C.J. 1996. *Stylosanthes* sp. aff *scabra*: A potential new forage plant for northern Australia. Tropical Grasslands 30, 133.

Date, R.A. & Norris, D.O. 1975. *Rhizobium* screening of *Stylosanthes* species for effectiveness in nitrogen fixation Australian Journal of Agricultural Research 30, 85–104.

Jansen, P.I. &. Edye, L.A. 1996. *Stylosanthes* sp. aff. *scabra*, a new stylo for heavy soils. Proceedings of the 8th Australian Agronomy Conference, Toowoomba.

Kale, K.D. & Konde, B.K. 1985. Nodulation and nitrogen fixation in stylo and Siratro. Journal of the Maharashtra Agricultural University 10, 347–348.

Maass, B.L. & Mannetje, L.'t. 2002. *Stylosanthes seabrana* (Leguminosae: Papilionoideae), a new species from Bahia, Brazil. Novon 12, 497–500.

Mannetje, L.'t. 1969. *Rhizobium* affinities and phenetic relationships within the genus *Stylosanthes*, Australian Journal of Botany 17, 553–564.

Mannetje, L.'t. 1977. A revision of varieties of *Stylosanthes guianensis* (Aubl.) Sw. Australian Journal of Botany 25, 347–362.

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I *Stylosanthes* development and utilisation in South America. Tropical Grasslands 31, 454–459.

Parkinson, J.A. & Allen, S.E. 1975. A wet digestion for determination of nitrogen and other mineral nutrients in biological materials. Communication Soil Science and Plant Analysis 6, 1–11.

Ramesh, C.R., Bhag Mal, Hazra, C.R., Sukanya, D.H., Ramamurthy, V & Chakraborty, S. 1997. Status of *Stylosanthes* in other countries. III. *Stylosanthes* development and utilization in India. Tropical Grasslands 31, 467–475.

Stace, H.M. & Edye, L.A. (eds) 1984. The Biology and Agronomy of *Stylosanthes*. Academic Press: Sydney, Australia.

Thompson, J.A., Bhromsiri, A., Shutsrirung, A. & Lillakan, S. 1991. Native root-nodule bacteria of traditional soybean-growing areas of northern Thailand. Plant and Soil 235, 53–66.

Tuley, P. 1968. *Stylosanthes gracilis*: A review. Herbage Abstracts 38, 87–94.

Vargas, M.A.T. & Suhet, A.R. 1980. Efecto de tipos y niveis de inoculantes na soja cultivada em um solos de cerrados. Pesquisa Agropecuaria Brasileiro 15, 343–347.

Section B

Anthracnose resistant *Stylosanthes*

B2: Pathogen biology and epidemiology

Chapter 15

Colletotrichum gloeosporioides diversity at centres of origin in Brazil and Colombia

S. Chakraborty[1], C.D. Fernandes[2], M.J. Charchar[3], P.L. Weeds[4] and S. Kelemu[5]

Summary

This chapter reviews research on the pathogenic and genetic diversity in the anthracnose pathogen at a centre of diversity in Brazil and Colombia and presents new information on pathogenic diversity in the Brazilian pathogen. Early research on pathogenic diversity focused on describing regional variation in the pathogen. More recent work has developed well-defined host differential sets to classify the pathogen population into distinct races. A large number of selectively neutral markers including isozyme and DNA-based polymorphisms have been used to describe the genetic structure of *Colletotrichum gloeosporioides.* These studies have shown extensive diversity in the pathogen population. A high level of genetic differentiation in the pathogen is associated with diverse host populations at sites where native *Stylosanthes* populations were present or where *Stylosanthes* has been cultivated over a long period of time. In contrast, a high level of pathogenic diversity is associated with sites where a diverse range of *Stylosanthes* with different levels of anthracnose resistance have been cultivated. Mostly simple races with specificity towards one to three host differentials are predominant on native plants where no deliberate selection for anthracnose resistance had been carried out. For a pathogen where the same races arise convergently from different genetic backgrounds, it is important to use both virulence and selectively neutral markers to understand pathogen population structure.

[1] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia
[2] EMBRAPA-CNPGC/Doctor student UNESP-FCA, Botucatu-SP 18609-490, Brazil
[3] EMBRAPA-CPAC, 73301-970, Planaltina, DF, Brazil
[4] Landcare Research, New Zealand
[5] Centro Internacional de Agricultura Tropical, Cali, Colombia

Introduction

The genetic plasticity in *Colletotrichum gloeosporioides*, the pathogen that causes anthracnose disease in the tropical pasture legume *Stylosanthes*, has been a major limiting factor affecting the productivity, persistence and utilisation of this legume. South and Central America is the principal centre of diversity of both the pathogen and *Stylosanthes*, although 4 of the 40 known species of *Stylosanthes* are native to parts of Asia and Africa (Williams et al 1984). In countries outside the centre of diversity, such as Australia, China, parts of India and South-East Asia, where it has been introduced as a forage and pasture legume, *Stylosanthes* has achieved commercial success under a range of cropping and pasture systems (Cameron & Chakraborty, this volume; Pengelly et al, this volume). Two important factors have contributed to this: in its new environment the introduced *Stylosanthes* has found less competition from companion grasses (Hall & Glatzle, this volume); and the anthracnose pathogen, often accidentally introduced along with its host, has failed to fully match the host resistance of some released cultivars, allowing them to flourish without serious anthracnose damage.

In Colombia, Peru and many other South American countries *S. guianensis* has shown the most promise as a pasture legume, and the majority of cultivars released for commercial use belong to this species. In addition to *S. guianensis*, in some regions of Brazil other species such as *S. capitata* and *S. macrocephala* have shown good anthracnose resistance and herbage yield under grazing. However, the commercial use of *Stylosanthes* in South America has been limited due to a number of biotic and abiotic constraints (Kelemu & Rao, this volume), and susceptibility to anthracnose has been a major cause of failure of cultivars to persist and maintain productivity beyond the first two years (Miles & Lascano 1997). So far nine *Stylosanthes* cultivars have been released in South America,

and all except a multiline cultivar 'Estilosantes Campo Grande' released in 2001 (Grof et al 2001) have had limited success. The extensive genetic and pathogenic diversity in *C. gloeosporioides* has allowed the pathogen to rapidly overcome the resistance of newly released cultivars.

Australian research has identified two biotypes of *C. gloeosporioides* infecting *Stylosanthes* spp. (Irwin & Cameron 1978). The presence of these two biotypes has been confirmed in Colombia, Brazil and some African countries, but there are strains that do not clearly belong to either biotype (Chakraborty et al 2002; Kelemu et al 1999; Munaut et al 1998). The two biotypes are distinguished by minor differences in morphology, symptomatology, host range and genetics. Biotype A infects all species of *Stylosanthes* and produces discrete lesions with grey centres and dark brown margins on all aerial plant parts. Biotype B mainly infects *S. guianensis* and causes a general necrosis and blighting of the terminal shoots, and lesions on leaves are not clearly defined.

Both biotypes have cellular proteins of similar molecular weight (Dale et al 1988) with some differences in extracellular proteins among biotype B isolates. The existence of two distinct pathogen populations in Australia has been confirmed by restriction fragment length polymorphism (RFLP) nucleotide sequence divergence estimates, double stranded RNA (dsRNA) banding patterns (Dale et al 1988), and 'mini-chromosome' types (Masel et al 1990). Using RFLP analysis, the genetic variation between the two biotypes was less than 6% (Braithwaite & Manners 1989). However, electrophoretic karyotype (EK) analysis has shown that biotype A isolates have five large chromosomes (2 to >6Mb) and eight to ten 'mini-chromosomes' (270 to 600kb) whilst biotype B isolates have three large chromosomes (4.7 to 6Mb) and two to five mini-chromosomes (300 to 1200kb) (Masel et al 1990).

Manners et al (1992) examined RFLP data to conclude that little or no recombination between biotypes has occurred, as isolates with RFLP patterns intermediate between the two biotypes have not been observed. This is also consistent with distinct dsRNA patterns of biotypes A and B, which suggest an absence of any cytoplasmic recombination between biotypes. However, recent evidence shows that some strains of *C. gloeosporioides* in Australia carry a 2Mb biotype A-like chromosome in a biotype B-like background, suggesting genetic recombination between the two biotypes (Masel et al 1996). Whether this recombination event is by sexual, parasexual or other mechanisms is not known. Studies of vegetative compatibility under laboratory conditions have demonstrated DNA transfers between isolates of biotypes A and B during anastomosis but this occurs at low frequency. Recent studies have demonstrated horizontal transfer of chromosomes (>2Mb) when the two biotypes are co-inoculated on growth media under severe selection pressure (He et al 1998).

Use of selectively neutral markers such as isozymes (Lenné & Burdon 1990), RFLP (Manners et al 1992), EK (Masel et al 1990), and random amplified polymorphic DNA (RAPD) (Chakraborty et al 1999; Kelemu et al 1999; Manners & He 1997; Weeds et al 2003) has shown that the two biotypes are genetically distinct although there is considerable variation within each biotype. It is clear from these studies that the range of genetic variation is much wider in South America than in Australia or South-East Asia. Pathogenic diversity among isolates within each biotype has also been recently determined for populations from Brazil and Colombia.

This paper summarises the current knowledge on genetic and pathogenic diversity within biotypes A and B of *C. gloeosporioides* populations at its centre of diversity in Brazil and Colombia and presents new information on pathogenic diversity in the Brazilian pathogen. For simplicity, in this paper we have considered pathogen isolates originating from species other than *S. guianensis* as putative biotype A and isolates from *S. guianensis* as putative biotype B.

Pathogenic Diversity

Early work had focused on the demonstration of pathogenic variation and on classifying and characterising groups according to their disease severity on a range of host species and accessions (Lenné 1994; Lenné & Calderon 1984; Miles & Lenné 1984). Many of these studies included pathogen isolates from several countries (including Brazil, Colombia, Peru and Venezuela) that were collected without following any specific sampling strategy that would have allowed a detailed analysis of variation between and within a country or a region. The studies have helped demonstrate that extensive variation exists in the population including qualitative and quantitative differences in pathogenicity among isolates obtained from *S. guianensis* (putative biotype B pathogen). One exception, a study by Lenné (1988) that examined variation in anthracnose resistance among native *S. capitata* populations between sites in Minas Gerais, Brazil, pointed to a complex pathogen population (putative biotype A). In an attempt to group pathogen isolates according to their host specificity and geographical origin, Lenné et al (1982) described eight different groups. Groups 1 and 2 were present in both Brazil and Colombia and attacked only *S. guianensis*; group 3 from Colombia only attacked *S. guianensis*; group 4, pathogenic on *S. capitata* and *S. scabra*, was from Brazil only; group 5 from Brazil was pathogenic on *S. capitata* and *S. hamat*a; group 6 from Colombia only attacked *S. capitata*; and groups 6a and 7 were pathogenic on *S. guianensis* and *S. capitata*. There have been other attempts at classifying pathogenic variation in South America and the development of an international host differential set (Davis, Lenné & Chakraborty, unpublished), including the use of the Australian biotype A differential set to classify Brazilian isolates originating from hosts other than *S. guianensis* (Chakraborty et al 1997), but these have not been successful.

Putative biotype A races

Work on the selection of well-defined host differential sets and rigorous and quantitative analysis of pathogenic specialisation have only recently started. A host differential set for Brazil has been developed and used to successfully classify 296 isolates collected from native and cultivated plants of *S. capitata, S. guianensis, S. scabra* and *S. macrocephala*

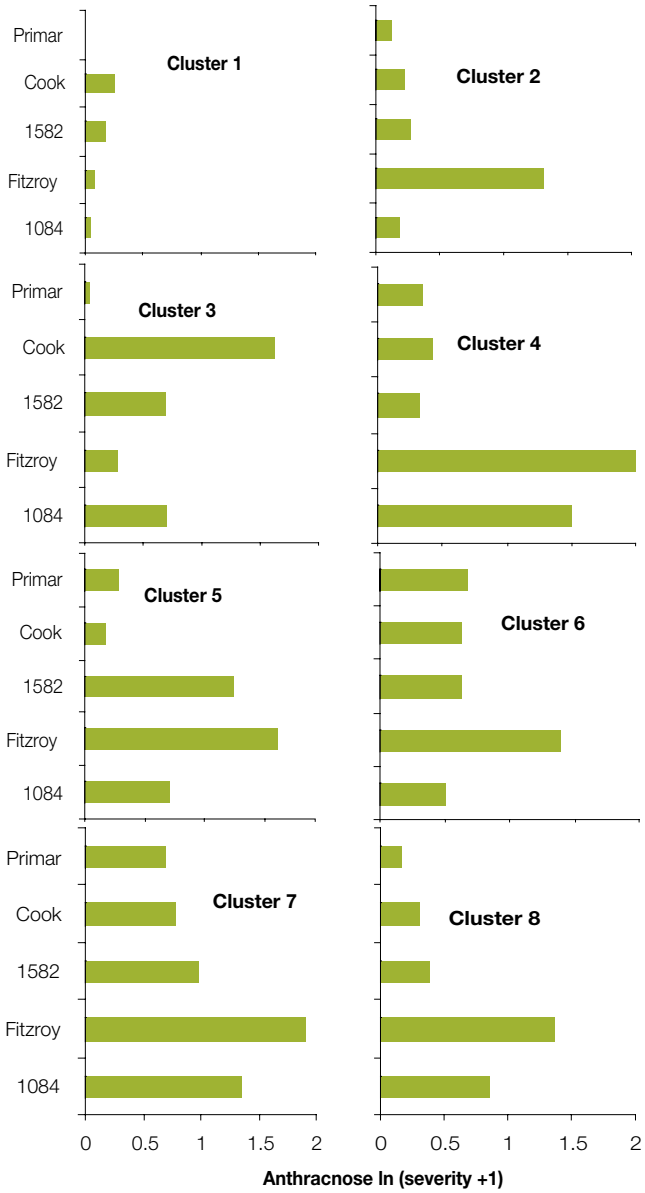


Figure 15.1 Mean disease severity (ln [severity+1]) on five host differentials *Stylosanthes capitata* 1084, *S. scabra* cv. Fitzroy, *S. macrocephala* 1582, *S. guianensis* cv. Cook and *S. seabrana* cv. Primar of eight race clusters used to classify 195 biotype A isolates of *Colletotrichum gloeosporioides* from Brazil.

in Brazil (Chakraborty et al 2002). The differential set of 11 accessions comprising two *S. macrocephala*, four *S. capitata*, two *S. scabra,* one *S. seabrana* and two *S. guianensis* accessions was selected from 19 host accessions after screening these with a subset of 18 diverse isolates in repeated infection assays. Screening the differentials with 195 isolates indicated that the Brazilian pathogen population may be less pathogenically diverse than initially expected, and an adequate summary of the variation of this population could be obtained using only five of the differentials. Eight race clusters adequately explained the pathogenic variation in the 195 isolates: cluster 1 was weakly pathogenic on all five differentials; clusters 2, 6 and 8 are only virulent on the highly susceptible *S. scabra* cv Fitzroy, but have different levels of aggressiveness towards the other four differentials; cluster 3 isolates are only virulent on *S. guianensis* cv. Cook; clusters 4 and 7 isolates are virulent on Fitzroy and *S. capitata* 1084, and cluster 7 isolates are more aggressive on *S. macrocephala* 1582, Cook and *S. seabrana* cv. Primar; and cluster 5 isolates are virulent on Fitzroy and 1582 (Figure 15.1). Ninety other isolates were successfully classified using linear discriminant functions developed for the eight clusters using the 195 isolates as the training data. A further 11 isolates remained unclassified and represent six potential new races with unique virulence combinations on the five differentials.

In Brazil cultivars with single gene resistance to the biotype A pathogen have not been deployed to any significant extent. Hence, the pathogen population does not carry large numbers of specific virulence factors due to the fitness cost associated with virulence (Leonard 1977). These races have dominated the pathogen population in the absence of widespread use of race-specific resistance in Brazil. Hot spots of races with specificity towards four or more host differentials have appeared around research stations in response to the extensive range of resistance present in the germplasm evaluated over a period of time (Chakraborty et al 2002). This situation may change as cultivars with anthracnose resistance are developed and used over large areas.

Since the Chakraborty et al (2002) publication, a further 170 isolates were collected during 2000 and race typed using the 11 differentials. Of these, 90 isolates came from four cultivated sites where promising *Stylosanthes* germplasm has been under evaluation, and the other 80 came from native *Stylosanthes* populations collected in Bahia and Minas Gerais, Brazil. Linear discriminant functions were developed for the existing eight race clusters using data on the disease severity of 355 isolates on the 11 differentials (*S. macrocephala* cv. Pioneiro and GC 1582; *S. capitata* GC 1081, GC 1084, GC 1086 and GC 1094; *S. scabra* cvv. Fitzroy and Seca; *S. seabrana* cv. Primar; and *S. guianensis* cv. Endeavour and Cook) and used to classify the 170 newly collected isolates. All isolates were successfully classified into the eight races, indicating that no new races have emerged during the two-year period. Race cluster 1 is the simplest race and cluster 7 is the most complex. Cluster 8 is similar to cluster 7 but has lower levels of differential aggressiveness towards some differentials (Figure 15.2). Cluster 7 contains isolates that are severe on *S. guianensis* cvv. Endeavour and Cook and may represent a new putative biotype. As before (Chakraborty et al 2002), simple races 1 and 2 dominated the pathogen population isolated from native *Stylosanthes* plants in Bahia and Minas Gerais, and the complex races 7 and 8 were absent from these sites (Figure 15.3). In contrast, the pathogen population was dominated by the complex race 7 at the four regional sites where *Stylosanthes* germplasm has been cultivated over the past several years.

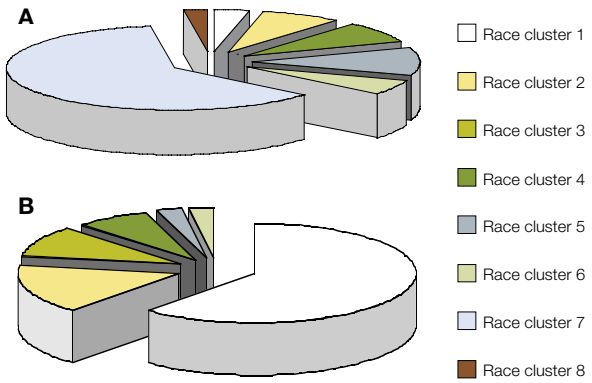


Figure 15.2 Frequency of *Colletotrichum gloeosporioides* putative biotype A race clusters at regional sites where *Stylosanthes* germplasm is cultivated (A) and at sites where the pathogen occurs naturally on a native *Stylosanthes* population (B).

Putative biotype B races

Kelemu et al (1996) used 12 *S. guianensis* genotypes, including four Australian biotype B differentials, to classify 42 isolates from Colombia, two from Peru and one from Brazil into 23 pathotypes. Using less than 5% necrotic tissue affected to denote resistance and over 5% for susceptibility, these authors developed a reaction matrix where the most virulent pathotype attacked all differentials and the least virulent failed to attack any (Figure 15.4). Using the 12 differentials, Kelemu et al (1999) classified a further 62 isolates, mostly from Colombia, and the total 104 isolates were grouped into 57 pathotypes. When only the four Australian differentials were used, the same isolates were grouped into just 11 pathotypes, which confirms earlier findings that the differentials currently used in Australia are not sufficient to classify the complex South American pathogen population. At least 11 putative biotype B isolates from *S. guianensis* infected *S. scabra* cv. Fitzroy which is not a host to the biotype B pathogen, indicating that this group of South American isolates may represent a third biotype in addition to the two biotypes described from Australian studies. In Colombia, Peru and many other South American countries *S. guianensis* has shown the most promise as a pasture legume and the majority of cultivars released for commercial use belong to this species. Therefore, it is not altogether surprising that pathogenic specialisation in the putative biotype B (57 races) is more extensive than that in the biotype A pathogen (8 races).

Diversity in selectively neutral markers

The number and size of supernumerary chromosomes (Masel et al 1990), RAPD profiles (Chakraborty et al 1999; Kelemu et al 1999; Munaut et al 1998) and RFLP (Braithwaite et al 1990; Kelemu et al 1999) can distinguish differences between and within each of the two biotypes. In addition, there is variation in the ITS region of the ribosomal DNA among some *Stylosanthes*-infecting *C. gloeosporioides* from Mexico, although the biotype of these isolates is unclear (Munaut et al 2001).

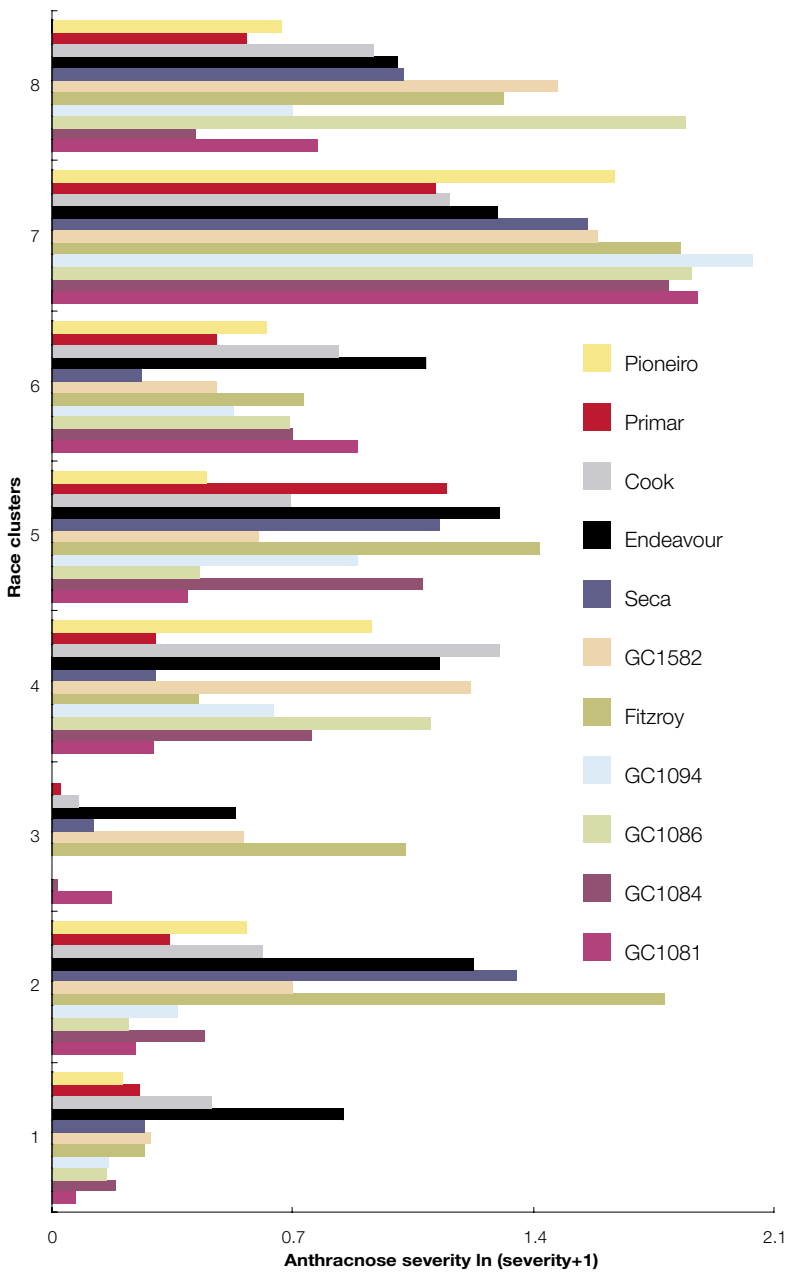


Figure 15.3 Mean disease severity (ln [severity+1]) of eight race clusters for 170 Brazilian biotype A isolates of *Colletotrichum gloeosporioides* on 11 differentials from four *Stylosanthes* species.

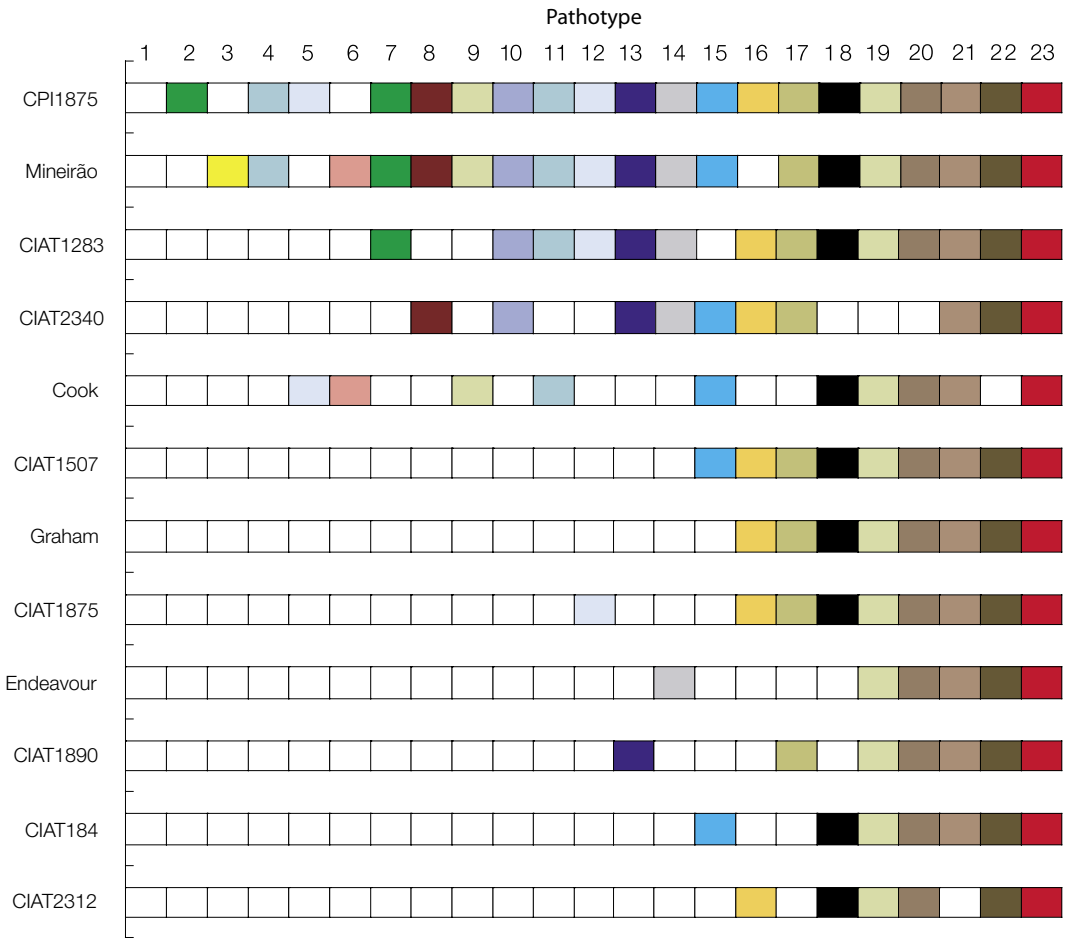


Figure 15.4 Reaction of 12 *Stylosanthes guianensis* host differentials to 23 putative biotype B pathotypes of *Colletotrichum gloeosporioides.* All filled-in patterns on the differential row indicate susceptibility/compatibility and empty boxes indicate resistance/ incompatibility.

Diversity within biotype A

Using RAPD markers, Weeds et al (2003) determined four major clusters among 144 mainly biotype A isolates from Brazil and Colombia. Cluster I contained Brazilian isolates mainly from *S. capitata*; cluster II isolates were from Brazil together with Australian biotype A isolates; cluster III contained isolates from Brazil, Colombia and Peru originating from *S. capitata, S. gracilis* or *S. guianensis*, and Australian biotype B isolates; and cluster IV isolates were from a number of *Stylosanthes* spp., predominantly from Brazil. Cluster IV contained 55 closely related isolates, of which 44 were clonal with identical fingerprints, and 35 of these were from six different race clusters (Chakraborty et al 2002). The variation within biotype A was extensive and isolates taken from the same leaf did not have identical haplotypes. Using RAPD markers, there was no clear relationship between haplotype clusters and the host species of origin, with isolates from *S. capitata* and *S. guianensis* being present in almost all clusters.

There were 87 haplotypes among the 144 isolates from Brazil and Colombia. This represents a genetic diversity of only 7% of the theoretical maximum (Weeds et al 2003), due to the high frequency of the clonal isolates; excluding these 44 isolates from the data produced a more realistic estimate of diversity (60% of the theoretical maximum). In Brazil there was higher genetic diversity at sites such as Buritis (63% of theoretical maximum), where isolates came from native *Stylosanthes* plants, than at sites such as Campo Grande and Chapadão do Sul (5–17% of theoretical maximum), where *Stylosanthes* accessions are cultivated.

Diversity within biotype B

The first ever study of genetic diversity in the anthracnose pathogen used isozyme markers to compare variation within and between five discrete *C. gloeosporioides* populations from native *S. guianensis* stands in Colombia and Peru (Lenné & Burdon 1990). This study revealed an extensive diversity that did not correlate with pathogenic diversity. Since then, DNA markers have been used by several workers to demonstrate the extensive range of variation that exists in Central and South America (Braithwaite et al 1990; Kelemu et al 1999; Manners & He 1997; Masel et al 1990; Weeds et al 2003). Using RFLP with a retrotransposon DNA sequence and RAPD analysis, Kelemu et al (1999) identified 13 distinct lineages among 63 Colombian isolates, which correlated with geographic origin and indicated a probable single introduction event. Where isolates

from different regions were clustered together, most came from identical host genotypes.

According to RAPD profiles, most study sites contained fairly homogeneous endemic populations that have undergone some diversification. However, about 20% of isolates at some sites were very different from the population at any given site, suggesting that multiple introductions had occurred. This pattern was evident at Campo Grande, Quilichao and Caquetá, where a diverse range of host germplasm had been grown over a long period of time. At Carimagua, where *S. guianensis* has been grown for the longest period of time, the *C. gloeosporioides* population was highly diverse (Kelemu et al 1999).

Implications

Given the extensive geographical distribution of *Stylosanthes* spp. in South America, most studies have only been able to sample a relatively small component of the overall pathogen population, and the diversity estimate for South America is therefore likely to be conservative. Ample opportunities for the pathogen population to diversify and adapt to previously resistant cultivars are offered by the extensive genetic diversity demonstrated in the two putative biotypes, an increasing interest in the *S. guianensis* cultivar Mineirão, and the recent release of a new *S. capitata* – *S. macrocephala* multiline cultivar. Such adaptation for increased aggressiveness and virulence has been of common occurrence in Australia (Chakraborty, this volume; Chakraborty & Perrott, this volume) and this has been demonstrated experimentally (Chakraborty & Datta 2003). Similarly, the endemic Colombian *C. gloeosporioides* population, possibly derived from several lineages, has undergone considerable diversification (Kelemu et al 1999), although there is a general correspondence between isolate groups and their geographic origin. Genetic diversity in the pathogen was more extensive at sites where the host had been grown for a long time, thus allowing the pathogen to diversify through migration and other means. Ongoing monitoring of the pathogen population covering a more extensive range of native and cultivated areas of *Stylosanthes* is necessary for an overall assessment of pathogen diversity and microevolution.

There is no obvious and clear-cut relationship between pathogenicity groups, as detected using host differentials, and groupings based on selectively neutral markers such as RAPD, RFLP or isozymes. This indicates that pathogenic races can arise convergently from different genetic lineages, and highlights the importance of using both virulence and selectively neutral markers to understand pathogen population structure. A similar lack of distinct correlation between RAPD or EK markers and pathogen races has been reported in the Australian biotype A population of *C. gloeosporioides* (Chakraborty et al 1999). These results are consistent with findings on many other pathogens where isolates of the same race are not necessarily closely related (eg Jacobson & Gordon 1990; Woo et al 1996). Leung et al (1993) observed, in summary, that association between molecular markers and virulence patterns in plant pathogens can be perfect, partial or absent.

Biotype A incites anthracnose on most, if not all, *Stylosanthes* species, and biotype A isolates which cause typical discrete leaf lesions on *S. guianensis* can be frequently isolated. These isolates would normally be expected to attack biotype A hosts such as *S. scabra* cv. Fitzroy. In addition, several blight-inducing putative biotype B isolates from *S. guianensis* also infected Fitzroy; these isolates represent new putative biotypes and require further study.

Acknowledgments

This work was funded by the Australian Centre for International Agricultural Research and CSIRO. The Cooperative Research Centre for Tropical Plant Protection provided infrastructure and operational support. Ross Perrott provided technical assistance. These assistances are gratefully acknowledged.

References

Braithwaite, K.S., Irwin, J.A.G. & Manners, J.M. 1990. Random fragment length polymorphisms in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp in Australia. Mycological Research 94, 1129–1137.

Braithwaite, K.S. & Manners J.M. 1989. Human hypervariable minisatellite probes detect DNA polymorphisms in the fungus *Colletotrichum gloeosporioides*. Current Genetics 16, 473–476.

Chakraborty, S. & Datta, S. 2003. How will plant pathogens adapt to host plant resistance at elevated CO_2 under a changing climate? New Phytologist 159, 733–742.

Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A. & Thomas, M.R. 2002. Pathogenic variation in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp. in a centre of diversity in Brazil. Phytopathology 92, 553–562.

Chakraborty, S., Perrott, R., Charchar, M.J.d'A., Fernandes, C.D. & Kelemu, S. 1997. Biodiversity, epidemiology and virulence of *Colletotrichum gloeosporioides*. II. Genetic and pathogenic diversity in *Colletotrichum gloeosporioides* isolates from eight species of *Stylosanthes.* Tropical Grasslands 31, 387–393.

Chakraborty, S., Perrott, R., Ellis, N. & Thomas, M.R. 1999. New aggressive *Colletotrichum gloeosporioides* strains on *Stylosanthes scabra* detected by virulence and DNA analysis. Plant Disease 83, 333–339.

Dale, J.L., Manners, J.M. & Irwin, J.A.G. 1988. *Colletotrichum gloeosporioides* isolates causing different anthracnose diseases on *Stylosanthes* in Australia carry distinct double stranded RNA. Transactions of the British Mycological Society 91, 671–676.

Grof, B., Fernandes, C.D. & Fernandes, A.T.F. 2001. A novel technique to produce polygenic resistance to anthracnose in *Stylosanthes capitata*. In Gomide, J.A., Wilson, W.R.S. & da Silva, S.C (eds) 'Proceedings of the XIX International Grassland Congress', p. 525. FEALQ: Brazil.

He, C., Rusu, A.G., Poplawski, A., Irwin, J.A.G. & Manners, J.M. 1998. Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the phytopathogen *Colletotrichum gloeosporioides*. Genetics 150, 1459–1466.

Irwin, J.A.G. & Cameron, D.F. 1978. Two diseases of *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in Australia, and pathogenic specialisation within one of the causal organisms. Australian Journal of Agricultural Research 29, 305–317.

Jacobson, D.J. & Gordon, T. R. 1990. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis*. Mycological Research 94, 734–744.

Kelemu, S., Badel, J.L., Moreno, C.X. & Miles, J.W. 1996. Virulence spectrum of South American isolates of *Colletotrichum gloeosporioides* on selected *Stylosanthes guianensis* genotypes. Plant Disease 80, 1355–1358.

Kelemu, S., Skinner, D.Z., Badel, J.L., Moreno, C.X., Rodriguez, M.X., Fernandes, C.D., Charchar, M.J. & Chakraborty, S. 1999. Genetic diversity in South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. European Journal of Plant Pathology 105, 261–272.

Lenné, J.M. 1988. Variation in reaction to anthracnose within native *Stylosanthes capitata* populations in Minas Gerais, Brazil. Phytopathology 78, 131–134.

Lenné, J.M. 1994. Diseases of *Stylosanthes*. In Lenné, J.M. & Trutmann, P. (eds) 'Diseases of tropical pasture plants', pp. 21–42. CAB International: Kent, UK.

Lenné, J.M. & Burdon, J.J. 1990. Preliminary study of virulence and isozyme variation in natural populations of *Colletotrichum gloeosporioides* from *Stylosanthes guianensis.* Phytopathology 80, 728–731.

Lenné, J.M. & Calderon, M.A. 1984. Disease and pest problems of *Stylosanthes*. In Stace, H.M. & Edye, L.A. (eds) 'The biology and agronomy of *Stylosanthes*', pp. 279–293. Academic Press: Sydney, Australia.

Lenné, J.M., de Alvarez, A.V. & Torres, G.C. 1982. Pathogenic variation among isolates of *Colletotrichum gloeosporioides* affecting *Stylosanthes* spp. Phytopathology 72, 123 (abstract).

Leonard, K.J. 1977. Selection pressures and plant pathogens. Annals of the New York Academy of Science 287, 207–222.

Leung, H., Nelson, R.J. & Leach, J.E. 1993. Population structure of plant pathogenic fungi and bacteria. Advances in Plant Pathology 10, 157–205.

Manners, J.M. & He, C. 1997. Recent advances in studies of anthracnose of *Stylosanthes*. IV. Molecular approaches to studies of *Colletotrichum gloeosporioides* causing anthracnose of *Stylosanthes* in Australia. Tropical Grasslands 31, 435–444.

Manners, J.M., Masel, A., Braithwaite, K.S. & Irwin, J.A.G. 1992. Molecular analysis of *Colletotrichum gloeosporioides* pathogenic on the tropical pasture legume *Stylosanthes*. In Bailey, J. A. & Jeger, J. M. (eds) '*Colletotrichum*: biology, pathology and control', pp. 250–266. C.A.B. International: Wallingford, U.K.

Masel, A., Braithwaite, K., Irwin, J.A.G. & Manners, J.M. 1990. Highly variable molecular karyotypes in the plant pathogen *Colletotrichum gloeosporioides*. Current Genetics 18, 81–86.

Masel, A.M., He, C., Poplawski, A.M., Irwin, J.A.G. & Manners, J.M. 1996. Molecular evidence for chromosome transfer between biotypes of *Colletotrichum gloeosporioides* Molecular Plant Microbial Interaction 9, 339–348.

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I. *Stylosanthes* development and utilization in South America. Tropical Grasslands 31, 454–459.

Miles, J.W. & Lenné, J.M. 1984. Genetic variation within a natural *Stylosanthes guianensis*, *Colletotrichum gloeosporioides* host-pathogen population. Australian Journal of Agricultural Research 35, 211–218.

Munaut, F., Hamaide, N., & Maraite, H. 2001. Molecular and morphological characterization of *Colletotrichum gloeosporioides* isolated from native Mexican *Stylosanthes* species. Plant Pathology 50, 383–396.

Munaut, F., Hamaide, N., Vander Stappen, J. & Maraite, H. 1998. Genetic relationship among strains of *Colletotrichum gloeosporioides* isolated from *Stylosanthes spp.* in Africa and Australia using RAPD and ribosomal DNA markers. Plant Pathology 47, 641–648.

Weeds, P.L., Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A., Ramesh, C.R., Kexian, Y. & Kelemu, S. 2003. Genetic Diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centres of origin and utilization. Phytopathology 93, 176–185.

Williams, R.J., Reid, R., Schultze-Kraft, R., Sousa Costa, N.M. & Thomas, B.D. 1984. Natural distribution of *Stylosanthes*. In Stace, H.M. & Edye, L.A. (eds.) 'The biology and agronomy of Stylosanthes', pp. 73–101. Academic Press: Sydney, Australia.

Woo, S. L., Zoina, A., Del Sorbo, G., Lorito, M., Nanni, B., Scala, F. & Noviello, C. 1996. Characterization of *Fusarium oxysporum* f. sp. *phaseoli* by pathogenic races, VCGs, RFLPs, and RAPD. Phytopathology 86, 966–973.

Chapter 16

Colletotrichum gloeosporioides diversity at centres of utilisation in Australia, China and India

S. Chakraborty[1], C.R. Ramesh[2] and Y. Kexian[3]

Summary

This paper summarises the pathogenic and genetic diversity in the anthracnose pathogen *Colletotrichum gloeosporioides* that infects species of the tropical pasture legume *Stylosanthes* at its centres of commercial utilisation in Australia, India and China. The pathogen has diversified at these centres of utilisation despite the overall limited genetic diversity and a relatively short history of *Stylosanthes* in these countries. There is both pathogenic diversity and genetic differentiation in all three locations, although the association between virulence and genetic groupings is weak. This points to convergent evolution of the same race from different genetic lineages, and helps to explain the number of races that have arisen in some countries away from the centre of diversity despite the pathogen being genetically less diverse. Under experimental conditions highly aggressive strains and novel haplotypes can evolve in as little as 25 sequential infection cycles. This plasticity in the pathogen population makes it necessary to monitor changes through regular field surveys in order to align plant breeding and cultivar development to dominant races of the pathogen.

[1] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia
[2] Indian Grassland and Fodder Research Institute Regional Station, Dharwad, Karnataka, India
[3] Chinese Academy of Tropical Agricultural Sciences, Badao Xinchum, Danzhou 571737, Hainan, PR China

Introduction

Despite its agronomic suitability, commercial utilisation of *Stylosanthes* spp. has been severely curtailed by anthracnose, caused by *Colletotrichum gloeosporioides*, which restricts its establishment, productivity and persistence. Worldwide, anthracnose has been the single most important factor in limiting the development and utilisation of *Stylosanthes* cultivars. The genetic plasticity of a *C. gloeosporioides* population has often resulted in the development of virulent strains following the release of anthracnose-resistant cultivars. Extensive pathogenic and genetic variation at the centre of host–pathogen diversity in South and Central America (Chakraborty et al 1997, 2002; Kelemu et al 1997; Weeds et al 2003) has continued to plague the sustainability of commercial cultivars (Miles & Lascano 1997).

In general, commercial utilisation of *Stylosanthes* has been more successful in countries away from the centre of host–pathogen diversity. Among the centres of commercial utilisation, *Stylosanthes humilis* was accidentally introduced into Australia from Brazil in the early 1900s. Introduction of other species and an active breeding program have continued since then and several new cultivars are now used in improved pastures, mainly in the dry tropics of northern Queensland and the Northern Territory (Cameron et al 1997; Edye 1997), with *Stylosanthes* spp. presently covering over 1 million ha. In its 28-year history the anthracnose pathogen has developed new genotypes and races in Australia, some devastating previously resistant cultivars. *S. humilis* cultivars once occupied over 500,000 ha but anthracnose eliminated the genus from these areas in the late 1970s, within three to four years of the disease being first reported in Australia (Pont & Irwin 1976). Similarly, *S. scabra* 'Fitzroy' was discarded within five years of its release in 1979 (Davis et al 1984) and 'Seca' was affected by a new race within five years of its release (Davis et al 1984).

Although Seca continues to show a moderate level of resistance in the field, strains with increasing aggressiveness towards Seca and new genotypes have appeared (Chakraborty et al 1999).

Stylosanthes spp. have been introduced to China and other parts of Asia from Australia, Africa and South America since the late 1960s, followed closely by the anthracnose pathogen. In India the first record of *Stylosanthes* introduction goes back to 1945 (Ramesh et al 1997). A similar pattern of devastation by anthracnose is gradually starting to emerge (Guodao et al 1997) following the release of cultivars in China, Thailand, the Philippines and other parts of South-East Asia.

The limited diversity in the pathogen population at centres of utilisation has been a key determinant of the commercial success of *Stylosanthes* in these countries (Lenné 1988; Miles & Lascano 1997; Weeds et al 2003). The aim of this chapter is to summarise the current knowledge and to offer a comparative assessment of the pathogenic and genetic variation among those populations of *C. gloeosporioides* in Australia, China and India that have been studied using similar experimental and analytical tools.

Pathogenic diversity in Australia

Australian research has identified two biotypes of *C. gloeosporioides* infecting *Stylosanthes* spp. (Irwin & Cameron 1978). Biotype A causes discrete lesions with a grey centre and dark brown margin on all aerial plant parts on all species of *Stylosanthes*, while biotype B causes a blight symptom on leaf and stem of mainly *S. guianensis*. The two biotypes are also present in South America (Chakraborty et al 2002; Kelemu et al 1996), Africa (Munaut et al 1998) and Asia (Chakraborty, unpublished) but there are strains in South America and Africa that do not clearly belong to either biotype.

In Australia the two genetically distinct biotypes have clonally descended from two separate introductions (Manners & He 1997), but the transfer of transposable elements in biotype B and of whole chromosomes from biotype A to B can occur in the field and laboratory. This, along with mutation and possible sexual recombination within the biotypes, may have contributed to the variability in the pathogen population, although ascospores from its sexual stage *Glomerella cingulata* are only weakly pathogenic (Ogle et al 1986).

Irwin and Cameron (1978) presented the first evidence of pathogenic specialisation within each of the two biotypes, describing isolates with specificity towards accessions of *S. guianensis*, *S. scabra* and *S. viscosa*. A few years later Davis et al (1984) reported a further specialisation in biotype A towards the *S. scabra* cv. Seca. Four biotype A races were recorded in the mid 1990s (Chakraborty et al 1997), but with expanding areas under *S. scabra, S. hamata* and, more recently, *S. seabrana* (all hosts of the biotype A pathogen), further specialisation has occurred in this biotype. Further details on the detection and classification of new biotype A races are given below. Four biotype B races have also been described in Australia using a host differential set comprising *S. guianensis* cvv. Endeavour, Graham, Cook and 18750 (Kelemu et al 1997). Cultivars of *S. guianensis,* the principal host of the biotype B pathogen, are only grown in a very small area and further specialisation in the biotype B has not been reported in Australia.

New cultivars of *S. hamata* (Amiga), *S. scabra* (Siran) and *S. seabrana* (Primar, Unica) were released in the 1990s and it was necessary to make an exhaustive search for appropriate host differentials to adequately describe specialisation in the biotype A pathogen. A selection of 14 biotype A isolates representing the existing four races and other more recently collected isolates were inoculated onto a set of 43 mainly *S. scabra* accessions, including the previously used differential set (*S. scabra* Fitzroy, Seca, Q10042 and 93116; *S. viscosa* 34904 and 33941) on two occasions (Chakraborty, Ellis & Thomas, unpublished). A principal component analysis of the 14x43 mean severity data matrix showed that the first three components explain 73%, 85% and 90% of the variation. The biplot shows that the 14 isolates, indicated by red arrows in Figure 16.1, are well separated into three clusters: (a) group 1 with SR4 and others, (b) group 2 with SR24 and (c) group 3 with indistinguishable WRS20 and WRS32. Overall, these groups represent the three biotype A races on *S. scabra*; however, the *S. viscosa* race, represented by isolate 21365, is also within the SR4 group. Assuming that the three clusters represent the natural grouping of isolates, we attempted to select a small set of host accessions that would best discriminate between the clusters. A stepwise discriminant analysis showed that about six differentials are needed to give prediction successes of around 90%, and reasonable prediction success is achieved by using just three accessions. The *S. scabra* accession 36260, previously used in race typing (Chakraborty et al 1999), was no different to Q10042, and only Q10042 was retained.

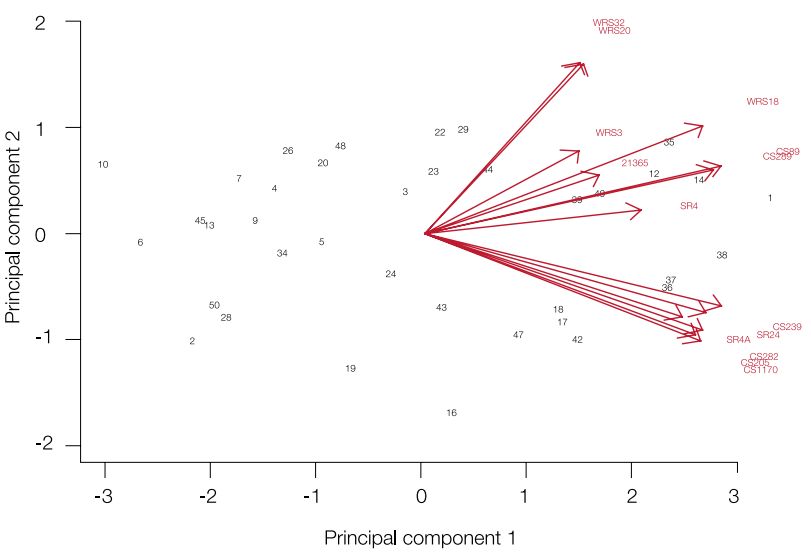


Figure 16.1 Biplot of the mean data matrix for 43 ***Stylosanthes*** **spp. accessions (in black) inoculated with each of 14 isolates (in red) on two different dates.**

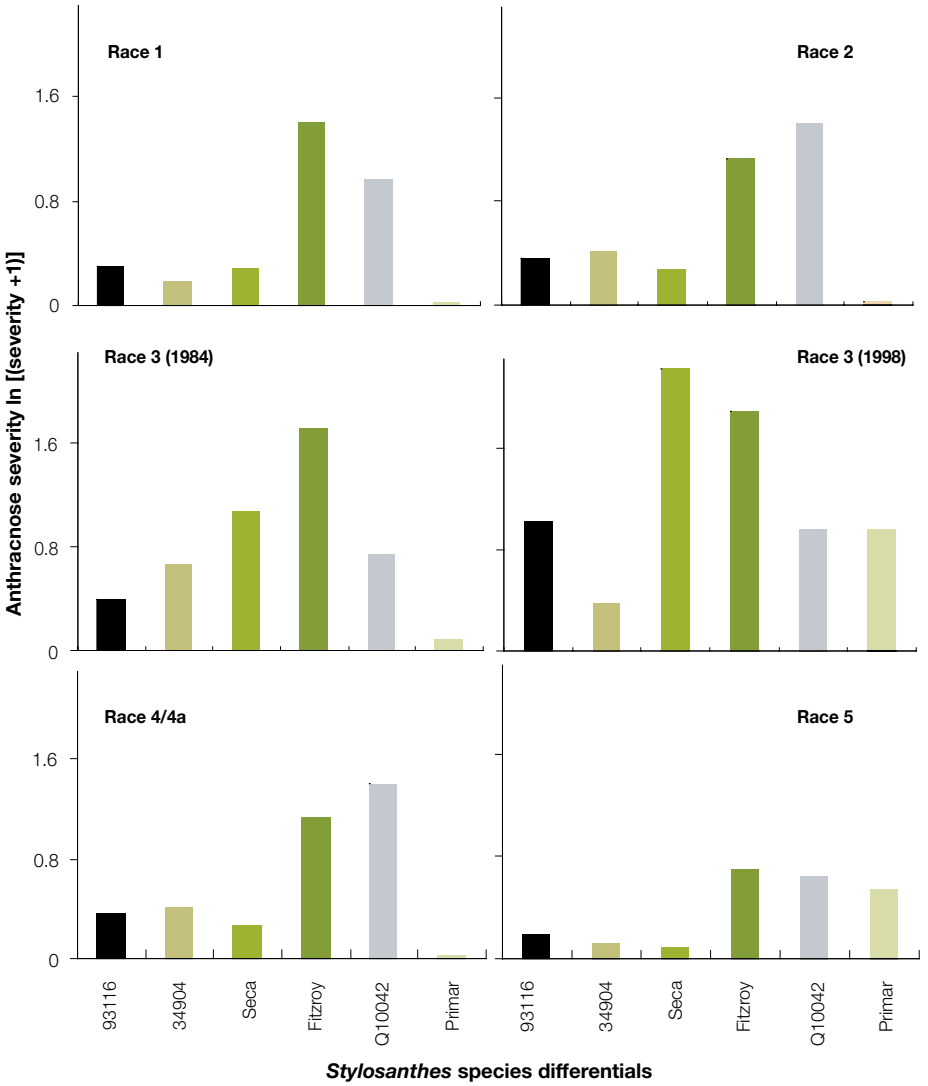


Figure 16.2 Response of Australian biotype A races to a set of *Stylosanthes* species differentials. The race 3 isolates have been grouped into early (1984) and recent (1998) populations to show that more recent isolates of this race are more damaging to Seca and 93116.

In 1998, only a few years after the release of *S. seabrana* Primar and Unica, a new race developed specificity towards these cultivars, although both cultivars were resistant to all known races at the time of their release (Trevorrow et al 1998). The new race on *S. seabrana* takes the total number of Australian biotype A races to five (Figure 16.2). A multivariate technique using linear discriminant function analysis has been developed to analyse pathogenic variation (Chakraborty et al 1996). In this analysis linear discriminant functions (LDFs) for existing race clusters are developed using data on existing isolates of known race classification, and the LDFs are then used to classify and assign field isolates to existing or new race clusters.

Of the five races, race 3, with specificity towards Seca, is the most economically important due to the widespread use of Seca and the increase in aggressiveness of race 3 on Seca throughout the past years (Chakraborty & Datta 2003). Consequently, isolates of race 3 collected in latter years are far more damaging to both Seca and 93116 (Figure 16.2). Whether more damaging strains of the new race 5 develop with increasing use of Primar and Unica remains to be seen.

Pathogenic diversity in India

In India *S. fruticosa* is endemic to the southern peninsular regions (Hooker 1879). South American species were first introduced in 1945 to evaluate their agronomic and commercial potential, and introductions from Australia, Africa, South America and the USA have continued to the present day (Ramesh et al 1997). Anthracnose has been a more recent concern in India than in Australia. Given the short history of introduction of *Stylosanthes* in India, the diversity in the Indian pathogen population should be less extensive than that in Australia, unless there had been a proliferation in the pathogen population on the native *S. fruticosa.* A detailed analysis on the role of *S. fruticosa* is given elsewhere (Ramesh et al, this volume).

Among its many uses, *Stylosanthes* is used as a cut-and-carry fodder and as a pioneering coloniser to stabilise watersheds and degraded land. Seeds of *S. scabra* Fitzroy, Seca and *S. hamata* Verano are produced commercially. Recent evaluation of *S. seabrana* has shown that this species is well adapted to the Indian agroecological conditions, nodulates with native strains of *Bradyrhizobium* spp. and has high biomass and seed yield. The differential set comprising *S. scabra* 93116, 36260, 33941, Fitzroy, Seca and Q10042, *S. seabrana* 105546B and *S. hamata* Verano was selected to match the promising species and to include the relevant Australian biotype A differentials.

A total of 277 putative biotype A isolates were available. Of these, at least 83 were collected from the native *S. fruticosa* population and the remaining 194 came from introduced *Stylosanthes* spp. A principal component analysis of all 277 Indian isolates showed that >95% of the variation could be explained by eight components, indicating that eight clusters should be adequate to explain most of the variation that existed among the Indian isolates. In a preliminary study, disease severity scores on the set of six common differentials, 93116, 36260, 33941, Fitzroy, Seca and Q10042, were analysed to examine whether LDFs developed from a large number of isolates of the Australian biotype A races could adequately classify the Indian isolates. Although the Indian isolates were similar to the Australian isolates in their response to the six common differentials, the Indian isolates were generally less aggressive on Seca and 93116 than the Australian isolates (Chakraborty et al, unpublished).

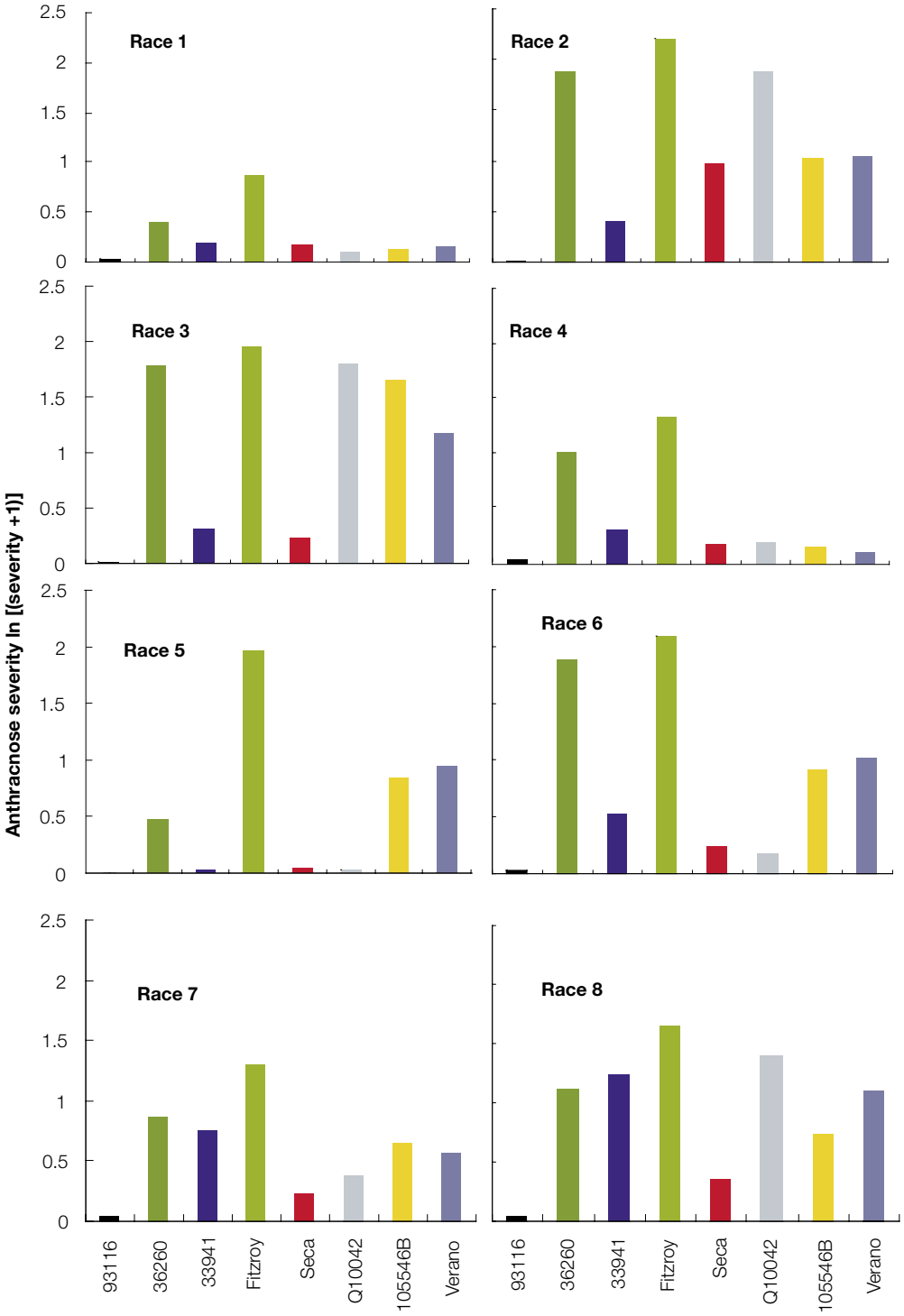


Figure 16.3 Mean severity of Indian biotype A races to a set of *Stylosanthes* species differentials (*S. scabra* 93116, Fitzroy, Seca, 36260, Q10042; *S. hamata* Verano; *S. viscosa* 33941; *S seabrana* 105546B).

A subset of 39 isolates was used as the training set to develop LDFs for the Indian isolates, and the remaining isolates were classified into one of the eight race clusters. The range of specificity among the clusters was spread across all host differentials except 93116, which was not infected to any extent by any of the isolates (Figure 16.3). Six of the eight race clusters were weakly pathogenic on Seca, but *S. seabrana* 105546B suffered low to moderate damage from races 1, 4, 5, 6, 7 and 8 and severe damage from races 2 and 3. Given the rapid pathogen adaptation to *S. seabrana* both in Australia and India, the long-term future of this otherwise promising species for India may be with leaf meal production, where it can be managed under more stringent conditions.

Pathogenic diversity in China

In southern China *S. guianensis* and *S. hamata* are used in a number of production systems, as a cut-and-carry fodder, as leaf meal and as hay in several animal-based industries including cattle, pigs, poultry and fish. There is heavy reliance on a single *S. guianensis* cultivar developed from CIAT 184 but in recent years this cultivar has shown increasing susceptibility to strains of the anthracnose pathogen (Guodao, unpublished information). Previous experience with cultivars such as *S. guianensis* Cook suggests that CIAT 184 may not remain a productive cultivar for much longer and three early flowering selections from CIAT 184 have been released as new cultivars. There is an urgent need to develop alternative cultivars to replace CIAT 184. Equally important is to sample the pathogen population for a detailed examination of the range of variation that exists in China. The durability and productivity of any potential future cultivars depend on this.

Colletotrichum gloeosporioides isolates were collected through regular surveys of the *Stylosanthes* growing regions. They were purified and inoculated onto a set of host differentials largely based on the Australian biotype A differential (*S. scabra* 93116, 36260, Q10042, Fitzroy and Seca) with three additional lines of *S. seabrana* (2523, 2534 and 2539) and

S. guianensis cultivar Endeavour. Due to difficulties with seed production of the host differentials, data were available for 25 isolates only. Although a cluster analysis indicated the presence of five clusters, most isolates were pathogenic on all differentials; only one isolate was non-pathogenic on 36260. It is impractical to draw major conclusions on the pathogen population in China based on the 25 biotype A isolates studied so far, and more extensive sampling of both biotypes is necessary.

Genetic diversity at centres of utilisation

Isolates of biotypes A and B are genetically distinct according to their double-stranded RNA profile, extra cellular proteins, RFLP and electrophoretic karyotype, among others (reviewed by Manners & He 1997). Among the limited number of studies on genetic diversity within a biotype at centres of utilisation, all except Lenné and Burdon (1990) have dealt with molecular markers for the Australian biotype A pathogen.

Recently Weeds et al (2003) have compared genetic diversity in the pathogen populations at centres of origin and utilisation using molecular markers, and a review of the pathogen diversity at centres of diversity appears elsewhere (Chakraborty et al, this volume). Isolates of both biotypes A and B of *C. gloeosporioides* from India, Australia and China are grouped into clusters representing the diversity in the South American pathogen population. Only a small number of the South American clusters are represented in these countries and the pattern for each country is distinctive. The Australian isolates showed very limited variation and all isolates could be allocated to one of three South American clusters.

The Chinese isolates had a high level of diversity; some isolates were allocated to two clusters but the majority was highly variable. Although the majority of the Indian isolates were grouped in two clusters, at least 17 highly variable isolates could not be placed in any of the South American clusters. Where two isolates were collected from the same lesion they were never identical and did not always group together in the same cluster, but many isolates from *S. fruticosa* and the cultivated *S. scabra* and *S. seabrana* had identical haplotypes. This indicated cross-infection of isolates between the naturalised and cultivated species.

Of the three countries utilising *Stylosanthes*, Shannon's index of diversity was the highest for India (h = 0.45), followed by China (h = 0.26) and Australia (h = 0.17). The pathogen population was least diverse in Australia, probably due to its geographical isolation and an effective quarantine, but the extensive diversity in India and China poses an additional quarantine risk to Australia. Most of the diversity found on cultivated hosts in Australia, India and China originated from a limited number of introduced pathogen haplotypes, resulting in a founder effect. In India some of the highly variable isolates could not be placed in any South American isolate clusters, pointing to the native *S. fruticosa* as a likely contributor to this diversity. In India the distribution of pathogen genetic groups was spatially heterogeneous and the high level of genetic differentiation in the pathogen was associated with sites where populations of the native *S. fruticosa* or other *Stylosanthes* spp. have been naturalised over a long period of time.

Conclusion

The anthracnose pathogen has diversified in Australia, India and China despite a relatively short history of *Stylosanthes* in these countries. Recent experimental evidence shows that highly aggressive strains and novel haplotypes can evolve after only 25 sequential infection cycles (Chakraborty & Datta 2003). There is an overall weak association between virulence and genetic groupings for *C. gloeosporioides* populations, indicating that the same race can arise convergently from different genetic lineages. This helps to explain the number of races that have arisen in some countries away from the centre of diversity despite the pathogen being genetically less diverse. The mechanisms that generate genetic variation in *C. gloeosporioides* are not well understood. RFLP and double-stranded RNA data suggest little or no recombination between biotypes (Manners & He 1997). The transfer of supernumerary chromosomes between the biotypes (He et al 1998) and a retrotransposon in the genome would offer ample opportunities to generate genetic variants.

Acknowledgments

Drs Nick Ellis and Mervyn R Thomas of CSIRO Mathematical and Information Sciences assisted with statistical analysis; Ms S. Mini, Ross Perrott, Toh he Wong, Leonard Ford and John Baisden provided technical assistance; and the Australian Centre for International Agricultural Research and the Cooperative Research Centre for Tropical Plant Protection provided funds for this research. These assistances are gratefully acknowledged.

References

Cameron, D.F., Trevorrow, R.M. & Liu, C.L. 1997. Recent advances in studies of anthracnose of *Stylosanthes.* II Approaches to breeding for anthracnose resistance in *Stylosanthes* in Australia. Tropical Grasslands 31, 424–429.

Chakraborty, S. & Datta, S. 2003. How will plant pathogens adapt to host plant resistance at elevated CO_2 under a changing climate? New Phytologist 159, 733–742.

Chakraborty S, Fernandes C.D., Charchar M.J.d'A. & Thomas M. 2002. Pathogenic variation in *Colletotrichum gloeosporioides infecting Stylosanthes* spp. at its center of diversity in Brazil. Phytopathology 92, 553–62.

Chakraborty, S., Perrott, R., Charchar, M.J.D'A., Fernandes, C.D. & Kelemu, S. 1997. Biodiversity, epidemiology and virulence of *Colletotrichum gloeosporioides*. II. Genetic and pathogenic diversity in *Colletotrichum gloeosporioides* isolates from eight species of *Stylosanthes.* Tropical Grasslands 31, 387–393.

Chakraborty, S., Perrott, R., Ellis, N. & Thomas, M.R. 1999. New aggressive *Colletotrichum gloeosporioides* strains on *Stylosanthes scabra* detected by virulence and DNA analysis. Plant Disease 83, 333–339

Chakraborty, S., Thomas, M.R. & Ellis, N. 1996. A multivariate analysis of pathogenic variation in *Colletotrichum gloeosporioides* infecting the tropical pasture legume, *Stylosanthes scabra*. Phytopathology 86, 283–289.

Davis, R.D., Irwin, J.A.G. & Cameron, D.F. 1984. Variation in virulence and pathogenic specialization of *Colletotrichum gloeosporioides* isolates from *Stylosanthes scabra* cvv. Fitzroy and Seca. Australian Journal of Agricultural Research 35, 653–662.

Edye, L.A. 1997. Commercial development of *Stylosanthes* pastures in northern Australia. I Cultivar development within *Stylosanthes* in Australia. Tropical Grasslands 31, 503–508.

Guodao, L., Phaikaew, C. & Stur, W.W. 1997. Status of *Stylosanthes* development in other countries: II. *Stylosanthes* development and utilisation in China and south-east Asia. Tropical Grasslands 31, 460–467.

He, C., Rusu, A.G., Poplawski, A., Irwin, J.A.G. & Manners, J.M. 1998. Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the phytopathogen *Colletotrichum gloeosporioides* Genetics 150, 1459–1466.

Hooker, J.D. 1879. Flora of British India. Vol 11, Sabiaceae to Cornaceae. L Reeve & Co, Ltd, Lloyds Bank Building: Bank Street, Ashford, Kent London, 148 pp.

Irwin, J.A.G. & Cameron, D.F. 1978. Two diseases in *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in Australia, and pathogenic specialization within one of the causal organisms. Australian Journal of Agricultural Research 29, 305–317.

Kelemu, S. Badel, J.L., Moreno, C.X. & Miles, J.W. 1996. Virulence spectrum of South American isolates of *Colletotrichum gloeosporioides* on selected *Stylosanthes guianensis* genotypes. Plant Disease 80, 1355–1358.

Kelemu, S., Badel, J.L., Moreno, C.X., Miles, J.W., Chakraborty, S., Fernandes, C.D. & Charchar, M.J.d'A. 1997. Biodiversity, epidemiology and virulence of *Colletotrichum gloeosporioides.* I. Genetic and pathogenic diversity in *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis.* Tropical Grasslands 31, 387–392.

Lenné, J.M. 1988. Variation in reaction to anthracnose within native *Stylosanthes capitata* populations in Minas Gerais, Brazil. Phytopathology 78, 131–134.

Lenné, J.M. & Burdon, J.J. 1990. Preliminary study of virulence and isozyme variation in natural populations of *Colletotrichum gloeosporioides* from *Stylosanthes guianensis.* Phytopathology 80, 728–731.

Manners, J.M. & He, C. 1997. Recent advances in studies of anthracnose of *Stylosanthes.* IV. Molecular approaches to studies of *Colletotrichum gloeosporioides* causing anthracnose of *Stylosanthes* in Australia. Tropical Grasslands 31, 435–444.

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I. *Stylosanthes* development and utilization in South America. Tropical Grasslands 31, 454–459.

Munaut, F., Hamaide, N., Vander Stappen, J. & Maraite, H. 1998. Genetic relationship among strains of *Colletotrichum gloeosporioides* isolated from *Stylosanthes spp.* in Africa and Australia using RAPD and ribosomal DNA markers. Plant Pathology 47, 641–648.

Ogle, H.J., Irwin, J.A.G. & Cameron, D.F. 1986. Biology of *Colletotrichum gloeosporioides* isolates from tropical legumes. Australian Journal of Botany 34, 281–292.

Pont, W. & Irwin, J.A.G. 1976. *Colletotrichum* leaf spot and stem canker of *Stylosanthes* spp. in Queensland. Australasian Plant Pathology Society Newsletter 5 (supplement): 35.

Ramesh C.R., Bhag Mal, Hazra, C.R., Sukanya, D.H., Rammamurthy, V. & Chakraborty, S. 1997. Status of *Stylosanthes* development in other countries: III. *Stylosanthes* development and utilisation in India. Tropical Grasslands 31, 467–475.

Trevorrow, R.M., Chakraborty, S. & Jansen, P.I. 1998. Anthracnose resistance in *Stylosanthes seabrana.* In Michalk, D.L. & Pratley, J.E. 'Agronomy – Growing Greener Future: Proceedings of the 9th Australian Society of Agronomy Conference', pp. 211–212. Wagga Wagga, NSW, 20–23 July 1998.

Weeds P.L., Chakraborty S., Fernandes C.D., Charchar M.J.d'A., Ramesh C.R., Kexian Y. & Kelemu S., 2003. Genetic diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centres of origin and utilization. Phytopathology 93, 176–185.

Chapter 17

Role of native *Stylosanthes fruticosa* in the diversity of *Colletotrichum gloeosporioides* in India

C.R. Ramesh[1], S. Chakraborty[2], and D.H. Sukanya[1]

Summary

Pathogenic variation in *Colletotrichum gloeosporioides* infecting species of the tropical pasture legume *Stylosanthes* in India was determined from 277 isolates. Of these, 83 were collected from native populations of *S. fruticosa* and 194 from selected germplasm of *S. scabra, S seabrana, S humilis, S. guianensis* and *S hamata* that have been introduced for use in India over the years. A host differential set comprising eight accessions or cultivars was selected to study pathogenic diversity. Of the eight differentials, *S. scabra* 93116 was immune, cv. Seca was resistant and cv. Fitzroy was highly susceptible. Q10042, 36260 and *S. seabrana* 105546B showed varying degree of resistance whereas *S. hamata* cv. Verano was moderately resistant and susceptible, respectively, to most isolates. A majority of the non-fruticosa isolates were aggressive on one to two host differentials and only one race cluster of 13 isolates was aggressive on six of the eight differentials. The level of diversity varied between field sites and regions. Although *S. seabrana* has been grown in India for just over five years, four race clusters were aggressive to highly aggressive on 105546B. The native *S. fruticosa* population has not contributed to the diversity or the aggressiveness of the *C. gloeosporioides* population in India, and there was little significant difference in anthracnose severity for any host differential among isolates collected from *S. fruticosa* and other *Stylosanthes* species. Given the imminent release of new varieties, ongoing monitoring of the pathogen population will be necessary to detect early appearance of new races.

[1] Indian Grassland and Fodder Research Institute, Regional Station, University of Agricultural Sciences Campus, Dharwad, Karnataka, India
[2] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia

Introduction

Anthracnose, caused by *Colletotrichum gloeosporioides,* is the most important disease of *Stylosanthes* and is of worldwide distribution (Lenné & Calderon 1984). The host is a diverse tropical and subtropical forage legume of great potential that is naturally distributed in Central and South America (Williams et al 1984). Because of their adaptation to acid and infertile soils in semi-arid environments, *Stylosanthes* spp. have been introduced to many countries including Australia, China, India, Thailand and the Philippines to improve animal production and restore soil nitrogen. The presumed centre of genetic diversity of *C. gloeosporioides* is South America, from where the pathogen has most likely been spread to centres of *Stylosanthes* utilisation through infected seed. Irwin and Cameron (1978) described two anthracnose diseases of *Stylosanthes* in Australia. Symptoms of type A comprise a distinct anthracnose lesion, with a light-coloured centre and dark margin, and occur on all species of *Stylosanthes.* Type B causes a general necrosis of mainly *S. guianensis*; blighting of the terminal shoots may lead to plant death. Considerable pathogenic and genetic diversity has been reported in the pathogen population from centres of both diversity and utilisation (Chakraborty et al 2002; Kelemu et al 1997, 1999; Weeds et al 2003). Miles and Lenné (1984) reported variation in both morphology and pathogenicity from a single site in Colombia. In general, commercial utilisation of *Stylosanthes* has been more successful in countries away from the centre of origin (Miles & Lascano 1997), partly due to a limited diversity in the pathogen population.

In India *Stylosanthes* is regarded as the most important tropical legume for the semi-arid and arid regions and is mainly used in wasteland reclamation, as cut-and-carry forage in horticultural systems and as a component of dryland mixed cropping (Ramesh et al 1997). Species of *Stylosanthes* have

been introduced from Australia, South America, Africa and the USA for use in India since 1945 and introductions continue to the present day (Ramesh et al 1997). There is a significant seed industry based on *Stylosanthes* in the Anantpur district of Andhra Pradesh servicing the various utilisation schemes. In addition to the introduced species, the perennial *Stylosanthes fruticosa* Alston is endemic to the southern peninsular regions of India (Hooker 1872–97). It has a wide distribution spanning coastal sand dunes to mangrove areas at Killai Karithurai, near Pitchavarum. It is common in red sandy soils derived from granite, sandstone or coral of low phosphorus status, and on laterite stones at Caper Mount near Cuddalore. We have conducted targeted surveys covering potential native distribution ranges of *S. fruticosa* that show a wide adaptation to most soil types except heavy clays. It occurs from sea level to 1800 m in Tamil Nadu (36 sites), Pondichery (7 sites), Andhra Pradesh (29 sites) and Karnataka (38 sites) covering 8–18°N latitude and 75–85°E longitude. Seed kept in storage for 18 months had 90% hard seed in the pod, but when dehulled and scarified, 60% of the seed germinated within 10 days at 26.5°C. Surveys indicate that anthracnose incidence and severity vary with the location; it is severe in areas adjoining Doddaballapur and the Chickballapur-Bangalore road, moderate in Chettinad (Tamil Nadu) and low in Mundanthuai, Dindigul and Courtalllum, while stands in other locations were free from disease or insect pests.

Stylosanthes fruticosa is taxonomically closely related to *S. scabra*, the species that has been widely adapted in Australia and India, and Mannetje (1984) found it difficult to distinguish the two species based on morphology. Using maternally inherited chloroplast DNA sequence analysis, Liu and Musial (2001) contended that *S. fruticosa* and *S. seabrana,* another species of great potential significance to India, are closely linked. The broad geographical distribution of the native *S. fruticosa* in India implies that a resident population of the anthracnose pathogen may have developed over a long period of time within the country. Genetic and pathogenic diversity in *C. gloeosporioides* from *S. fruticosa* may therefore provide additional challenges to the development and use of resistant cultivars of *S. scabra* and *S. seabrana* in particular. Although understanding the pathogenic and genetic variability is the key to creating effective breeding programs for anthracnose resistance and deployment of resistance, very little is known about the extent of variability in India (Ramesh et al 1997) and certainly no information is available on the nature and extent of pathogenic variation on the native *S. fruticosa.* The objectives of this study were to determine the variability in pathogenicity of *C. gloeosporioides* originating from *S. fruticosa* and to relate this to the overall diversity present in the pathogen population from all species of *Stylosanthes* in India.

Materials and Methods

Source of isolates

A total of 277 Indian isolates, 194 from five *Stylosanthes* species with commercial potential and the remainder from *S. fruticosa,* were used in this study (Table 17.1). Isolates mostly originated from five field sites: Rahuri (Maharastra), Hyderabad (Andhra Pradesh), Dharwad (Karnataka), Coimbatore (Tamil Nadu) and Trivandrum (Kerala), representing different agroecological zones in India where promising germplasm of *Stylosanthes* spp. have been under evaluation for the past four to five years. Other isolates were obtained by surveying areas in the same provinces where *S. scabra, S. hamata, S. seabrana, S. guianensis* and *S. humilis* are grown for wasteland management, soil conservation etc. In addition, surveys were conducted of areas where native populations of *S. fruticosa* are known to exist. These areas were selected using descriptions of the flora of Karnataka, Tamil Nadu, Kerala and Andhra Pradesh published by the Botanical Survey of India. A hierarchical sampling was followed for field surveys, allowing diversity between regions, sites, stands, plants and plant parts to be compared. Data on latitude and longitude were recorded using a global positioning system (GPS 12, Garmin Corporation, Olathe, KS), and assessments of the stand size, level of anthracnose infection and other pest and disease incidence were obtained during the survey. Small pieces of surface sterilised anthracnose-infected plant tissue were plated on oatmeal agar (OMA), and monoconidial cultures were obtained by streaking a spore suspension onto 2% water agar plates and picking up single conidia under a stereoscopic microscope. All isolates were stored under sterile distilled water.

Selection of host differentials, screening and analysis of pathogenic variation

Given the history of *Stylosanthes* in India, our working hypothesis was that the diversity in the Indian pathogen population is not likely to be more extensive than that in Australia. Hence we selected a host differential set similar to the biotype A differential set used in Australia (*S. scabra* Fitzroy and Seca accessions 36260, Q10042 and 93116; *S. viscosa* 33941); and added two new accessions of particular significance to India, *S. hamata* Verano and *S. seabrana* 105546B.

Isolates were screened for pathogenic variation using a seedling bioassay (Chakraborty & Jones 1993), which consisted of growing each of the eight differentials in a sandy loam soil in 30 cavity trays in a greenhouse for six weeks. Each tray, containing three to five replicate seedlings of each differential, was inoculated with a single isolate by spraying to runoff with a conidial suspension of 10^6 conidia/mL Inoculated seedlings were maintained at near 100% relative humidity at 25°C for 48 hours. Ten days after inoculation, plants were visually assessed for disease severity using a 10-point rating scale (0 = disease free, 1 = 1–3% leaf area diseased, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–94% and 9 = 95–100%).

Table 17.1 Location and host of origin of *Colletotrichum gloeosporioides* isolates obtained from *Stylosanthes* spp. of potential commercial significance and the native *S. fruticosa* in India.

Location	*Stylosanthes* species		
	Species other than *S. fruticosa*	*S. fruticosa*	Total
Chikkaballapur, Karnataka	8	17	25
Chitradurga, Karnataka	3	–	3
Doddaballpur, Karnataka	20	43	63
Dharwad-IGFRI, Karnataka	3	–	3
Tegur Farm, Karnataka	40	–	40
Ghati sub., Karnataka	5	5	10
Savanur, Karnataka	1	–	1
RSFPD, Hasser Gatta, Karnataka	9	–	9
MPKV, Rahuri, Maharashtra	15	–	15
Mamidipally, Andhra Pradesh	1	7	8
Gorantala, Palasamudra, Andhra Pradesh	21	2	23
Dhoni Farm KLDB, Kerala	13	–	13
Manjilmadi Kalam, Kerala	9	–	9
Vannamala, Kerala	7	–	7
Trivandram, Vellayanai, Kerala	34	–	34
Coimbatore, Tamil Nadu	1	–	1
Chettinad farm, Tamil Nadu	2	6	8
Dindigul, Tamil Nadu	2	2	4
Ullakuravi, Tamil Nadu	–	1	1
Total	194	83	277

A discriminant function analysis was used to assign isolates into races (Chakraborty et al 1996). In this analysis a training set of data on individuals with known race membership is used to develop linear discriminant functions (LDFs). These functions were then used to classify individuals of unknown grouping. Before commencing any analysis, the multivariate structure of the 277 Indian isolates was examined by principal component analysis. Disease ratings on each host accession were regarded as variables, and each isolate was regarded as an observation. Principal component analysis was used to generate composite scores, which are linear combinations of the ratings on each accession. Principal component scores have the maximum variance of any set of uncorrelated normalised linear combinations of the original ratings.

For race classification of the 194 non-fruticosa isolates, the number of natural clusters was determined by varying cluster numbers from 2 to 40 and the proportional reduction in residual sum of squares (R^2) was plotted against the number of clusters. For this, data on the 194 isolates were averaged over replicates and inoculation dates, log-transformed, and subjected to cluster analysis with a complete linkage algorithm. This showed incremental improvement in R^2 for up to 40 clusters, indicating that 40 clusters were needed to explain 90%, and >30 clusters to explain 80%, of the variation. When 20 clusters were selected as the number of putative race groups, several clusters had zero severity for some differentials; and although between 10 and 15 cluster solutions explained >75% of the variation, the real difference between clusters was only in the level of aggressiveness. Consequently, an 8-cluster solution was used to classify the 194 non-fruticosa isolates. Of these, 39 isolates selected at random were used as the training set to classify the remaining 155 isolates; therefore, the training data set was independent of the test set. Data were log transformed and averaged across replicates and inoculation dates before allocation to race clusters. Isolates within the 95th percentile of the distribution of within-cluster Mahalanobis distance for a given race cluster were classified as a member of that race cluster. Isolates that did not meet this distance criterion for any cluster remained unclassified.

To analyse and assign race clusters to the 83 *S. fruticosa* isolates, LDFs were developed using all 194 non-fruticosa isolates. Finally, all Indian isolates were subjected to general linear model (GLM) analysis of variance, and means were compared by Duncan grouping to examine whether the *S. fruticosa* isolates are overall more severe on any or all of the host differentials.

Results

Multivariate structure of the 277 Indian isolates

The severity data for 83 *S. fruticosa* and 194 non-fruticosa isolates indicate that the first six principal components explain 85% of the total variance, revealing that six dimensions are required to explain the variation amongst the eight differentials. The first component has positive weight on each differential (Figure 17.1a). Variation in this component reflects changes in the overall level of severity over all differentials rather than variation in the pattern of severity across differentials. Eigen values for all other principal components (Figure 17.1b–f) show variation in the pattern of severity across the differentials in various combinations. For instance, the second component has a large negative coefficient on 93116 and a large positive coefficient on Fitzroy (Figure 17.1b); the third shows a large positive coefficient on 33941 but a negative coefficient for 105546B (Figure 17.1c); the fourth indicates a strong positive coefficient for 33491 but a strong negative coefficient for 36260 (Figure 17.1d); the fifth has large positive weight for Q10042 and negative for Verano (Figure 17.1e); and the sixth has a large positive coefficient for both Fitzroy and Seca (Figure 17.1f).

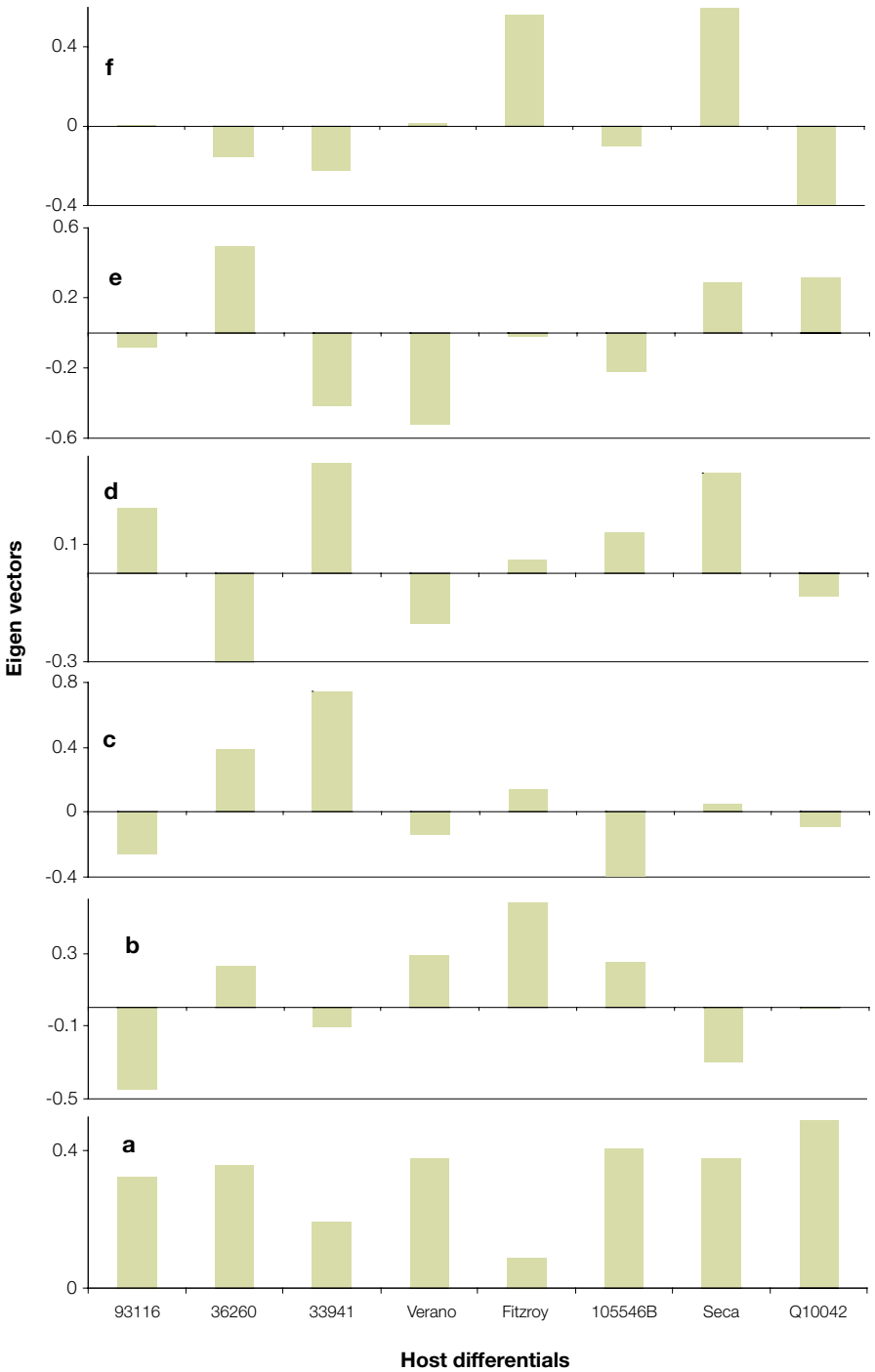


Figure 17.1 Weighting of the first six principal components (a–f) from an analysis of anthracnose severity of 277 Indian *Colletotrichum gloeosporioides* isolates inoculated on 8 host differentials of *Stylosanthes* spp.

Race assignment of non-fruticosa isolates

The eight clusters of the 194 non-fruticosa isolates can be summarised by their mean severity on the eight differentials (Table 17.2). As expected, the pathogenic diversity among the Indian pathogen race clusters was similar to that of the Australian pathogen population. All isolates were weakly aggressive on 93116 and Seca, which are resistant to highly resistant to Australian isolates, and only two clusters were highly aggressive on Q10042 and 33941. This can be clearly visualised by using ln(severity+1) transformed severity >0.8 to denote a high level of aggressiveness. Race cluster 1 with 45 isolates are weakly aggressive on all eight differentials, and isolates in all other clusters were more aggressive on the susceptible Fitzroy. The 19 cluster 2 isolates were aggressive only on Fitzroy, while isolates in clusters 3, 4, 5, 6, 7 and 8 were aggressive on at least another differential in addition to Fitzroy. Clusters 4 and 7 were aggressive on three differentials, cluster 8 was aggressive on four, cluster 6 was aggressive on five, and cluster 5 was aggressive on six of the eight differentials. At least four race clusters were aggressive to highly aggressive on *S. seabrana* 105546B, despite this species being grown in India for just over five years.

Diversity between and within sites was compared using the Shannon's index of diversity (Brown et al 1990). The Shannon index for pathogenic diversity ranged between 0.81 for sites in Karnataka; 0.59 for Kerala; 0.55 for Maharashtra; 0.46 for Tamil Nadu and 0.44 for Andhra Pradesh. Of these, the native *S. fruticosa* was only collected from sites in Karnataka and Tamil Nadu. Race clusters 1, 4 and 7 were found at field sites in all five states, whereas clusters 2 and 8 were only found in Karnataka. Where more than one isolate was obtained from the same lesion, these often grouped under different race clusters.

Race assignment of *S. fruticosa* isolates

LDFs were developed for the eight clusters using the 194 non-fruticosa isolates as the training data set, which were then used to classify the 83 *S. fruticosa* isolates. This showed that non-fruticosa isolates could be easily classified into seven of the eight existing race clusters (Table 17.3) and none remained unclassified. Thirty isolates were grouped in cluster 1, two in cluster 2, 11 in cluster 3, six in cluster 4, and 28 and five in clusters 5 and 6, respectively. There were no cluster 7 isolates but one isolate was classified as race cluster 8. As with non-

Table 17.2 Mean and standard error (in parenthesis) of anthracnose severity of 194 non-*fruticosa* isolates of *Colletotrichum gloeosporioides* on eight host differentials of *Stylosanthes* spp. showing the characteristics of the eight race clusters.

Race cluster (isolates)	Anthracnose severity (ln[severity+1]) on host differentials							
	93116	36260	33941	Fitzroy	Seca	Q10042	105546B	Verano
1 (45)	0.06 (0.01)	0.59 (0.05)	0.48 (0.06)	0.69 (0.05)	0.23 (0.03)	0.20 (0.03)	0.28 (0.05)	0.23 (0.05)
2 (19)	0.03 (0.03)	0.46 (0.10)	0.12 (0.05)	1.81 (0.08)	0.00 (0.00)	0.08 (0.04)	0.02 (0.02)	0.06 (0.04)
3 (43)	0.05 (0.01)	1.17 (0.03)	0.57 (0.06)	1.21 (0.05)	0.24 (0.03)	0.41 (0.05)	0.55 (0.05)	0.51 (0.04)
4 (34)	0.01 (0.01)	0.31 (0.06)	0.25 (0.07)	1.80 (0.08)	0.07 (0.04)	0.04 (0.02)	0.97 (0.08)	0.91 (0.10)
5 (13)	0.06 (0.02)	1.03 (0.17)	1.12 (0.09)	1.45 (0.09)	0.45 (0.17)	0.96 (0.15)	1.01 (0.12)	1.02 (0.13)
6 (17)	0.02 (0.01)	1.86 (0.03)	0.27 90.06)	2.12 (0.04)	0.58 (0.12)	1.82 (0.06)	1.29 (0.12)	1.07 (0.09)
7 (12)	0.03 (0.03)	1.16 (0.14)	0.91 (0.12)	2.18 (0.07)	0.11 (0.06)	0.24 (0.11)	0.37 (0.10)	0.53 (0.11)
8 (11)	0.00 (0.00)	1.92 (0.10)	0.10 (0.06)	2.12 (0.05)	0.28 (0.11)	0.15 (0.08)	0.85 (0.15)	1.08 (0.16)

Table 17.3 Mean and standard error (in parenthesis) of anthracnose severity of 83 isolates of *Colletotrichum gloeosporioides* obtained from *Stylosanthes fruticosa* on eight host differentials of *Stylosanthes* spp. showing the characteristics of the seven race clusters.

Race cluster (isolates)	Anthracnose severity (ln[severity+1]) on host differentials							
	93116	36260	33941	Fitzroy	Seca	Q10042	105546 B	Verano
1 (30)	0.67 (0.11)	0.47 (0.10)	0.53 (0.11)	0.32 (0.07)	0.65 (0.10)	0.40 (0.09)	0.36 (0.10)	0.39 (0.11)
2 (2)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.01 (0.22)	0.35 (0.12)	0.00 (0.00)	0.12 (0.12)	0.00 (0.00)
3 (11)	0.51 (0.12)	1.25 (0.08)	0.41 (0.16)	0.88 (0.04)	0.86 (0.19)	0.70 (0.12)	0.65 (0.10)	1.01 (0.13)
4 (6)	0.44 (0.21)	0.15 (0.08)	0.41 (0.14)	1.77 (0.17)	0.45 (0.29)	0.49 (0.16)	1.19 (0.29)	0.79 (0.33)
5 (28)	0.87 (0.10)	1.10 (0.10)	0.87 (0.11)	1.16 (0.12)	0.89 (0.12)	1.36 (0.11)	1.37 (0.11)	1.01 (0.12)
6 (5)	0.14 (0.06)	1.91 (0.13)	0.45 (0.19)	1.73 (0.28)	0.52 (0.26)	1.89 (0.11)	1.26 (0.27)	1.08 (0.26)
8 (1)	0.00	2.30	0.00	1.15	0.00	0.23	0.23	2.16

Table 17.4 An analysis of variance to test differences in anthracnose severity between *Colletotrichum gloeosporioides* isolates collected from *Stylosanthes fruticosa* and those from species other than *S. fruticosa*.

Origin of isolates	No of isolates	Anthracnose severity (ln[severity+1]) on host differentials							
		93116	36260	33941	Fitzroy	Seca	Q10042	105546B	Verano
Stylosanthes fruticosa	83	0.64 A	0.86 A	0.59 A	0.91 A	0.72 A	0.85 A	0.84 A	0.76 A
Other *Stylosanthes* species	194	0.03 B	0.91 A	0.45 A	1.46 A	0.22 A	0.40 A	0.61 A	0.59 A

Means within a column followed by the same letter are not significantly (P<0.05) different according to Duncan's multiple range test.

fruticosa isolates, two isolates collected from the same lesion did not always group together in the same cluster.

It appears that the native *S. fruticosa* population has not contributed greatly to the diversity or the aggressiveness of the *C. gloeosporioides* population in India since there was no specific adaptation towards this or other species/accessions among isolates collected from this species. Also, there was little significant difference in anthracnose severity for any host differential among isolates collected from *S. fruticosa* and the other *Stylosanthes* species (Table 17.4). This trend is also obvious from the mean severity data for the race clusters of isolates collected from these species (Tables 17.2 and 17.3).

Discussion

This is the first published work on the pathogenic variation in *C. gloeosporioides* infecting species of *Stylosanthes* in India. Eight host differentials were used to classify pathogenic variation in 194 isolates collected from *S. humilis, S. hamata, S. scabra, S. guianensis* and *S. seabrana* and 83 isolates collected from the native *S. fruticosa*. All non-fruticosa and *S. fruticosa* isolates were successfully grouped into one of eight clusters according to their aggressiveness on a set of eight host differentials, which was largely based on the host differential for the Australian biotype A. *Stylosanthes scabra* 93116 and Seca were resistant and *S. scabra* Fitzroy was susceptible to most race clusters. There was no evidence of pathogen adaptation towards the native *S. fruticosa* population or of this native host species contributing to an increased aggressiveness or specialisation in the pathogen to attack other commercially adapted species of *Stylosanthes.* This is consistent with the hypothesis that, in the absence of race-specific resistance, host–pathogen coevolution may favour simple races due to fitness cost associated with virulence (Burdon 1993; Leonard 1977). However, we have only surveyed a very small portion of the native host and pathogen populations and more extensive surveys may reveal specific adaptation not detected in this study. Also, the native *S. fruticosa* collections have not been widely tested for differences in their level of race-specific or non-specific resistance, and more extensive studies are needed for a realistic assessment of host–pathogen coevolution.

Stylosanthes seabrana has performed extremely well at several field sites in India, and with its high herbage and seed yield and the capacity to nodulate with native Indian *Bradyrhizobium* strains (Ramesh et al this volume), this species may prove to be a commercial success for India. At least four race clusters were aggressive to highly aggressive on the *S. seabrana* accession 105546B, despite this species being introduced to India for the first time in 1996. This is similar to the rapid adaptation observed in the *C. gloeosporioides* population towards this species in Australia, where new races have evolved within five years of release of two new *S. seabrana* cultivars (Trevorrow et al 1998). However, widespread damage to *S. seabrana* has not been recorded at field sites in India, and careful and continued monitoring of the pathogen population is necessary to detect early signs of severe damage following the deployment of potential new cultivars. The host differential set and the summary analysis of race clusters described in this paper will provide valuable tools and baseline reference information for future work.

Although there is variability between sites in the composition and prevalence of race clusters, the level of pathogenic diversity in India appears to be less extensive than what may be expected from the extensive genetic diversity previously detected using selectively neutral molecular markers (Weeds et al 2003). Areas where *S. fruticosa* is naturally distributed generally harboured a more diverse pathogen population than sites with a relatively short history of commercial *Stylosanthes* cultivation, although only 10 of the 95 Indian isolates were from *S. fruticosa* (Weeds et al 2003). According to the small number of isolates for which both genetic (Weeds et al 2003) and pathogenic diversity data are available, there is no apparent correlation between genetic and pathogenic groups. Although this is in common with other findings (Chakraborty et al 2002), the relatively limited pathogenic specialisation does not appear to be congruent with the long history of *Stylosanthes* cultivation in India (Ramesh et al 1997). In the current study the highest Shannon's index was for Karnataka and the more complex race clusters (4, 5, and 6) were generally more prevalent at sites in Karnataka, where large resident populations of the native *S. fruticosa* have been detected. However, the sampling of *S. fruticosa* in the other states has not been as thorough or extensive and this needs to be addressed through more comprehensive surveys.

Acknowledgments

The Australian Centre for International Agricultural Research, the Indian Council for Agricultural Research and the Cooperative Research Centre for Tropical Plant Protection funded much of the research reported in this work. This assistance is gratefully acknowledged. We are indebted to Drs P.S. Pathak, Gangaiah, Sridhar, N. Birader and Ms Mini from IGFRI and Dr P. Weeds and Mr R. Perrott from CSIRO for input into ideas, discussions and technical assistance.

References

Brown, J.E., Zar, J.H. & van Ende, C. 1990. Field and laboratory methods for general ecology. Third edition, W.C. Brown Publishers: Dubuque, Indiana, USA.

Burdon, J.J. 1993. The structure of pathogen populations in natural plant communities. Annual Review of Phytopathology 31, 305–323.

Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A. & Thomas, M.R. 2002. Pathogenic variation in *Colletotrichum gloeosporioides infecting Stylosanthes* spp. in a centre of diversity in Brazil. Phytopathology 92, 553–562.

Chakraborty, S. & Jones, P.N. 1993. A rapid bioassay for the assessment of pathogenic variation in Colletotrichum *gloeosporioides* infecting *Stylosanthes scabra.* Plant Disease 77, 1016–1020.

Chakraborty, S., Thomas, M.R. & Ellis, N. 1996. A multivariate analysis of pathogenic variation in *Colletotrichum gloeosporioides* infecting the tropical pasture legume, *Stylosanthes scabra.* Phytopathology 86, 283–289.

Hooker, J.D. 1872–1897. The Flora of British India. Vol 2. L. Reeve and Co. Ltd.: Kent, England.

Irwin, J.A.G. & Cameron, D.F. 1978. Two diseases of *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in Australia and pathogenic specialization within one of the causal organisms. Australian Journal of Agricultural Research 29, 305–317.

Kelemu, S., Badel, J.L., Moreno, C.X., Miles, J.W., Chakraborty, S., Fernandes, C.D. & Charchar, M.J.d'A. 1997. Biodiversity, epidemiology and virulence of *Colletotrichum gloeosporioides*. I. Genetic and pathogenic diversity in *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis.* Tropical Grasslands 31, 387–392.

Kelemu, S., Skinner, D.Z., Badel, J.L., Moreno, C.X., Rodriguez, M.X., Fernandes, C.D., Charchar, M.J. & Chakraborty, S. 1999. Genetic diversity in South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. European Journal of Plant Pathology 105, 261–272.

Leonard, K.J. 1977. Selection pressures and plant pathogens. Proceedings New York Academy of Science 287, 207–222.

Lenné, J.M. & Calderon, M.A. 1984. Diseases and pest problems of *Stylosanthes*. In Stace, H.M. & Edye, L.A. (eds) 'The Biology and agronomy of *Stylosanthes*', pp. 279–293. Academic Press: Sydney, Australia.

Liu, C.J. & Musial, J.M. 2001. The application of chloroplast DNA clones in identifying maternal donors for polyploid species of *Stylosanthes*. Theoretical and Applied Genetics 102, 73–77.

Mannetje, L.t' 1984. Considerations on the taxonomy of the genus *Stylosanthes*. In Stace, H.M. & Edye, L.A. (eds) 'The Biology and agronomy of *Stylosanthes*', pp. 1–21. Academic Press: Sydney, Australia..

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I. *Stylosanthes* development and utilisation in South America. Tropical Grasslands 31, 454–459.

Miles, J.W. & Lenné, J.M. 1984. Genetic variation within a natural *Stylosanthes guianensis*, *Colletotrichum gloeosporioides* host–pathogen population. Australian Journal of Agricultural Research 35, 211–218.

Ramesh, C.R., Bhag Mal, Hazra, C.R., Sukanya, D.H., Rammamurthy, V. & Chakraborty, S. 1997. Status of *Stylosanthes* development in other countries: III. *Stylosanthes* development and utilisation in India. Tropical Grasslands 31, 467–475.

Trevorrow, R.M., Chakraborty, S. & Jansen, P.I. 1998. Anthracnose resistance in *Stylosanthes seabrana.* In Michalk, D.L. & Pratley, J.E. 'Agronomy – Growing Greener Future: Proceedings of the 9th Australian Society of Agronomy Conference', pp. 211–212. Wagga Wagga, NSW, 20–23 July 1998.

Weeds, P.L., Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A., Ramesh, C.R., Kexian, Y. & Kelemu, S. 2003. Genetic Diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centres of origin and utilization. Phytopathology 93, 176–185.

Williams, R.J., Reid, R., Schultze-Kraft, R., Sousa Costa, N.M. & Thomas, B.D. 1984. Natural distribution of *Stylosanthes*. In Stace, H.M. & Edye, L.A. (eds) 'The biology and agronomy of *Stylosanthes'*, pp. 73–102. Academic Press: Sydney, Australia.

Chapter 18

Host–pathogen adaptation in *Colletotrichum gloeosporioides* on Seca in Australia

Sukumar Chakraborty[1] and Ross Perrott[1]

Summary

We have analysed race frequency and aggressiveness of *Colletotrichum gloeosporioides* collected from commercial pastures over a 22-year period to document changes in virulence and aggressiveness. In addition we have studied different commercial seed sources of *Stylosanthes scabra* cultivar Seca to examine whether morphologically and genetically distinct subpopulations exist within commercial Seca in Australia and, if so, whether these host populations support specific aggressiveness or genetic groups of the pathogen. We show that the frequency of race 3, which has specificity to Seca, has increased over the years; while during the same period, the frequency of races 1 and 2, which are either non-pathogenic or weakly pathogenic on Seca, have either declined or remained at a low level. Increasing frequency of race 3 is associated with significant increases in aggressiveness, and highly aggressive strains have arisen from different sources of commercial Seca in geographically separated areas. Since its release in 1976, morphologically and genetically distinct subpopulations have arisen in commercial Seca and its resistance has increased from that of the original accession 40292. There were differences in randomly amplified polymorphic DNA fingerprints, morphological attributes and anthracnose resistance between and within the different sources of commercial Seca, but the subpopulations do not support specific aggressiveness or genetic groups of the pathogen.

[1] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia

Introduction

Nearly a million hectares of tropical and subtropical Australian grazing land is covered by *Stylosanthes* spp. and *S. scabra* cv. Seca dominates over 70% of this area. Seca originates from a germplasm accession (40292) collected by Mr R.J. Williams of CSIRO in 1965 from near Gravata, Pernambuco, in northeastern Brazil (Oram 1990). Since its release in Australia in 1976, Seca has developed vigorous legume-dominant stands with several pasture grasses despite its slow establishment. It has been a strong perennial that can withstand drought. Both *S. hamata* cvv. Verano and Seca are suited to areas with annual average rainfall of 600–1700 mm, but Seca is higher yielding in regions with highly variable rainfall (Edye et al 1975).

Following a single accidental introduction, damaging strains of the anthracnose pathogen *Colletotrichum gloeosporioides* have evolved to devastate commercial *Stylosanthes* cultivars in Australia (Chakraborty 1997). Although largely asexual, horizontal transfer of genetic materials has occurred via retrotransposon and supernumerary chromosomes (Manners et al 2000) to generate variability. Consequently, the once productive *S. scabra* cv. Fitzroy was discarded within five years of its release when stands were severely damaged by race 1 anthracnose strains in the early 1970s, and its susceptibility to all four races in Australia (Chakraborty et al 1996) has seen it disappear completely from commercial pastures. Seca was released with two resistance genes (Cameron et al 1988) but the new race 3 struck within six years of its release (Davis et al 1984), and this race now causes a significant level of damage in some high rainfall areas such as the tablelands in northern Queensland. The heavy reliance on Seca for the vast majority of the Australian tropical sown pastures carries with it an inherent risk. Should a more damaging strain of the pathogen evolve locally or be

accidentally introduced from overseas, this could spell disaster for the entire area that relies on Seca. Similar devastation has occurred in the past, when over 500,000 ha of naturalised *S. humilis* pastures were decimated by an accidental introduction of the pathogen in the 1970s.

There are indications that over the past 22 years aggressiveness of *C. gloeosporioides* has increased towards Seca and to a lesser extent towards the resistant accession 93116, but not towards the susceptible Fitzroy or the partially resistant accession Q10042 (Chakraborty & Datta 2003). During the same period the pathogen has developed new genotypes, but these changes are not directly linked to changes in aggressiveness (Chakraborty et al 1999). The literature on the selective effect of host resistance on aggressiveness is limited, and confusion over the concept and nomenclature of terms relating to pathogenicity (Shaner et al 1992) has contributed to this. In this work we have used 'virulence' as the genetic ability of a pathogen race to overcome genetically determined host resistance that is effective against other races; and 'aggressiveness' as a property of the pathogen that reflects the relative amount of damage caused to the host without regard to resistance genes (Shaner et al 1992). Since aggressiveness is the non-specific disease-causing ability of a pathogen (Vanderplank 1968), it should not be influenced by host resistance genes (Shaner et al 1992). But in the handful of studies on fungal pathogens, host resistance has always significantly influenced aggressiveness, although the relationship between resistance and aggressiveness has not been consistent even for the same host–pathogen combination. For instance, with *Mycosphaerella graminicola* some studies have shown an association between host susceptibility and aggressiveness (Ahmed et al 1996; Mundt et al 1999), while a more recent study (Cowger & Mundt 2002) indicated that resistant hosts selected for more aggressive isolates. Although experimental evidence from polycyclic epidemics in controlled environments shows that aggressiveness increased on both resistant and susceptible cultivars over 25 sequential infection cycles (Chakraborty & Datta 2003), the overall level of aggressiveness was significantly higher on Seca than on Fitzroy. These results may indicate a stronger selection for increased aggressiveness by the resistant cultivar.

There is anecdotal evidence of variation in Seca populations grown in the various regions of Queensland, with commercial seed lots varying in morphology, phenology and agronomic characteristics including flowering time and dry matter yield. Whether this level of variation is underpinned by genetic changes in the Seca population, and whether the variation is indicative of the range of resistances to anthracnose, is not known. If there is a range of anthracnose resistance in the commercial Seca populations, this raises important questions regarding host–pathogen coevolution and whether a certain host population is more likely to support highly aggressive strains of *C. gloeosporioides.*

In this work we further document the changing virulence and aggressiveness of the pathogen population collected from *Stylosanthes* pastures over the past 22 years. In a targeted study we also examine whether morphologically and genetically distinct subpopulations exist within the various sources of commercial Seca in Australia and, if so, whether these host populations support specific aggressiveness or genetic groups of the pathogen.

Materials and Methods

Changing virulence and aggressiveness of *Colletotrichum gloeosporioides*

Infected plants were collected from surveys of commercial *Stylosanthes* pastures in Queensland conducted since 1986 covering more than 20 sites in eastern and northern Queensland between Southedge (17°0'S, 145°20'E) and Samford (27°22'S, 152°53'E). Several samples were collected from a number of points along one to two transects at each site. Small pieces of surface sterilised infected plant tissue was plated on oatmeal agar (OMA) and monoconidial cultures were obtained by streaking a spore suspension onto 2% water agar plates and picking up single conidia under a stereoscopic microscope. In 2000 we sampled the pathogen population by exposing glasshouse-grown disease-free seedlings of the susceptible cultivar Fitzroy for 48 hours as a trap plant within a commercial pasture at the Cedarvale site (24°18'S, 151°38'E), which had been regularly sampled. Monoconidial cultures of the pathogen were obtained on OMA from infected exposed plants. Monoconidial isolates collected before 1986 were obtained from culture collections of Mr Robert Davis of the Queensland Department of Primary Industries and Professor John Irwin of the University of Queensland. A total of 1774 isolates were available from the various years: 4 (1978), 10 (1982), 6 (1984), 11 (1986), 42 (1987), 143 (1991), 119 (1992), 509 (1993), 395 (1994), 454 (1998), 51 (1999) and 30 (2000).

Variation in virulence and aggressiveness of isolates was tested on a host differential set comprising cultivars Fitzroy and Seca and accessions Q10042 and 93116. Fitzroy is susceptible to all races; Q10042 is susceptible to races 1 and 3 but partially resistant to race 2; Seca is resistant to races 1 and 3 but partially resistant to race 3; and 93116 is resistant to all races. Isolates were tested in a bioassay using 6-week-old seedlings of each host differential grown in a naturally illuminated glasshouse at the CSIRO Tropical Pasture Research Centre at Samford, Queensland. Seedlings were grown in 50-mm^2 plastic pots (Kwik pots, Arthur Yates & Co., Australia) in a sandy loam soil treated with 0.4 g/L Ridomil (CIBA-GEIGY Aust. Ltd., Lane Cove, New South Wales) to protect against damping off. Plants were fertilised with a soluble fertiliser (Aquasol, Hortico Australia Pty. Ltd., Laverton North, Victoria) every two weeks. Three to four replicate seedlings of each differential were inoculated with a 10^6 conidia/mL suspension of an isolate grown on OMA for seven days. Inoculated seedlings were maintained in a dew chamber for 48 hours and assessed for percentage leaf area diseased using a 0–9 scale (Chakraborty et al 1999) ten days after inoculation.

Table 18.1 The source of commercial seed lots of *Stylosanthes scabra* Seca used in this study and the 117 *Colletotrichum gloeosporioides* isolates obtained from the seed lots and other sources.

Year	Sample No[a]	Type	Isolates obtained from	
			Seed	Plant material
1996	NQCS1	Commercial seed crop	12	3
1996	NQCS2	Commercial seed crop	16	
1996	NQCS3	Commercial seed crop	3	
1996	NQCS4	Commercial seed crop	3	13
1996	NQCS5	Experiment station plots	3	16
1996	CQCS6	Commercial seed crop	9	25
1993	CQCS7	Commercial pasture		3
1996	CQCS8	Commercial pasture	11	
1993	NQCS9	Commercial seed crop		
1991	NQCS10	Commercial seed crop		
1990	NQCS11	Commercial seed crop		
1993	NQCS12	Commercial seed crop		
1993	CQCS13	Commercial seed crop		
1991	CQCS14	Commercial seed crop		
1987	NQCS15	Commercial seed crop		

[a]NQ = northern Queensland, CQ = central Queensland; exact location and property details have been withheld for commercial reasons.

Host–pathogen adaptation in Seca seed crops

Seed of Seca was obtained from 12 commercial seed lots, 10 in northern Queensland and 2 in central Queensland (Table 18.1). In addition, seed of the original accession 40292, released as the cultivar Seca in 1976 (Oram 1990), was obtained from the CSIRO tropical forage germplasm collection for comparison.

A total of 120 *C. gloeosporioides* isolates were used in this work: 84 of these were recovered from either seed lots or plants collected from five commercial seed crops; 33 were isolated from commercial or experimental Seca pastures (Table 18.1); and three, representing the current pathogenic diversity in the Australian *C. gloeosporioides* population on *S. scabra*, were obtained from the CSIRO isolate collection. The pathogen could not be recovered from six commercial seed samples that had been in storage for over three years. The exact details of the property/field for the source of Seca seed lots and isolates have been withheld for commercial consideration. Monoconidial isolates were obtained by plating surface sterilised seeds (20–30) from each lot and/or infected plant tissue using methods described in the earlier section.

To examine the potential adaptation of pathogen groups to commercial Seca seed sources, all 120 isolates were inoculated onto 15 *Stylosanthes* genotypes. These included a selection of eight commercial Seca sources (NQCS1, NQCS2, NQCS6, NQCS9, NQCS10, NQCS11, NQCS12 and NQCS13); the cultivar Fitzroy; *S. scabra* accessions 40292, Q10042, 36260 and 93116; and *S. seabrana* accessions 92838B and 110361. The *S. scabra* accessions and cultivar are part of a host differential set and the *S. seabrana* are among promising anthracnose-resistant accessions. Six-week-old seedlings of each host were raised in a greenhouse and three to four replicate seedlings of each host were inoculated with a 10^6 conidia/mL suspension for each of the 120 isolates following methods described earlier (Chakraborty & Jones 1993). Following inoculation, seedlings were maintained in a dew chamber for 48 hours and the percentage of leaf area diseased on each seedling and the number and type of lesions were determined from three young infected leaves for each replicate seedling ten days after inoculation. Four types of lesions were recognised: 1) minute brown specks (highly resistant), 2) lesions <0.5 mm in diameter with a dark margin and grey centre (susceptible), 3) lesions 0.5–1 mm in diameter with a dark margin and grey centre (susceptible) and 4) lesions >1.0 mm in diameter with a dark margin and grey centre (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 size and relative frequency (Chakraborty et al 1990). The highest and lowest rankings, respectively, were assigned to the most and least prevalent types and y_w was calculated as follows:

$y_w = [\sum (\text{rank x lesion type})] / \sum \text{rank}$.

To examine whether the level of resistance in the commercial Seca seed sources correlated with other characters, morphology, flowering time and randomly amplified polymorphic DNA (RAPD) profiles were studied for selected commercial seed sources. Plants were raised in the glasshouse using methods described above. Reddening of the stem (1 = trace to no redness of internodes, 2 = redness of most internodes), extent

of branching (1 = minimal branching, 2 = medium level of branching, 3 = extensive branching), days to flowering, number of veins off the leaf midrib, number of fully expanded leaves for the whole plant, and dry weight were determined from three to ten replicate plants for each seed source.

RAPD fingerprints were studied for two replicate plants of each of nine selected seed lots and accession 40292 using six arbitrary primers (Operon Technologies Inc., Alameda, CA 94501). DNA was extracted from the youngest fully expanded *Stylosanthes* leaves as follows: three leaflets were frozen in liquid nitrogen and ground in a mortar and pestle; the ground leaves were placed in 10-mL centrifuge tubes and placed on ice; 4 mL of an extraction solution [41.5 mL of 0.6% sodium sulphite in 208.5 mL of extraction buffer (200mM TrisHCL pH 7.5 + 50mM EDTA + 2M NaCl + 2% CTAB, and adjusted to pH 7.5)] was added to each tube and mixed gently with 1 mL of 5% Sarkosyl; the tubes were incubated at 65°C for one hour, after which 50 µg/mL of proteinase K was added and incubated at 37°C for 30 minutes; an equal volume (5 mL) of phenol/chloroform/iso-amyl alcohol (25:24:1) was added, mixed gently and centrifuged for 30 minutes; supernatant was transferred to fresh tubes to which an equal volume of chloroform/iso-amyl alcohol was added and centrifuged for 15 minutes; a half volume of 7.5M ammonium acetate was added to each tube and incubated for 15 minutes at room temperature, and centrifuged for 15 minutes; a 0.7–0.8 volume of isopropanol was added to each tube and left at room temperature for 10–30 minutes; the tubes were centrifuged for 10 minutes and pellet washed with 70% ethanol, dried and suspended in 1M TE buffer.

Data analysis

A discriminant function analysis was used to assign field isolates to the three Australian races. Existing data on >500 isolates of known race assignment were used as a training set to develop linear discriminant functions (LDFs). These LDFs were used to classify the 1774 isolates collected from field sampling. Data on disease severity on each of the host differentials were ln(y+1) transformed before analysis. We have previously used this technique to classify and identify anthracnose races in Australia (Chakraborty et al 1996) and Brazil (Chakraborty et al 2002) and further details can be obtained from these sources.

Host–pathogen specialisation of isolates from the commercial Seca seed sources were analysed using cluster analysis. The number of optimum clusters for grouping isolates and *Stylosanthes* genotypes was determined separately from the R^2 values for an increasing number of clusters. In addition, for the host population all morphological attributes were combined with their anthracnose severity and y_w data and examined using cluster analysis. The overall relationship between host and pathogen clusters was examined using correlation.

Duplicate gel images were combined and consistent scorable bands identified based on amplicon size. The presence or absence of bands was coded into binary matrices for cluster analysis. Scorable bands were identified from an initial analysis of 29 diverse isolates selected from a previous study (Chakraborty et al 1999). Binary data were analysed for band similarity using the unweighted pair group method with arithmetic averaging, and analyses were based on a paired association matrix derived using Jaccard's coefficient. SAS (SAS Institute, Carey, NC) and the multivariate statistical package MVSP (Kovach Computing Services, Pentraeth, Wales, UK) were used for statistical analysis and to generate dendrograms.

Results

Changing virulence and aggressiveness of *Colletotrichum gloeosporioides*

All 1774 isolates collected from field surveys were classified as one of the three existing Australian races using LDFs developed from their severity on host differentials (Figure 18.1). The seedling bioassay used to detect pathogenic variation determines the virulence and avirulence on a given host differential. However, virulence, as estimated using disease severity measures, is also a measure of the relative aggressiveness of a pathogen isolate, and the race assignment of isolates is complicated by the inherent variability within bioassays. The specificity of the three races can be easily summarised using thresholds, by designating ln(severity+1) <0.1 as weakly pathogenic, >0.1<1.0 as pathogenic, and >1.0 as highly pathogenic. Race 1 is weakly pathogenic on Fitzroy and Q10042 and shows only slight symptoms on 93116 and Seca; race 2 is pathogenic on Fitzroy, weakly pathogenic on Q10042 and non-pathogenic on Seca and 93116; and race 3 is highly pathogenic on Fitzroy, Seca and Q10042, but only weakly pathogenic on 93116. Race 3 is also the most complex of the three races, with specificity towards most of the differentials. Several isolates exhibited a disease severity on Seca that was higher than that of previously sampled race 3 isolates. These represent race 3 isolates with increased aggressiveness on Seca and were left unclassified (Figure 18.1). However, when calculating race frequencies, these isolates were designated as race 3.

Isolates collected in each year were classified separately and the changing frequency of the three races over time was examined using regression analysis. This showed a steady and significant increase in the frequency of race 3 isolates over time (Figure 18.2). Race 2, non-pathogenic on Seca, generally had the lowest frequency, which gradually declined over time, but no race 2 isolates were detected in the field population sampled after 1999. The frequency of race 1 isolates also remained low throughout the survey period, its weak pathogenicity on many differentials and its ability to cause minor symptoms on Seca keeping the small population from disappearing. An increase in its frequency in latter years probably reflects its ability to infect *S. hamata* and *S. seabrana* cultivars.

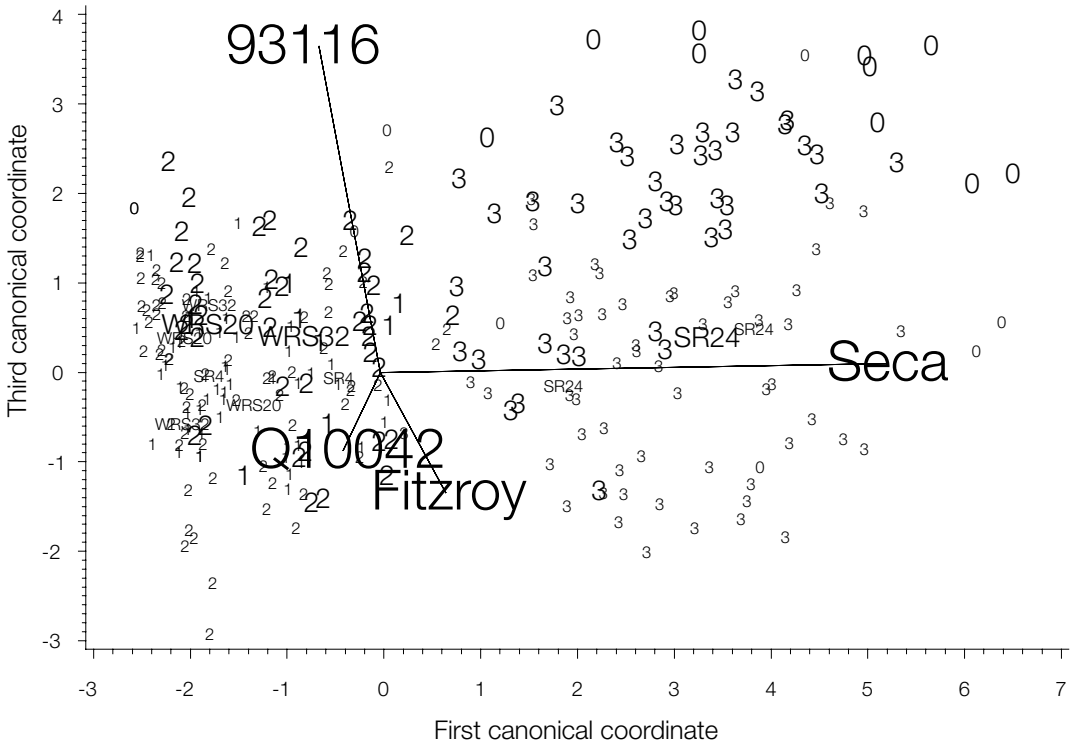


Figure 18.1 Race assignment (large numerals) of *Colletotrichum gloeosporioides* isolates collected from field surveys in 1998 based on their severity on four host differentials (Seca, 93116, Fitzroy and Q10042) and classified using linear discriminant functions developed from a training dataset of existing isolates of known races (small numerals). Unclassified isolates are represented by a zero.

As a measure of aggressiveness on the original Seca, we have plotted the level of disease severity of the 1774 isolates on 40292 (Figure 18.3). In common with the increasing frequency of race 3, aggressiveness has increased steadily and significantly over the 22-year period and there was a four-fold difference between isolates with the lowest and highest severity levels. The large standard errors in the mean for the 40292 severity data prior to 1987 indicate that the pathogen population consisted of both weakly (severity <0.6) and moderately aggressive (severity 0.6–0.8) isolates during this period. However, populations sampled between 1991 and 1999 were more uniform and only aggressive (severity 0.8–1.2) to highly aggressive (severity >1.2) isolates made up the pathogen population. Although the sample size prior to 1987 was relatively small, the generally consistent trend in aggressiveness over the entire period offers a realistic view of changing pathogen aggressiveness. The trend towards increasing aggressiveness was broken in 2000 when the susceptible Fitzroy plants were used to sample the pathogen population. Isolates in 2000 were not highly aggressive towards 40292, indicating that less aggressive isolates were still persisting as a component of the pathogen population. However, this may have been influenced by the small sample size (30 isolates) in 2000.

Host–pathogen adaptation in Seca seed crops

The 84 isolates from the various commercial sources of Seca produced a broad range of anthracnose severity, with a >25-fold difference between the most and the least aggressive isolates. Highly aggressive isolates were associated with seed samples from both northern and central Queensland sites. However, when only the 84 isolates obtained from the five commercial seed sources were considered separately, there was a significant (P<0.05) difference among the commercial seed sources. Overall, isolates obtained from sample NQCS3 were most pathogenic, followed by isolates from samples NQCS2, NQCS4, CQCS6 and NQCS1, respectively.

Potential differentiation among the pathogen isolates and commercial Seca seed lots was examined separately. A cluster analysis of the untransformed disease severity data for the 120 isolates on 15 *Stylosanthes* genotypes showed that five clusters explained >84% and eight clusters explained >93% of the variation among isolates. Only minor increments in R^2 were observed by increasing the number of clusters beyond eight. For the five-cluster solution, cluster 5 contained only the reference isolate wrs32 (race 4/4a), while the remaining 119 isolates, a large majority from commercial Seca, were grouped in one of the remaining four clusters. Of these, cluster 3 isolates were highly pathogenic on all sources of commercial Seca, followed by clusters 2 and 1, respectively, while cluster 4 isolates were weakly pathogenic on all Seca (Figure 18.4).

The 15 *Stylosanthes* genotypes showed different levels of resistance to the 120 isolates. As expected, Fitzroy was the most susceptible genotype and *S. scabra* accession 93116 was among the most resistant (Figure 18.5). Among the eight commercial Seca seed sources, all except NQCS6 showed a moderate level of resistance and all were significantly more resistant than 40292, the original accession released as commercial Seca in Australia.

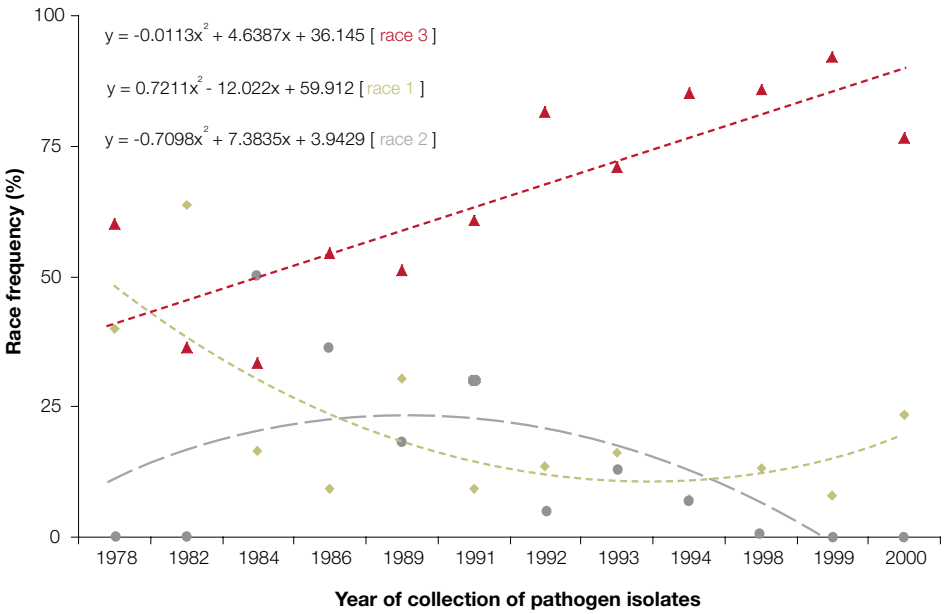


Figure 18.2 Changing frequency of three *Colletotrichum gloeosporioides* races 1 (♦), 2 (•) and 3 (▲) collected from commercial *Stylosanthes* spp. pastures during a 22-year period.

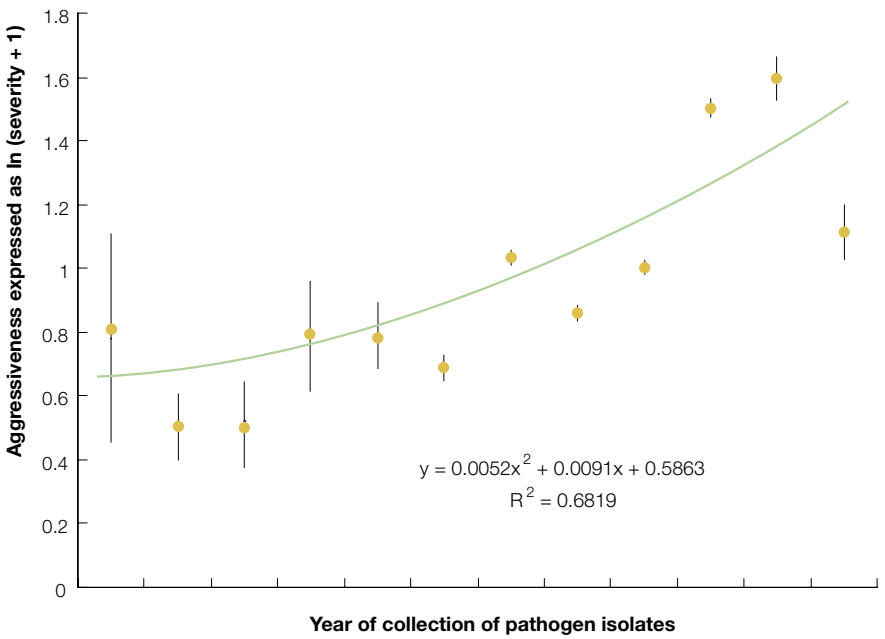


Figure 18.3 Changes in aggressiveness of the *Colletotrichum gloeosporioides* population collected from the field during a 22-year period and tested on *Stylosanthes scabra* accession 40292, the source of commercial Seca, in a glasshouse.

There were only minor differences in morphology and the phenological attributes considered in this work. The number of days to flowering, ranging between 120 and 140 days, was the most useful character separating the eight commercial Seca sources. Contrary to expectations, the commercial seed sources did not flower early, 40292 flowered around the same time as the majority of the commercial Seca, and at least two sources of commercial Seca flowered later than 40292. All morphological, phenological and disease resistance measures were combined in a cluster analysis where five clusters explained >90% of the variation among the eight commercial Seca and 40292. Of these, cluster 1 contained NQCS1, NQCS2, NQCS9, NQCS12 and NQCS13 and this was the only cluster with more than one source of commercial Seca. The other four were distinct clusters, each containing either only one source of commercial Seca (clusters 3, 4 and 5) or 40292 (cluster 2). Cluster 2 was the most susceptible and cluster 1 the most resistant, indicating that many commercial Seca from northern Queensland had developed higher anthracnose resistance than that of the original 40292.

The RAPD profile of duplicate samples of the commercial Seca showed genotypic differences between and within sources. A representative gel is shown for the primer AS5 (Figure 18.6). All samples of the accession 40292 showed the same identical fingerprint. This clearly indicates that genetic changes have occurred in the commercial Seca population following the release of the original accession 40292. However, there was no clear relationship between RAPD clusters and those developed using the morphology or disease severity data for these genotypes.

Discussion

By monitoring the pathogen population over the past 22 years we have shown that aggressiveness of *C. gloeosporioides* has steadily and significantly increased towards the partially resistant cultivar Seca. This is also reflected in the steady and significant increase in the frequency of race 3 (that has specificity to Seca) while, during the same period, the frequency of races 1 and 2 (that are either non-pathogenic or weakly pathogenic on Seca) have either declined or remained at a low

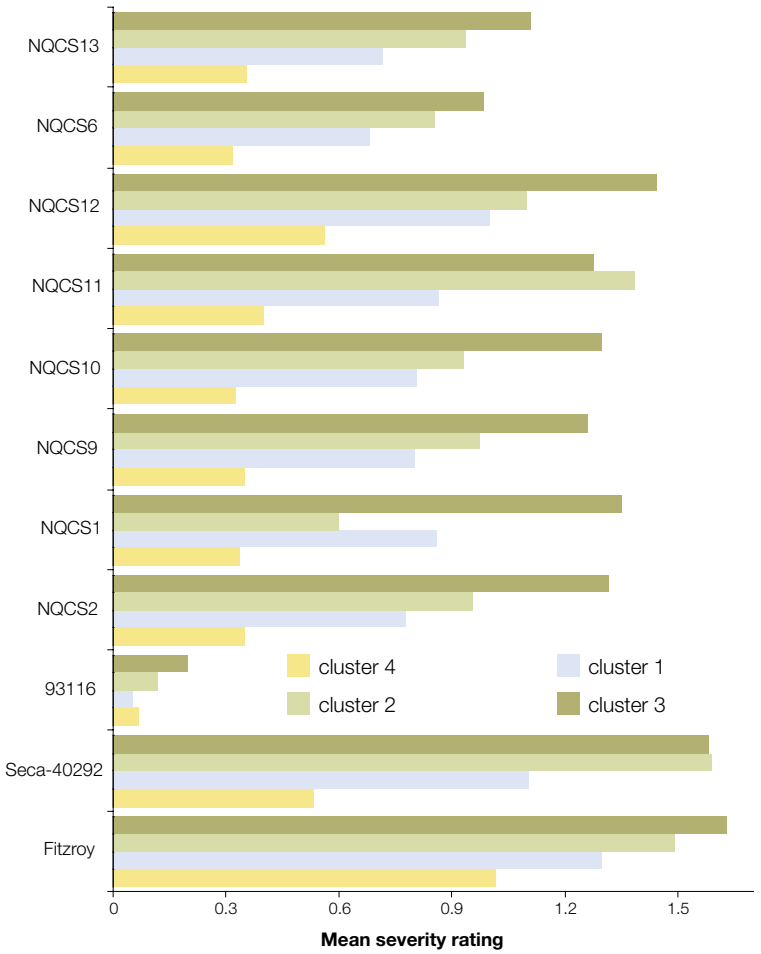


Figure 18.4 Mean anthracnose severity of the four main clusters of 120 *Colletotrichum gloeosporioides* isolates obtained from Seca on eight commercial Seca seed sources and other *Stylosanthes scabra* genotypes.

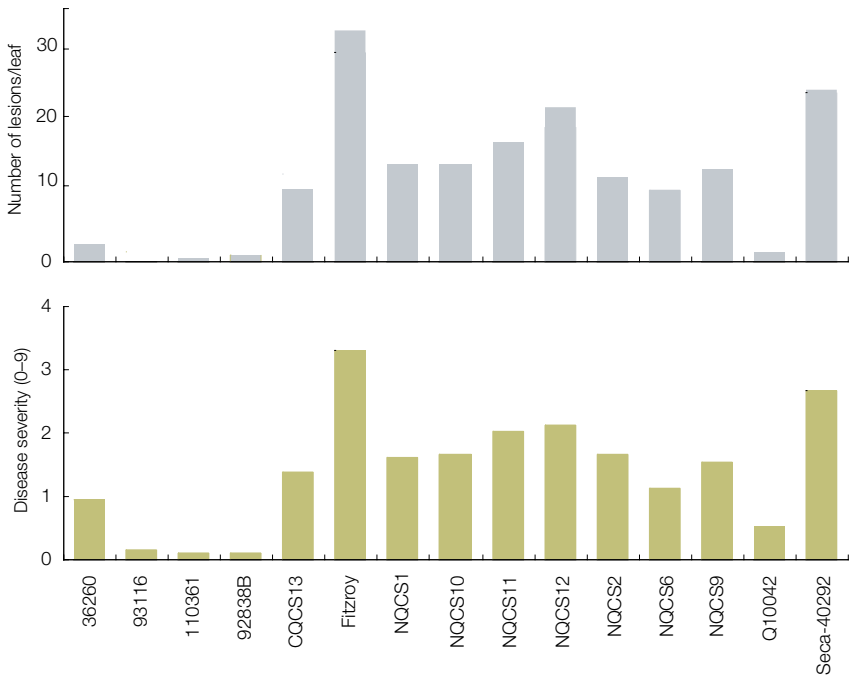


Figure 18.5 Mean anthracnose severity and the number of lesions caused by 120 *Colletotrichum gloeosporioides* isolates on 15 *Stylosanthes genotypes* including the eight commercial Seca seed sources.

level. Since its release in 1976, morphologically and genetically distinct subpopulations have arisen in the commercial Seca population, as is evident from our targeted study of host–pathogen interaction from a selection of commercial sources of Seca. The host population has certainly increased its level of resistance since its release as a commercial cultivar as all commercial sources were significantly more resistant than the original 40292. Although there were differences in RAPD fingerprint, morphological attributes and anthracnose resistance between and within the different sources of commercial Seca, there is no evidence to indicate that these host subpopulations support specific aggressiveness or genetic groups of the pathogen. Highly aggressive strains of the anthracnose pathogen have developed on many different sources of commercial Seca in geographically separated areas.

Despite the differences in morphology, flowering time and RAPD fingerprint, all commercial sources of Seca were more resistant to anthracnose than the original 40292. Host life-history traits are often pleiotropic with disease resistance; a good example is the heat shock proteins that affect the response of a cell to acute stress including those induced by disease (Kischner & Roy 2001). The lack of direct correlation between anthracnose resistance and any of the morphological traits examined suggests that the relevant life-history traits were not considered in this work. However, our results clearly show that the host population developed more resistant genotypes in response to challenges from an evolving and aggressive pathogen population and this bodes well for the future of this cultivar. Although mainly self-pollinating, rates of outcrossing in some species of *Stylosanthes* such as *S. guianensis* can be as high as 21% in Australia (Cameron & Irwin 1986). Genetic changes in *S. scabra* have also occurred through outcrossing and changes in the commercial Seca have been noted since its release. The recently released cultivar Siran is a composite of three *S. scabra* selections, 'Bahia', 'Recife' and 'Feira' (Anon. 1990). Of these, Recife is a selection of Seca with improved anthracnose resistance, early flowering and improved dry matter yield.

As shown previously (Chakraborty et al 1999), the pathogen has developed new genotypes during the same period, but changes in the aggressiveness phenotype are not directly linked to genotypes as determined using selectively neutral

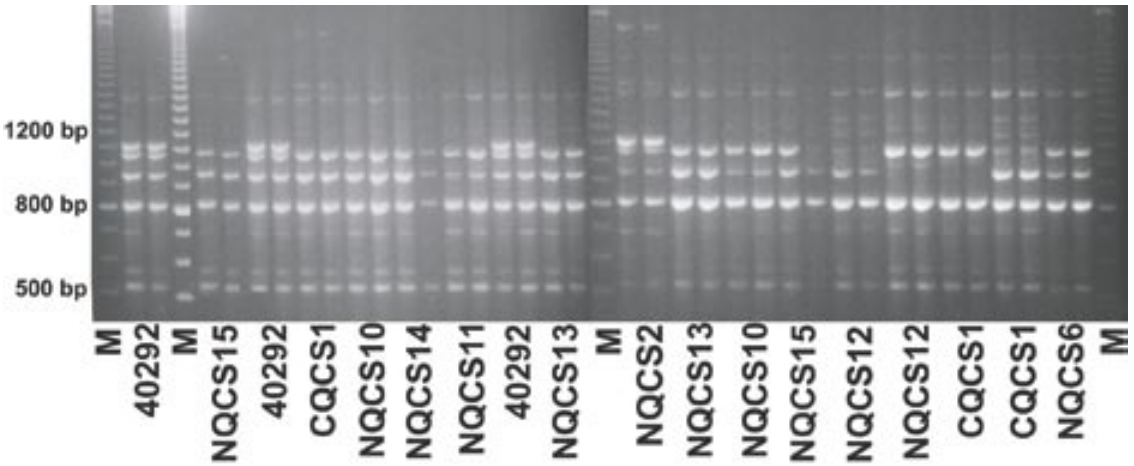


Figure 18.6 Randomly amplified polymorphic DNA profile of duplicate samples of eight sources of commercial Seca and the *S. scabra* accession 40292 showing different genotypes between and within the different commercial sources. The lanes marked as M contain a 100 base-pair ladder DNA (Pharmacia Biotech, Sydney, Australia) as a size marker.

markers. This indicates that pathogenic races have arisen independently within each genetic group. For a specialised necrotroph like *C. gloeosporioides,* both virulence and aggressiveness are important fitness components, and virulence estimates from disease severity measures are confounded by relative aggressiveness. Being sensitive to small changes in the environment such as temperature, aggressiveness data are generally variable (Newton 1989). The confusion in the literature over the concept and nomenclature of terms relating to pathogenicity (Shaner et al 1992), developed largely from studies on biotrophic pathogens with well-understood host–pathogen genetics, has contributed to this. For instance, aggressiveness is the non-specific disease-causing ability of a pathogen (Vanderplank 1968) and should not be influenced by host resistance genes (Shaner et al 1992). In the handful of studies on necrotrophic fungal pathogens, host resistance has always significantly influenced aggressiveness but the relationship between resistance and aggressiveness has not been consistent, even for the same host–pathogen combination (Ahmed et al 1996; Cowger & Mundt 2002; Mundt et al 1999). In our field survey the pathogen population originating from partially resistant Seca has developed increasing levels of aggressiveness over time. A similar trend of increased aggressiveness through infection cycles also occurred under experimental conditions, but there the pathogen increased aggressiveness on both Seca and the susceptible cultivar Fitzroy (Chakraborty & Datta 2003).

Although an overall increased level of anthracnose among the commercial Seca populations was detected in more than one source of commercial seeds, individual plants within each population showed different levels of resistance and were polymorphic for the morphological attributes examined. Also, no specific seed source was overwhelmingly associated with the appearance of pathogen strains showing increased aggressiveness. These results support the finding that frequency-dependent selection can act to maintain polymorphism in host life-history traits and not just host resistance (Kirchner & Roy 2001). Ongoing monitoring of host and pathogen populations is necessary to confirm these relationships.

Acknowledgments

This work was supported by the Australian Centre for International Agricultural Research, the Cooperative Research Centre for Tropical Plant Protection and CSIRO. Raylee Trevorrow, Richard Hopkinson and Peter Wilson provided technical assistance. These assistances are gratefully acknowledged.

References

Ahmed, H.U., Mundt, C.C., Hoffer, M.E. & Coakley, S.M. 1996. Selective influence of wheat cultivars on aggressiveness of *Mycosphaerella graminicola* (Anamorph *Septoria tritici*). Phytopathology 86, 454–458.

Anon. 1990. Shrubby stylo (*Stylosanthes scabra*) 'Siran'. Plant Varieties Journal 3, 33–34.

Cameron, D.F. & Irwin, J.A.G. 1986. Use of natural outcrossing to improve the anthracnose resistance of *Stylosanthes guianensis*. New Zealand Agronomy Society special publication No. 5, 224–227.

Cameron, D.F., Irwin, J.A.G. & Chakraborty, S. 1988. Strategies for anthracnose resistance breeding in *Stylosanthes.* In Proceedings of the Ninth Australian Plant Breeding Conference, pp. 221–222. Wagga Wagga, Australia.

Chakraborty, S. 1997. Recent advances in epidemiological research in Australia. Tropical Grasslands 31, 445–453.

Chakraborty, S. & Datta, S. 2003. How will plant pathogens adapt to host plant resistance at elevated CO_2 under a changing climate? New Phytologist 159, 733–742.

Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A. & Thomas, M.R. 2002. Pathogenic variation in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp. in a centre of diversity in Brazil. Phytopathology 92, 553–562.

Chakraborty, S. & Jones, P.N. 1993. A rapid bioassay for the assessment of pathogenic variation in *Colletotrichum gloeosporioides* infecting *Stylosanthes scabra.* Plant Disease 77, 1016–1020.

Chakraborty, S., Perrott, R., Ellis, N. & Thomas, M.R. 1999. New aggressive *Colletotrichum gloeosporioides* strains on *Stylosanthes scabra* detected by virulence and DNA analysis. Plant Disease 83, 333–339.

Chakraborty, S., Pettitt, A.N., Boland, R.M. & Cameron, D.F. 1990. Field evaluation of quantitative resistance to Anthracnose in *Stylosanthes scabra*. Phytopathology 80, 1147–1154.

Chakraborty, S., Thomas, M. R. & Ellis, N. 1996. A multivariate analysis of pathogenic variation in *Colletotrichum gloeosporioides* infecting the tropical pasture legume, *Stylosanthes scabra*. Phytopathology 86, 283–289.

Cowger, C. & Mundt, C.C. 2002. Aggressiveness of *Mycosphaerella graminicola* isolates from susceptible and partially resistant wheat cultivars. Phytopathology 92, 624–630.

Davis, R.D., Irwin, J.A.G. & Cameron, D.F. 1984. Variation in virulence and pathogenic specialisation of *Colletotrichum gloeosporioides* isolates from *Stylosanthes scabra* cvv. Fitzroy and Seca. Australian Journal of Agricultural Research 35, 653–662.

Edye, L.A., Field, J.B. & Cameron, D.F. 1975. Comparison of some Stylosanthes species in the dry tropics of Queensland. Australian Journal of Experimental Agriculture and Animal Husbandry 15, 655–662.

Kirchner, J.W. & Roy, B.A. 2001. Evolutionary implications of host-pathogen specificity: the fitness consequences of host life history traits. Evolutionary Ecology 14, 665–692.

Manners, J.M., Stephenson, S.A., He, C. & Maclean D.J. 2000. Gene transfer and expression in *Colletotrichum gloeosporioides* causing anthracnose on *Stylosanthes*. In Prusky, D., Freeman, S. & Dickman, M.B. (eds). '*Colletotrichum* Host specificity, pathology and host-pathogen interaction', pp. 180–194. American Phytopathological Society: St. Paul, Minnesota, USA.

Mundt, C.C., Hoffer, M.E., Ahmed, H.U., Coakley, S.M., DiLeone, J.A. & Cowger, C. 1999. Population genetics and host resistance. In Lucas J.A., Bowyer P., Anderson H.M. (eds) 'Septoria on cereals: a study of pathosystems', pp. 115–130. CAB International: Wallingford, UK.

Newton, A.C. 1989. Genetic adaptation of *Erysiphe graminis* fsp *hordei* to barley with partial resistance. Journal of Phytopathology 126, 133–148.

Oram, R.N. 1990. Register of Australian herbage plant cultivars, 3rd edition. CSIRO: Australia, 304 pp.

Shaner, G., Stromberg, E.L., Lacy, G.H., Barker, K.R. & Pirone, T.P. 1992. Nomenclature and concepts of aggressiveness and virulence. Annual Review of Phytopathology 30, 47–66.

Vanderplank, J.E. 1968. Disease Resistance in Plants. Academic Press: New York, USA.

Chapter 19

Yield losses caused by *Colletotrichum gloeosporioides* in three species of *Stylosanthes* in India

C.R. Ramesh[1] and B. Gangaiah[2]

Summary

Yield losses from the fungal disease anthracnose caused by *Colletotrichum gloeosporioides* on three species of *Stylosanthes* was estimated in a field trial at Tegur in Karnataka. Naturally infected control plots of *S. scabra* cvv. Seca and Fitzroy and *S. hamata* cv. Verano were compared with plots protected by three fungicide spray schedules. In Fitzroy the disease incidence and severity initially reached a peak and thereafter remained at this level or slightly decreased as plants matured and weather conditions were unfavorable for disease development. Fitzroy lost 86.9% seed and 73% dry matter yield and Verano lost 60.3% seed and 31.8% dry matter yield compared to plots where the disease was controlled by fungicide sprays every 15 days. In comparison, the resistant Seca lost only 10.7% dry matter and 21.4% seed yield. Compared with unsprayed plants, a fungicidal spray every 15 days reduced anthracnose severity by 52.6% in Fitzroy, 27.9% in Seca and 26.9% in Verano. Despite a similar level of severity, Seca suffered less dry matter and seed yield loss compared to Verano. A benefit–cost analysis shows that spraying fungicide every 15 days offers adequate protection against anthracnose and increases economic returns on the additional cost for disease management.

[1] Indian Grassland and Fodder Research Institute, University of Agricultural Sciences Campus, Dharwad, Karnataka 580005, India
[2] Department of Agronomy, Indian Agricultural Research Institute, New Delhi 110011, India

Introduction

The pasture legume *Stylosanthes* is increasingly being used to supply forage and restore soil fertility and vegetation cover of degraded land in India (Phaikaew et al, this volume; Ramesh et al 1997). With an estimated annual seed production of over 1000 t (Ramesh et al, this volume), *Stylosanthes* already plays a vital role in mitigating fodder shortages, improving soil fertility and restoring degraded lands; and its importance will increase several-fold if the full potential of *Stylosanthes* leaf meal as a component of poultry rations is realised (Guodao et al, this volume). In common with most countries where *Stylosanthes* is grown (Lenné 1994), anthracnose disease, caused by *Colletotrichum gloeosporioides*, is the most serious threat to the economic utilisation of *Stylosanthes* in India. Since its first record in Brazil in the 1930s (Anon. 1937), anthracnose has spread to all countries where germplasm of *Stylosanthes* spp. have been introduced from the centre of diversity in South and Central America. Anthracnose destroyed some 500,000 ha in Australia in the 1970s and has been responsible for the demise of many productive cultivars in many countries (Andrade et al, this volume; Irwin & Cameron 1978; Irwin et al 1984; Lenné 1993; Miles & Lascano 1997). New damaging races of the pathogen have developed in countries such as Australia (Chakraborty et al 1999). A recent review of the anthracnose disease appears in Chakraborty (this volume).

Anthracnose causes serious loss to both forage and seed production in all species of *Stylosanthes*. Lenné & Sonoda (1982) have determined up to 58% loss in dry matter production in *S. hamata* from field studies in southern Florida between 1977 and 1979 by comparing plots sprayed or unsprayed with fungicides to control anthracnose. Comparable yield loss figures have been obtained for Australia, where Davis et al (1987) reported 22% loss of dry

matter yield in the susceptible *S. scabra* cv. Fitzroy, 67% in the partially-resistant *S. hamata* cv. Verano and 53% in the susceptible *S. guianensis* cv. Graham. Anthracnose also caused reductions of 16–49% in seed yield in these three species. In Colombia, however, yield loss to *S. guianensis* from anthracnose was far greater (64 to 100%), as associated reductions in nutritive value rendered some crops totally useless (Lenné 1994).

In India by far the largest commercial seed production occurs in the Ananthapur district of Andhra Pradesh. Since 1974 the number of smallholder farm families engaged in seed production has grown in the Hindupur and Pennugonda Divisions of this district, where in 2002 some 600 families produced seeds on more than 400 ha (Ramesh et al, this volume). Most of the Indian *Stylosanthes* seed industry is based on what appears to be naturalised *S. hamata* cv. Verano and *S. scabra* cv. Fitzroy, and both suffer from anthracnose damage. Severe anthracnose epidemics during 1999 seriously undermined farmer confidence in Fitzroy (Ramesh et al, unpublished). Since then, new species such as *S. seabrana* and new improved germplasm of existing species have shown promise in India (Ramesh et al, this volume). Although reports of serious losses in forage and seed production from anthracnose can be found since the early 1970s, there has been no systematic study of yield loss from this disease in India. It has often been difficult to persuade farmers to grow the new resistant and high-yielding materials in the absence of proven yield loss data and a comparative assessment of the impact of anthracnose on dry matter and seed yield for the commonly used varieties. This paper reports on a field study to establish the extent of losses in dry matter and seed yield due to anthracnose in *S. scabra* cvv. Fitzroy and Seca and *S. hamata* cv. Verano.

Materials and Methods

During 2001–02 a field experiment was established at the research farm of the Indian Grassland and Fodder Research Institute's Regional Research Station at Tegur (15°29'0.11"N, 74°58'0.77"E; altitude 570 m) in Karnataka. The experiment consisted of a factorial arrangement of four treatments x three cultivars in a randomised block design with four replicates. Seedlings were raised in a polythene house and each 5X3 m plot was established by transplanting 6-week-old plants in the field with 30 cm between rows and 25 cm between plants. Scarified seeds of *S. scabra* cvv. Fitzroy and Seca and *S. hamata* cv. Verano were sowed in a peat–sand (1:1) mix in Rite-grow Kwik trays (30 cells per tray). The seedlings were thinned to one plant per cell and grown for six weeks in disease-free conditions in the polythene house with weekly applications of a complete nutrient solution.

After transplanting, anthracnose was induced by artificially inoculating plants with a 10^6 spores/mL suspension of a virulent isolate of *C. gloeosporioides* grown on potato dextrose agar medium in the laboratory. Plants were inoculated by spraying to runoff in the evening to ensure adequate surface wetness for infection to occur. To compare yield loss, plots were either sprayed with the fungicide Benomyl at 280 g/ha every 15, 45 or 60 days using a knapsack-type (ASPEE Ltd, Bangalore) spray unit commencing seven days after transplanting, or they were left unsprayed.

Ten stems were cut from ten randomly chosen plants in each plot for detailed anthracnose assessment at 50, 60, 80, 110 and 140 days after transplanting. Anthracnose severity was recorded using a 10-point rating scale (Chakraborty et al 1990). Five randomly selected plants per plot were cut at ground level, bulked and dried at 80°C for 25 hours, and weighed to determine the dry matter yield. In order to better estimate the maximum yield of each cultivar, plants were sampled and pooled at 80, 110 and 140 days after transplanting, and the total yield was determined from the cumulative dry matter yield. At seed maturity, five random plants from each plot were cut and dried at 40°C for 72 hours. The seed was threshed, cleaned, further dried at 35°C for 48 hours, and weighed for each replicate.

The data on disease severity, and forage and seed yield were analysed using an analysis of variance. The severity data were ln(severity+0.05) transformed before analysis to stabilise variance. The relationship between early anthracnose severity and other parameters and yield was examined using linear regression analysis using SPSS Version 11.2. The benefit:cost ratio for the different spray schedules was also computed.

Results and Discussion

Disease incidence and severity

In all three varieties anthracnose severity increased slightly in the early part of the season, up to 60 days after transplanting, declined by day 80 and then remained unchanged for the remainder of the growing season (Figure 19.1). At each of the five assessments severity was significantly ($P<0.05$) higher on Fitzroy than on Seca or Verano, and there was very little difference between Verano and Seca (data not shown). In all cultivars spraying Benomyl every 15 days reduced anthracnose severity; however, there were a few lesions in each treatment. Overall, anthracnose severity in all treatments was low due to a very dry growing season, and differences between fungicide treated and untreated results were only noticeable in the susceptible Fitzroy, where spraying every 15 days kept severity lower than the other treatments throughout the trial (Figure 19.1).

Compared with unsprayed plants, a fungicidal spray every 15 days reduced anthracnose severity by 52.6% in Fitzroy, 27.9% in Seca and 26.9% in Verano. Despite a similar level of severity, Seca suffered less dry matter and seed yield loss compared to Verano. This ability of Seca to produce high yield despite moderate anthracnose damage has made it a successful cultivar in situations where other moderately resistant varieties have failed to perform or persist. In Australia strains with increasing aggressiveness towards Seca have evolved in pastures and these cause significant damage to Seca under glasshouse inoculations (Chakraborty

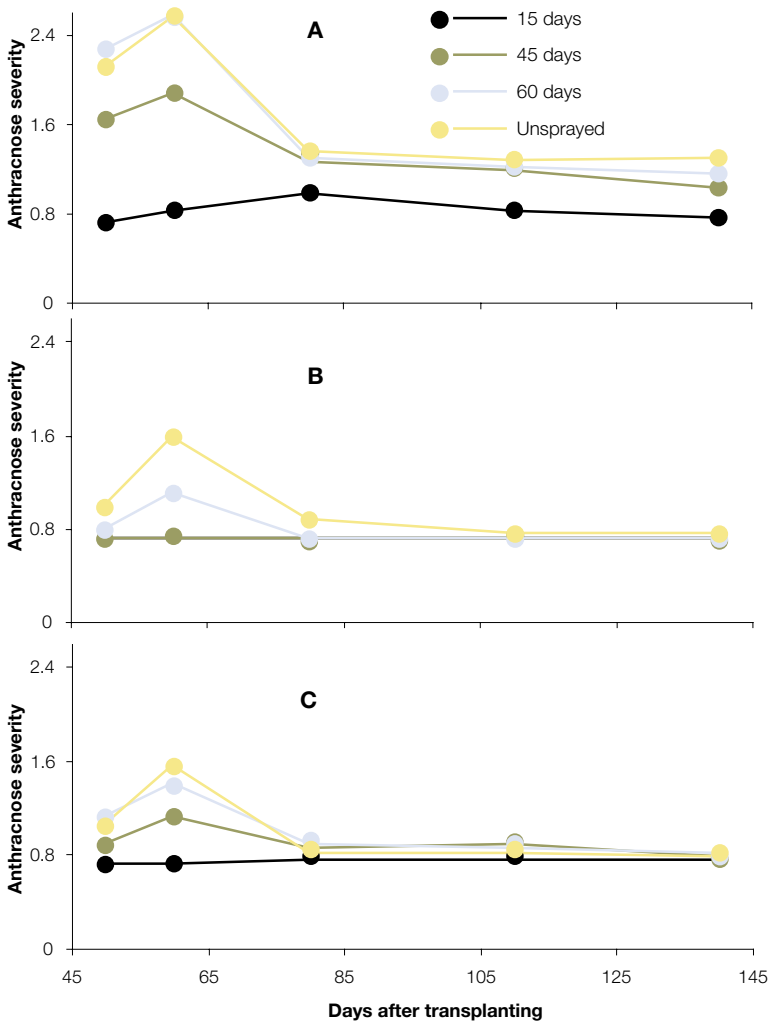


Figure 19.1 Anthracnose progress curves for *Stylosanthes scabra* cv. Fitzroy (A), Seca (B) and *S. hamata* cv. Verano (C) sprayed with Benomyl every 15, 45 or 60 days at Tegur during 2001–02.

et al 1999). Despite this, Seca has by and large performed and persisted well in Australian commercial pastures.

Forage and seed yield

There were significant differences in forage yield among varieties with and without fungicide protection. Green forage and dry matter yield of Fitzroy showed a typical pattern for a susceptible cultivar, where yield progressively decreased with increasing disease severity as the effectiveness of the fungicide decreased with a decreasing frequency of application (Figure 19.2). In Seca, although there was a reduction in green forage yield with increasing frequency of fungicide application similar to that of Fitzroy, this did not translate to a concomitant loss in dry matter yield (Figure 19.2), clearly demonstrating the influence of its high level of anthracnose resistance. The partially resistant *S. hamata* cv. Verano suffered loss in green and dry matter yield due to anthracnose, but fungicide application at all three frequencies reduced this loss (Figure 19.2).

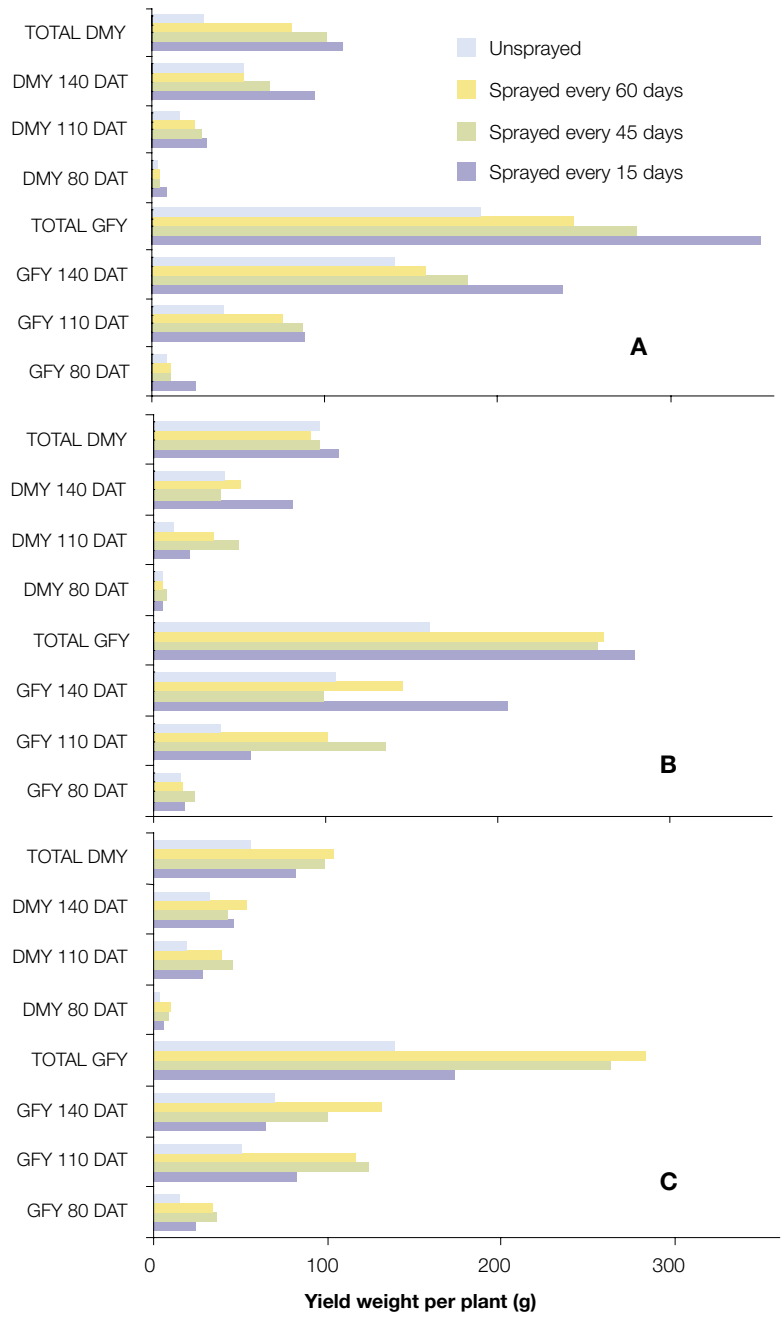


Figure 19.2 Green forage yield (GFY) and dry matter yield (DMY) harvested at 80, 110 and 140 days after transplanting (DAT) of *Stylosanthes scabra* cv. Fitzroy (A), Seca (B) and *S. hamata* cv. Verano (C) infected with *Colletotrichum gloeosporioides* and sprayed with the fungicide Benomyl every 15, 45 and 60 days.

Table 19.1 Percentage reductions in anthracnose severity, and loss of green forage, dry matter and seed yield, in *Stylosanthes scabra* cvv. Fitzroy and Seca and *S. hamata* cv. Verano due to anthracnose compared with treatments where a fungicide was sprayed every 15 days.

Cultivar	Reduction in anthracnose severity (%)	Reduction in green forage yield (%)	Reduction in dry matter yield (%)	Reduction in seed yield (%)
Fitzroy	52.6	45.9	73.0	86.9
Seca	27.9	42.5	10.7	21.4
Verano	26.9	20.4	31.8	60.3

Table 19.2 Net return in Rupees (Rs) from increased seed yield from fungicide sprays at various intervals and the benefit: cost ratio of anthracnose management for three cultivars of *Stylosanthes*

	Fungicide spray schedule				
Cultivar	Every 15 days (Rs)	Every 45 days (Rs)	Every 60 days (Rs)	No spray (Rs)	Mean (Rs)
Fitzroy	24969.8	10268.4	2848.2	–2304.1	8856.3
Seca	33311.4	32631.7	31969.6	27947.1	31465.6
Verano	34082.2	24014.7	13057.0	10957.8	20527.3
Mean	30788.6	22185.8	15958.3	12200.5	

	Benefit:cost ratio		
Spray schedule	Cost (Rs)	Benefit (Rs)	Benefit: cost ratio
15 days	5761.9	18588.1	3.23
45 days	2039.7	9985.3	4.90
60 days	1154.7	3757.8	3.25

Overall, Fitzroy suffered the highest percentage loss in green fodder, dry matter and seed yield (Table 19.1). Although both Fitzroy and Seca showed similar losses in green forage, higher than in Verano, dry matter loss in Seca was just over 10% compared with nearly 32% for Verano. Seca also suffered the least losses to its seed yield due to anthracnose whereas Verano lost over 60% of its seed yield. Although Verano has a level of partial resistance (Iamsupasit et al 1993), the resistance is not as effective as that of Seca. Dry matter yield loss of 26–58% has been reported for two accessions of *S. hamata* in Florida (Lenné & Sonoda 1982). A loss of 33% of dry matter and 51% of seed yield were reported from Australia (Davis et al 1987) and our results of 31 and 60%, respectively, closely match these figures.

The overall extent of losses to forage (22% in Australia, 86% in India) and seed (16% in Australia, 73% in India) yield in India is considerably higher than in Australia for Fitzroy. The trend in seed yield is similar for Verano but dry matter yield loss in Australia (67%) is more than twice that in India (32%). This is most likely a reflection of differences in the composition of the pathogen population (Chakraborty et al, this volume; Weeds et al 2003) and climatic conditions (Chakraborty et al, this volume) in the two countries. The Tegur site was selected for its proximity and similarity in soil and climatic conditions to the largest commercial seed production areas in India, so that findings from this site could be easily and directly applied to the seed production areas. *Stylosanthes* seed production is profitable in these drought-prone areas as other crops, such as groundnut and cotton, have higher requirements for water and other resources (Ramesh et al, this volume). However, *Stylosanthes* is grown widely throughout India including several high rainfall areas where the risk of severe anthracnose damage is much greater (White et al 2001). Losses to dry matter and seed yield can be expected to be much higher in these areas.

Relationship between anthracnose and yield loss

The relationship between green fodder, dry matter and seed yield in diseased treatments at 60 days was explored to assess early disease incidence as a predictor of yield in the three varieties. The regression coefficient ranged from 0.003 to 0.89 for GFY, 0.06 to 0.55 for DMY and 0.86 to 0.99 for seed yield. These indicate significantly high reductions per unit disease in Fitzroy for green fodder yield and in Seca for seed yield.

Benefit:cost ratio of fungicide application

We have calculated the benefit of using fungicidal sprays to control anthracnose in *Stylosanthes* seed crops to examine their economic viability. The benefit calculation included the prices of seeds and fodder. Estimates for the cost of using sprays, including the cost of fungicide and labour and revenues from the increased seed yield, were based on current market price. Net returns were positive for all cultivars; the highest were for Seca (Rs 31465.6), followed by Verano (Rs 20527.3), with the least return for Fitzroy (Rs 8856.3). Details are given in Table 19.2.

Acknowledgments

The Australian Centre for International Agricultural Research and the Indian Council for Agricultural Research funded this research. Drs P.S. Pathak, Sridhar, N. Birader and Ms Mini provided input into ideas, discussions and technical assistance. All input and assistance are gratefully acknowledged.

References

Anon. 1937. Informaçoes sobre algumas plantas forrageiras. Ministerio da Agricultura: Rio de Janeiro.

Chakraborty, S., Perrott, R., Ellis, N. & Thomas, M.R. 1999. New aggressive *Colletotrichum gloeosporioides* strains on *Stylosanthes scabr*a detected by virulence and DNA analysis. Plant Disease 83, 333–339.

Chakraborty, S., Pettitt, A.N., Boland, R.M. & Cameron, D.F. 1990. Field evaluation of quantitative resistance to Anthracnose in *Stylosanthes scabra.* Phytopathology 80, 1147–1154.

Davis, R.D., Irwin, J.A.G., Shepherd, R.K. & Cameron, D.F. 1987. Yield losses caused by *Colletotrichum gloeosporioides* in three species of *Stylosanthe*s. Australian Journal of Experimental Agriculture 27, 67–72.

Iamsupasit, N., Cameron, D.F., Cooper, M., Chakraborty, S. & Edye, L.A. 1993. Inheritance of anthracnose resistance and yield in the tropical pasture legume *Stylosanthes hamata.* Australian Journal of Agricultural Research 46, 1353–1364.

Irwin, J.A.G & Cameron, D.F. 1978. Two diseases in *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in Australia and pathogenic specialization within one of the causal organisms. Australian Journal of Agricultural Research 29, 305–317.

Irwin. J.A.G., Cameron, D.F. & Lenné, J.M. I 984. Response of *Stylosanthes* to anthracnose. In Stace, H. M. &. Edye, L. A. (eds) 'The biology and agronomy of *Stylosanthes'*, pp. 295–310. Academic Press: Sydney.

Lenné, J.M. 1993. *Colletotrichum* diseases of legumes. In Bailey, J.A. & Jeger, M.J. (eds) '*Colletotrichum:* Biology, pathology and control', pp. 134–166. CAB International: Wallingford, UK.

Lenné, J.M. 1994. Diseases of *Stylosanthes.* In Lenné, J.M. & Trutmann, P. (eds) 'Diseases of tropical pasture plants', pp. 21–42. CAB International: Kent, UK.

Lenné, J.M. & Sonoda, R.M. 1982. effect of anthracnose on yield on the tropical forge legume, *Stylosanthes hamata*. Phytopathology 72, 207–209

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I *Stylosanthes* development and utilisation in South America. Tropical Grasslands 31, 454–459.

Ramesh, C.R., Bhag Mal, Hazra, C.R., Sukanya, D.H., Ramamurthy, V. & Chakraborty, S. 1997. Status of Stylosanthes development in other countries. III. Stylosanthes development and utilization in India. Tropical Grasslands 31, 467–475.

Weeds, P.L., Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A., Ramesh, C.R., Kexian, Y. & Kelemu, S. 2003. Genetic Diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centres of origin and utilization. Phytopathology 93, 176–185.

White, N., Chakraborty, S., Fernandes, C.D., Charchar, M.J., Melkania, N.P., Ramesh, C.R., Gangaiah, B., Guodao, L., Cameron, D.F. & Miles, J.W. 2001. Selection of high yielding and anthracnose resistant *Stylosanthes* for Brazil India and China. Proceedings of XIX International Grassland Congress, 10–23 February, Piracicaba, Brazil, pp. 232–234.

Chapter 20

Weather dependency of anthracnose and risk mapping

S. Chakraborty[1] R. Ghosh[2], M. Ghosh[2], A.K. Maji[3], N. White[1], C.D. Fernandes[4], M.J. Charchar[5], C.R. Ramesh[6] and S. Kelemu[7]

Summary

In this work on the effects of weather and severity of anthracnose caused by *Colletotrichum gloeosporioides* on the tropical pasture legume *Stylosanthes scabra,* data were collected from seven field sites in Australia, India, Brazil and Colombia. Artificial neural network (ANN) models were developed using data from some or all field sites to predict anthracnose severity at other sites. The ANN models trained on data from all sites within one continent correctly predicted disease severity in another continent with varying degrees of success; the overall prediction error was 28% for the Australian model, 27% for the South American model and 48% for the Indian model. Rainfall, radiation and leaf wetness were the most important weather variables in all ANN models. The overall risk of anthracnose differed among the seven field sites. The sites in South America were generally more prone to severe disease development, but this was not directly related to the average annual rainfall. A hazard map for India was developed after combining data on soil type, climatic suitability and anthracnose risk. The agroclimatic conditions that make parts of peninsular India highly suitable for *S. scabra* also render them conducive to the development of severe anthracnose. Ideally, cultivars with high level of anthracnose resistance should only be planted in these areas.

[1] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia
[2] School of Information Technology and Mathematical Sciences (ITMS), University of Ballarat, PO Box 663, Victoria 3353, Australia
[3] National Bureau of Soil Survey and Land Use Planning, Nagpur 440010, India
[4] EMBRAPA-CNPGC/Doctor student UNESP-FCA, Botucatu-SP, 18609-490 Brazil
[5] EMBRAPA-CPAC, 73301-970, Planaltina, DF, Brazil
[6] Indian Grassland and Fodder Research Institute Regional Station, Dharwad, Karnataka, India
[7] Centro Internacional de Agricultura Tropical, Cali, Colombia

Introduction

Conidia of the anthracnose pathogen *Colletotrichum gloeosporioides* are produced in a mucilaginous matrix (Louis & Cook 1985) that requires surface wetness for splash dispersal and subsequent infection (Fitt & McCartney 1985). Under controlled conditions, anthracnose development is favoured by a leaf surface wetness period of 12 hours or longer, maximum severity is reached after 36 hours wetness, and severity is unaffected by brief interruptions of 2–4 hours in wetness if relative humidity (RH) is maintained over 85% (Chakraborty et al 1990). A temperature range of 20–30°C favours severe disease development if 24-hour leaf wetness is maintained (Irwin et al 1984).

In the field, wetness periods of <2 hours duration are the most frequent and <10% of all wetness periods are of 10 hours or longer duration (Chakraborty et al 1990). Although the roles of free surface water, temperature and moisture on the infection of *C. gloeosporioides* are well known, field studies have not always produced clear-cut quantitative relationships between specific sequences of weather events and anthracnose (Davis et al 1987). Chakraborty and Billard (1995) used multiple regression (REG) analysis to demonstrate that mean daily temperature and RH above certain thresholds were necessary for successful infection at a field site in Australia, but the REG models did not always correctly predict infection events at the same site in a different season. Subsequently, another independent study also showed that although REG models were adequate to predict site-specific and season-specific infection, these models did not have the ability to generalise across seasons and/or sites (Pangga 2002).

The role of weather in plant disease development has been studied using other analytical approaches besides multiple regression analysis. These include the use of stochastic

models (Chakraborty & Smyth 1995; Shaw 1994), fuzzy numbers (Scherm 2000) and artificial neural networks (ANNs), among others. Using a conditional ordinal logistic regression model, Chakraborty and Smyth (1995) showed that weather variables that directly influence the availability of moisture were the key determinants of anthracnose severity in the field, but the success or otherwise of this model to predict severity at other sites/seasons has not been ascertained. In recent years ANNs have been used in plant protection research to model leaf wetness (Francl & Panigrahi 1997), disease dynamics (Yang et al 1995), disease forecasting (Crisci et al 1998; De Wolf & Francl 1997) including the anthracnose disease of *Stylosanthes* (Chakraborty et al 2003), and pesticide in soil (Yang et al 1997).

New virulent strains of *C. gloeosporioides* have decimated many promising varieties of *Stylosanthes* spp. in all parts of the world where this legume is grown for commercial use. The extensive genetic variation in host and pathogen populations at the centre of diversity in South and Central America (Chakraborty et al 2002; Weeds et al 2003) has given rise to new pathogen races that severely affect many previously resistant varieties and, so far, no commercial variety has escaped serious anthracnose damage (Miles & Grof 1997). *Stylosanthes* has been more successfully used in countries away from centres of host–pathogen diversity (eg Australia and China) due largely to a less-diverse pathogen population (Weeds et al 2003). However, severe devastations have occurred in these countries, including the destruction of over 500,000 ha of *S. humilis* in Northern Australia in the 1970s. Besides host–pathogen diversity, there are differences in ecosystems and farming systems between and within the centres of origin and utilisation. For instance, antagonistic bacteria that suppress *C. gloeosporioides* on leaf surfaces in the humid tropics of Peru do not have any effect on the pathogen in the savannah ecosystems (Lenné & Brown 1991). However, since the fundamental quantitative relationship that demonstrates the dependence of anthracnose on physical weather is not expected to change, it should be possible to develop, validate and apply weather-based anthracnose models using data from across sites, countries and continents.

In a research project funded by the Australian Centre for International Agricultural Research (ACIAR), disease development and weather were monitored at selected field sites in Australia, Brazil, Colombia and India to improve knowledge of anthracnose epidemiology for effective disease management. Using data from Australian, Indian and South American field sites, this paper examines the usefulness of ANN to develop robust weather-based models to predict anthracnose development at sites within or outside the continent. In addition, weather-based anthracnose risk developed using existing REG model outputs are combined with soil and climatic suitability of *S. scabra* to map anthracnose risk for India using a geographical information system (GIS).

Materials and Methods

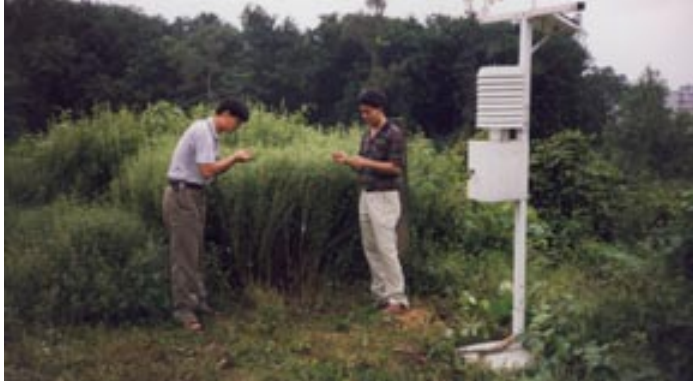
Weather and severity data

Data were collected from three sites in Australia, two sites in Brazil and one site each in Colombia and India. All three Australian sites were in Queensland: the CSIRO research station at Samford (27°22'S, 152°53'E), and the Queensland Department of Primary Industries stations at Southedge (17°0'S, 145°20'E) and Springmount (17°13'S, 145°18'E). The Centro de Pesquisa Agropecuária dos Cerrados research station of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) at Planaltina (15°36'S, 47°42'W) and the Centro Nacional de Pesquisa de Gado de Corte of EMBRAPA in Campo Grande (20°26'S, 54°42'W), Mato Grosso do Sul, were the Brazilian sites. The Centro Internacional de Agricultura Tropical field station in Carimagua (4°30'N, 71°19'W) was the Colombian site. Dharwad (15°29'0.26"N, 74°58'49.3"E) in Karnataka state was used as the Indian site. Details on site layout, instrumentation, disease assessment and analysis have been given earlier (Chakraborty et al 2003).

At each site ten weather attributes were collected from automated weather stations: maximum (RMX) and minimum (RMN) air RH; maximum (TMX) and minimum (TMN) air temperature; sunshine hours (SUN); total radiation (RAD); rainfall (RAIN); hours of leaf wetness (LWP); wind daily total (WND) and gust (GST). Anthracnose severity on the susceptible *S. scabra* cultivar Fitzroy was assessed based on the proportion of leaf area diseased (Chakraborty 1990), and classified as: disease free, very low (<0.2), low (0.2–0.4), medium (0.4–0.6), high (0.6–0.8) and very high (>0.8). A total of 200 days of disease severity data were used, comprising 40 days from Samford (1987–92), 22 from Southedge (1989–97), 15 from Springmount (1989–91), 34 from Campo Grande (1995–98), 28 from Planaltina (1995–2000), 12 from Caquetá (1994–96), 11 from Carimagua (1994–96) and 38 from Dharwad (1999–2002). Data from the day of disease assessment and the previous 24-hour period were considered for each weather variable in developing ANN models. For climate matching and risk mapping, six days of weather data were averaged for analysis.

ANN analysis

ANNs are parallel computing systems with large numbers of simple, highly interconnected processing nodes or neurons. ANN input neurons obtain values of input variables, weather attributes in this case, and standardise these values; hidden neurons perform internal computations to provide non-linearity; and output units compute predicted values and compare these with values of the target variables, severity classes in this case. Output is compared with the target value to compute error that is minimised by an iterative process. Fundamentals of ANNs and computational implementations can be found in textbooks such as Bishop (1995) and Hertz et al (1995). One front node (8 X Ultra Sparc III processor 750MHz, 8GB memory) and 8 back nodes (each containing 8 X Ultra Sparc III processors 900MHz, 8GB memory) in a cluster formation



An automated weather station near stylo plots for regular anthracnose monitoring at CATAS.

on a HPC super computer at Griffith University, Gold Coast, Australia was used as the processing unit. A fully connected feed forward network with one hidden and one output layer were used. Codes were written in MATLAB (The MathWorks Inc., Natick, MA 01760-2098, USA).

'Continent-wise' ANN models were trained on data for all sites within a continent and tested to predict the daily severity class for each site in another continent. In 'cross-continent' models all sites were pooled together and models were trained on data for all except the site being predicted. Each site was dropped in turn and the severity class of the dropped site predicted using models developed from the six remaining sites. The sensitivity of weather attributes in the ANN models and the overall anthracnose risk were determined using analytical tools described earlier (Chakraborty et al 2003).

Climate matching and risk mapping

For each disease severity data point, the weather data for the six preceding days were averaged to account for the week-long incubation period. Initial difficulties with the automated weather station in India prevented us from obtaining long-term weather data, and only weather data from Samford and Southedge in Australia and Campo Grande and Planaltina in Brazil were considered in this work. Data were plotted for visual examination and subjected to a principal component analysis. Outputs of the analysis were combined with information from published literature to develop climate-matching models for *S. scabra* and *C. gloeosporioides.* The CLIMEX software (Sutherst & Maywald 1985) was used for principal component analysis. The 'ecoclimatic index' produced by CLIMEX gives an overall measure of the potential of a given location to support a permanent population, and this was used to compute a suitability rating between 0 and 1 for both the host and pathogen populations. Model outputs were saved as ASCII files and linked into GIS as a grid of values with a cell size of 0.6 degree. The soil requirements for *Stylosanthes* species were obtained from the literature to build a predictive model using available water content, pH, texture and depth. These characteristics were modelled initially for one district (Bankura in the state of West Bengal) using Arc/Info (ESRI Inc., Redlands, CA, USA) and finally expanded to the whole of India using a Spatial Model Builder (in Arcview). The soil suitability index was compared with the generalised CLIMEX model for the growth potential of *Stylosanthes.* Finally, the potential distribution was modified by the risk of serious anthracnose developing.

Table 20.1 Classification error of artificial neural network models developed using data from Australian, South American or Indian field sites as the training set to predict anthracnose severity class at sites in another country

Training data	Testing site	Type I error (%)[a]	Type II error (%)[b]	Total error (%)
Australian sites	Campo Grande	11.8	8.8	20.6
	Planaltina	10.7	7.1	17.8
	Carimagua	9.1	27.3	36.4
	Dharwad	36.8	0	36.8
South American sites	Samford	5	15	20
	Southedge	9.1	18.2	27.3
	Springmount	6.6	13.3	20
	Dharwad	34.2	5.3	39.5
Indian sites	Samford	5	37.5	42.5
	Southedge	22.7	31.8	54.5
	Springmount	6.6	20	26.6
	Campo Grande	64.7	0	64.7
	Planaltina	3.6	21.4	25
	Carimagua	72.7	0	72.7

[a] Days when the predicted severity class was lower than the actual severity class

[b] Days when the predicted severity class was higher than the actual severity class

Results and Discussion

ANN analysis

Overall, the continent-based ANN models were not very accurate in predicting disease severity class at field sites in another continent (Table 20.1). The overall prediction error was 28% (ranging 18–37%) for the Australian model, 27% (20–39%) for the South American model and 48% (25–73%) for the Indian model. The Australian ANN model varied in its prediction of the three South American and the Indian sites. The South American model predicted severity at all the Australian sites equally well, but the prediction error for the Dharwad site was nearly 40%. The overall type I error percentage (under-prediction) was higher (21.3%) for the three continent-wise ANN models than the type II error (14.7%). At Dharwad technical difficulties with the operation of an automatic weather station had often led to erroneous weather data and thus the results from this site must be considered preliminary.

In the cross-continent ANN models data from all Australian, Indian and South American sites were pooled and models were trained on data for six sites to predict the severity class for the remaining seventh site. Of the seven models developed in this way, those without data from Campo Grande or Carimagua successfully predicted severity on almost 80% of days for both Campo Grande and Carimagua. The other five models

Table 20.2 Classification errors of artificial neural network models developed using pooled data from six Indian, Australian and South American field sites as the training set to predict anthracnose severity class for the seventh site.

Training data	Testing site	Type I error (%)[a]	Type II error (%)[b]	Total error (%)
All except Samford	Samford	2.5	32.5	35.0
All except Southedge	Southedge	31.8	0	31.8
All except Springmount	Springmount	6.6	26.6	33.3
All except Campo Grande	Campo Grande	2.8	17.7	20.5
All except Planaltina	Planaltina	25.0	7.1	32.1
All except Carimagua	Carimagua	0	18.2	18.2
All except Dharwad	Dharwad	31.6	7.9	39.5

[a] Days when the predicted severity class was below the actual severity class

[b] Days when the predicted severity class was above the actual severity class

were accurate on >60% of days (Table 20.2). The mean prediction error of the seven cross-continent models (30.1%) was lower than of the continent-based models (36%).

This is one of a growing number of reports demonstrating the usefulness of ANN models for weather and plant disease prediction (Chtioui et al 1999; DeWolf & Francl 1997; Francl & Panigrahi 1997) and other related analysis (Hajmeer & Basheer 2003). DeWolf & Francl (1997) trained an ANN model on data from a field site that accurately predicted 81–87% of wheat tan spot (caused by *Pyrenophora tritici-repentis*) infection for the same site. In our work ANN models predicted anthracnose severity for sites that were never used in model training/development. Training and testing sites were geographically distant, often located in another continent. Despite this rigorous and stringent validation, accuracy of the ANN model prediction was as high as 82%, and models developed from the multi-site data may prove useful over a broad range of field sites.

A sensitivity analysis showed that RAIN, LWP and RAD are the three most important weather attributes in both the continent-based and cross-continent models (Table 20.3). The most important weather attribute in the continent-based, ie Australian, South American and Indian, models was LWP, RAD and RAIN, respectively. RAIN on the day of disease severity assessment and RAD for the previous 24-hour period were by far the most important variables in the cross-continent models.

Previous greenhouse work had established the importance of moisture related variables to anthracnose infection (Irwin et al 1984; Chakraborty et al 1990), and field-based research had quantitatively linked moisture related weather variables to daily infection of *Stylosanthes*. Of these, Chakraborty and Billard (1995) and Pangga (2002) used multiple linear regression and Chakraborty and Smyth (1995) used a stochastic logistic regression where rainfall, relative humidity and temperature were important predictors, but none has been validated using data from an independent source. Among other factors, radiation, evaporation and high temperature were significant terms in some models due to their influence on the extent and duration of free water on plant surfaces (Chakraborty & Smyth 1995). In common with earlier research, all

Table 20.3 Sensitivity (5% or higher) of weather attributes [minimum air RH (RMN); maximum air temperature (TMX); total radiation (RAD); rainfall (RAIN); hours of leaf wetness (LWP); wind daily total (WND) and gust (GST)] on the day of disease severity assessment and for the previous 24-hour period in artificial neural network models, where continent-based models were trained on data from one continent to predict severity in another and cross-continent models were trained on data from six Australian, Indian and South American sites to predict severity at the seventh site.

	Sensitivity 5% or higher									
	Continent-based model			Cross-continent model						
Weather attribute	Australia	South America	India	Samford	Southedge	Springmount	Campo Grande	Planaltina	Carimagua	Dharwad
Weather on day of disease severity assessment										
RMN	8	–	–	–	–	–	–	–	–	–
RAD	8	33	6	10	26	8	9	13	8	7
RAIN	5	8	51	26	12	19	22	8	30	18
LWP	33	–	6	–	9	12	20	23	–	8
WND	–	–	6	5	–	–	–	6	7	5
GST	–	–	–	–	–	–	–	–	5	–
Weather for the day before disease severity assessment										
RMN	–	–		–	–	–	–	–	5	–
TMX	–	–	–	6	5	–	5	6	7	6
RAD	10	24	10	15	11	23	15	11	3	15
RAIN	13	–	5	9	17	7	5	11	11	10
GST	–	–	–	–	–	–	–	–	–	5

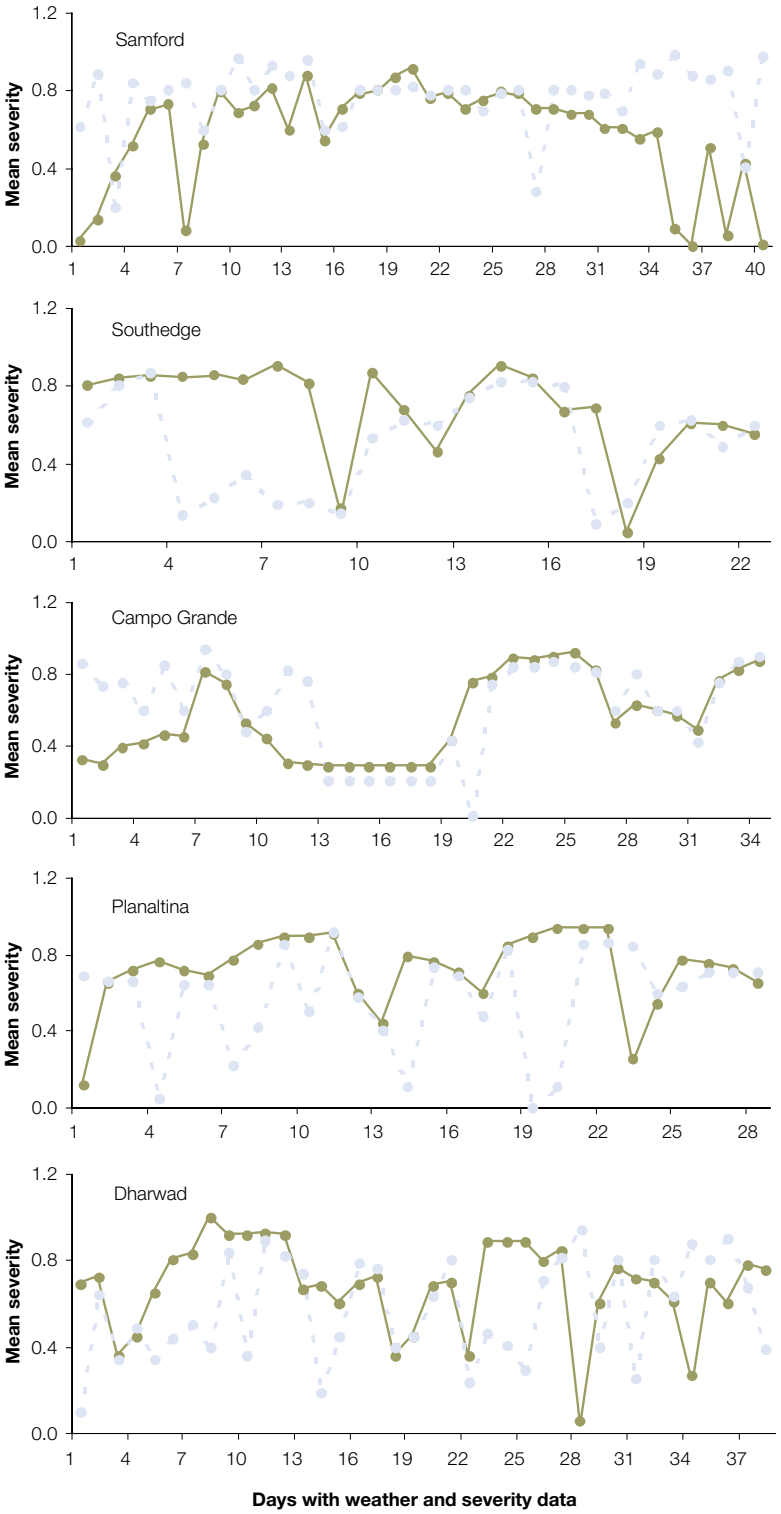


Figure 20.1 Actual (●) and predicted ( ) mean disease severity at selected field sites in Australia (Samford, Southedge), India (Dharwad) and South America (Campo Grande, Planaltina) according to artificial neural network models developed using pooled data for six sites to predict disease severity for the seventh site.

continent-based and cross-continent ANN models included weather variables that were directly related to moisture availability and the duration of leaf wetness, or variables such as radiation and wind that influence moisture and leaf wetness.

On a day-to-day basis the prediction success of cross-continent ANN models varied with the site. Predicted severity for Campo Grande and Southedge generally closely followed the actual severity, but predictions were not as accurate for Planaltina and Dharwad (Figure 20.1). In general, however, the predicted severity from ANN models tracked the actual severity reasonably closely for all sites.

With standard back-propagation ANN models, prediction is often more important than explanation and model construction is not easily understood (Frasconi et al 1993); this has created the perception of a 'black box' that has hindered the widespread use of these models. Prediction limited to local minima during gradient descent, and optimisation of the number of hidden layers, units, learning coefficients, momentum etc, are among other weaknesses of ANN models (Frasconi et al 1993). In this work we have increased transparency and improved the explanatory power of the ANN models by using sensitivity analysis, where the contributions of input weather variables can be seen easily.

Anthracnose risk

The risk of serious anthracnose was calculated for each site using weights from the ANN models. The greatest overall anthracnose risk was at Planaltina, followed by Carimagua, Campo Grande, Springmount, Samford, Southedge and Dharwad. The average annual rainfall at the seven field sites (Campo Grande – 1526 mm, Carimagua – 2337 mm, Dharwad – 900 mm, Samford – 1050 mm, Southedge – 1112 mm, Springmount – 804 mm and Planaltina – 1540 mm) does not alone explain the risk of anthracnose at these sites. However, the distribution and intensity of rainfall are factors that might have a major impact on disease development (Chakraborty & Billard 1995). Spatial heterogeneity in the geographical distribution of pathogenic races has been documented (Chakraborty et al 2002), and molecular haplotypes of *C. gloeosporioides* (Weeds et al 2003) are known in Brazil and India and may influence the risk of anthracnose. Some races may have significant advantages in terms of fitness components such as infection efficiency and fecundity (Chakraborty et al 1988; Chakraborty & Datta 2003). Interaction between *C. gloeosporioides* and antagonistic microorganisms in certain ecosystems may be another element influencing anthracnose risk (Lenné & Brown 1991). Using data from all except the Dharwad

site, we had previously shown that the Australian Southedge site was second only to the Brazilian Planaltina site for its high anthracnose risk (Chakraborty et al 2003). The work also indicated that centres of diversity and utilisation cannot be separated according to anthracnose risk as sites with high and low risk are present in both the Australian and South American continents (Chakraborty et al 2003).

Climate matching and mapping

Regression and principal component analysis showed that increases in anthracnose severity were almost always associated with rainfall events and both rain intensity and total rainfall were equally important. Over 96% of the variation could be explained by four principal components. The first component had a large positive coefficient for all variables except minimum RH, and the second component had positive coefficients for rain, minimum temperature and minimum RH but negative coefficients for average and maximum temperature.

Suitability indices were estimated using CLIMEX for *C. gloeosporioides* and *S. scabra* in India. The different climatic requirements, mainly in temperature and rainfall, for these two species were included in mapping their potential distribution using GIS. *Stylosanthes scabra* appears to be moderately suited to a wide area of the Indian subcontinent (Figure 20.2). Much of the area suitable for the cultivation of three *Stylosanthes* species is also climatically suitable for anthracnose. This implies that in the central southern areas there is the potential for high levels of disease in susceptible cultivars (Figure 20.3).

The work on risk mapping demonstrates the concept that it is possible to provide a meaningful summary of areas potentially suitable for *Stylosanthes* spp., which may serve as a useful first step towards targeting germplasm to the various agroecological regions. For instance, cultivars of *S. scabra* with low levels of resistance may be grown in some parts of peninsular India without significant economic damage. However, these hazard maps were developed using a highly susceptible cultivar; maps developed using commercially available cultivars with some level of anthracnose resistance would be more useful. More detailed district-level soil and weather data are necessary to target specific varieties at the regional scale and personal computer based GIS packages used in this work means that such specific targeting can be done by agricultural or other professional agencies at the local or regional level. Similar hazard mapping for pathogens with respect to soil suitability has appeared in the literature (Brasier & Scott 1994; Noble et al 2002; Pettit & Parry 1996).

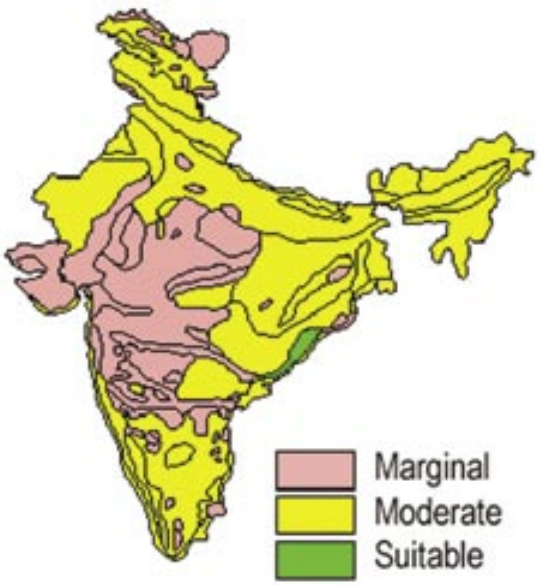


Figure 20.2 Soil and climate suitable for *Stylosanthes scabra* in India

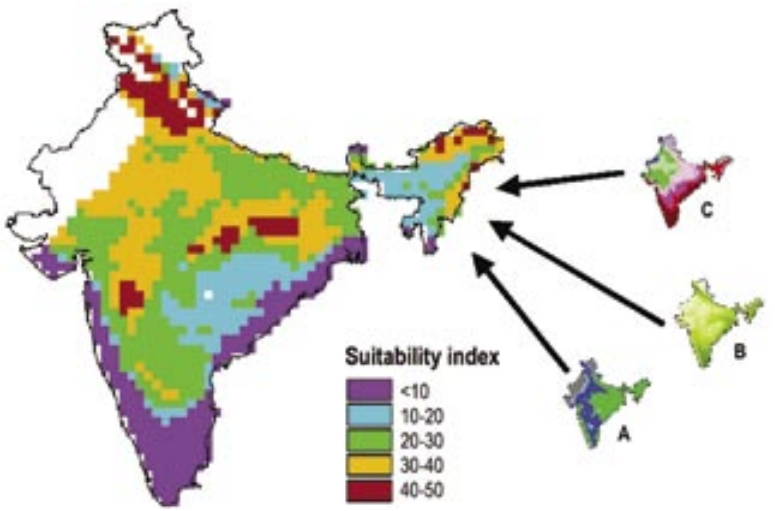


Figure 20.3 Overall suitability of *Stylosanthes scabra* determined from a combined suitability of this species based on individual risk assessments for climate (A), soil type (B) and risk of severe anthracnose damage (C).

Acknowledgments

We thank Ross Perrott, Arthur Kenji Akimoto, Roberto Monoel Gonçalves, Antonio Carlos Gomez, Jorge Badel, Raylee Trevorrow, Murray Anderson and Richard Ross for technical assistance. Financial assistance from the Australian Centre for International Agricultural Research, the Cooperative Research Centre for Tropical Plant Pathology and the CSIRO is gratefully acknowledged.

References

Bishop, C.M. 1995. Neural Networks for Pattern Recognition. Oxford University Press: Oxford, U.K.

Brasier, C.M. & Scott, J.K. 1994. European oak decline and global warming a theoretical assessment with special reference to the activity of Phytophthora cinnamomi. EPPO Bulletin 24, 221–232.

Chakraborty, S. 1990. Expression of quantitative resistance to *Colletotrichum gloeosporioides in Stylosanthes scabra* at different inoculum concentrations and day-night temperatures. Australian Journal of Agricultural Research 41, 89–100.

Chakraborty, S. & Billard, L. 1995. Quantitative relationships between Colletotrichum gloeosporioides infection of *Stylosanthes scabra* and weather factors under field conditions. Plant Pathology 44, 63–72.

Chakraborty, S., Cameron, D.F., Irwin, J.A.G. & Edye, L.A. 1988. Quantitatively expressed resistance to anthracnose (*Colletotrichum gloeosporioides*) in *Stylosanthes scabra*. Plant Pathology 37, 529–537.

Chakraborty, S. & Datta, S. 2003. How will plant pathogens adapt to host plant resistance at elevated CO_2 under a changing climate? New Phytologist 159, 733–742.

Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A. & Thomas, M.R. 2002. Pathogenic variation in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp. in a centre of diversity in Brazil. Phytopathology 92, 553–562.

Chakraborty, S., Ghosh, R., Ghosh, M., Fernandes, C.D., Charchar, M.J. & Kelemu, S. 2004. A comparison of artificial neural network and multiple regression analysis for weather-based prediction of anthracnose. Plant Pathology (in press).

Chakraborty, S., Ratcliff, D. & McKay, F.J. 1990. Anthracnose of *Stylosanthes scabra:* effect of leaf surface wetness on disease severity. Plant Disease 74, 379–384.

Chakraborty, S. & Smyth, G.K.S. 1995. A stochastic model incorporating the effect of weather conditions on anthracnose development in *Stylosanthes scabra*. Journal of Phytopathology 143 495–499.

Chtioui, Y., Panigrahi, S. & Francl, L. 1999. A generalized linear regression neural network and its application for leaf wetness prediction to forecast plant disease. Chemometrics and Intelligent Laboratory Systems 48, 47–58.

Crisci, A., Moriondo, M. & Orlandini, S. 1998. Analysis of downy and powdery mildew infection: modelling and neural network approach. Proceedings 7th ICCTA-International Congress for Computer Technology in Agriculture, Florence, Italy.

Davis, R.D., Irwin, J.A.G., Cameron, D.F. & Shepherd, R.K. 1987. Epidemiological studies on the anthracnose diseases of *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in North Queensland and pathogenic variation within the natural fungal populations. Australian Journal of Agricultural Research 38, 1019–1032.

De Wolf, E.D. & Francl, L.J. 1997. Neural networks that distinguish infection periods of wheat tan spot in an outdoor environment. Phytopathology 87, 83–87.

Fitt, B.D.L. & McCartney, H.A. 1985. Spore dispersal in splash droplets. In Ayres P.G. & Boddy, L. (eds) 'Water, fungi and plants', pp. 87–104. Oxford University Press: Cambridge, UK.

Francl, L.J. & Panigrahi, S. 1997. Artificial neural network models of wheat leaf wetness. Agricultural and Forest Meteorology 88, 57–65.

Frasconi, P., Gori, M. & Tesi, A. 1993. Successes and failures of back-propagation: a theoretical investigation. In Omidvar, O. (ed.) 'Progress in neural networks'. Ablex Publishing: Toronto, Canada.

Hajmeer, M. & Basheer, I. 2003. Comparison of logistic regression and neural network-based classifiers for bacterial growth. Food Microbiology 20, 53–55.

Hertz, J., Krogh, A. & Palmer, R.G. 1995. Introduction to the Theory of Neural Computation. Addison-Wesley: Reading, UK.

Irwin, J.A.G., Cameron, D.F. & Ratcliff, D. 1984. Influence of environmental factors on the development of anthracnose diseases of Stylosanthes spp. Australian Journal of Agricultural Research 35, 473–478.

Lenné, J.M. & Brown, A.E. 1991. Factors influencing the germination of pathogenic isolates of *Colletotrichum gloeosporioides* on leaf surfaces of Stylosanthes guianensis. Mycological Research 95, 227–232.

Louis, I. & Cook, R.C. 1985. Conidial matrix and spore germination in some plant pathogens. Transactions of the British Mycological Society 84, 661–667.

Miles, J.W. & Grof, B. 1997. Recent advances in studies of anthracnose of *Stylosanthes*. III. *Stylosanthes* breeding approaches in South America. Tropical Grasslands 31, 430–434.

Noble, A.D., Middleton, C., Nelson, P.N. & Rogers, L.G. 2002. Risk mapping of soil acidification under *Stylosanthes* in northern Australian rangelands. Australian Journal of Soil Research 40, 257–267.

Pangga, I. 2002. Influence of climatic factors on plant architecture of *Stylosanthes scabra and the epidemiology of the anthracnose disease.* PhD thesis, University of Queensland: Brisbane, Australia.

Pettit, T.R. & Parry, D.W. 1996. Effects of climate change on Fusarium foot rot of winter wheat in the United Kingdom. In Frankland, J.C., Magan, N. & Gadd, G.M. (eds) 'Fungi and environmental change', pp. 20–31. Cambridge University Press: Cambridge UK.

Scherm, H. 2000. Simulating uncertainty in climate change models with fuzzy numbers. Environmental Pollution 108, 373–379.

Shaw, M.W. 1994. Modelling stochastic processes in plant pathology. Annual Review of Phytopathology 32, 523–544.

Sutherst, R.W. & Maywald, G.F. 1985. A computerized system for matching climates and ecology. Agriculture Ecosystems and Environment 13, 281–299.

Weeds, P.L., Chakraborty, S., Fernandes, C.D., Charchar, M.J.d'A., Ramesh, C.R., Kexian, Y. & Kelemu, S. 2003. Genetic Diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centres of origin and utilization. Phytopathology 93, 176–185.

Yang, X.B., Batchelor, W.D. & Tshanz, A.T. 1995. A neural network model to predict soybean rust. Phytopathology 85, 1172.

Yang, C.C., Prasher, S.O., Sreekanth, S., Patni, N.K. & Masse L. 1997. An artificial neural network model for simulating pesticide concentrations in soil. Trans-ASAE 40, 1285–1294.

Chapter 21

Dispersal of *Colletotrichum gloeosporioides* conidia

I.B. Pangga[1], L. Geagea[2], L. Huber[2], S. Chakraborty[1,3] and D. Yates[4]

Summary

The spread and infection by *Colletotrichum gloeosporioides* conidia of *Stylosanthes scabra* Fitzroy were determined from single drop impaction, simulated rain and field experiments. Single drops, 3.4 and 4.9 mm in diameter, were released from heights of 25 and 100 cm above a diseased leaflet, and conidia were collected on microscope slides at different distances from the leaflet. Simulated raindrops, 3.4 and 4.9 mm in diameter, were made to fall for 15 and 30 minutes onto a line source of diseased seedlings and conidia were collected on microscope slides and Fitzroy seedlings at different distances from the source. Fall height and raindrop diameter significantly affected dispersal in single drop impaction and simulated rain, respectively. Half-distance (d_{50}), the distance at which the number of conidia decreases by half, was 1.6 to 2.6 cm under single drop impaction, and between 6.3 and 11.5 cm under simulated rain conditions. Equations based on raindrop kinetic energy were developed from single drop and rain simulation studies in an attempt to predict the dispersal of conidia in the field. Predictions were compared to actual distances travelled by conidia, as determined by exposing microscope slides and seedlings at different distances from a clumped point source of diseased seedlings on different dates during 1998 and 1999. A maximum d_{50} of 4.1 cm and 15.8 cm, respectively, were predicted using equations from the single drop and simulated rain studies. This contrasted with actual half-distances of between 1.1 and 9.6 m for conidial dispersal in the field. While a negative exponential model better explained conidia dispersal gradients under single drop impaction and simulated rain than an inverse power law model, the reverse was true for dispersal in the field. Lesions were detected up to 10 cm away from inoculum source under simulated rain, but up to 5 m under field conditions. The lack of success in predicting dispersal in the field is expected given the significant influence of weather on dispersal. Between 74% and 90% of the variability associated with conidia dispersal in the field was due to weather, of which hours of relative humidity over 95% and rain intensity were the most significant variables. On occasions, conidia were spread up to 10 m in the field without rain. This suggests mechanisms in addition to splash dispersal are responsible; however, the small range of wind speed and gust encountered during the study did not significantly influence dispersal.

[1] Cooperative Research Centre for Tropical Plant Protection, University of Queensland, St. Lucia, Queensland 4072, Australia
[2] Unité de Recherche en Bioclimatologie, Institut National de la Recherche Agronomique, Thiverval-Grignon, France 78850
[3] CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia
[4] Department of Botany, University of Queensland, St. Lucia, Queensland 4072, Australia

Introduction

The anthracnose disease, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., causes serious loss to herbage and seed production in the tropical pasture legume *Stylosanthes* worldwide. In grass–legume mixed pastures the disease affects production and persistence of the legume component to initiate a gradual decline in pasture productivity. Use of resistant varieties has not always provided sustainable protection against anthracnose, as new virulent and aggressive strains of the pathogen have arisen following the release of these varieties. Several promising commercial cultivars in Australia (Davis et al 1984), Colombia and Brazil (Miles & Lascano 1977) have been abandoned as a result. In Australia highly aggressive pathogen strains have continued to develop and spread, damaging the commercial cultivar Seca and threatening other promising new resistant cultivars (Chakraborty et al 1999).

In countries such as Brazil, several cultivars have failed to sustain a high level of production when adopted over a broad geographical area (Miles & Lascano 1977). Although many factors including poor seed production, seedling vigor and competition from a dominant grass species have contributed to this, anthracnose has been the single most common element associated with the decline of many cultivars. Recently, racial diversity in the pathogen population has been published from a study of nearly 300 isolates from native and cultivated *Stylosanthes* spp. in Brazil (Chakraborty et al 2002). The results show that in the absence of widespread use of cultivars with specific resistance genes, complex pathogen races are generally restricted to field sites adjoining those research stations where promising germplasm had been screened for resistance. This suggests that if a new cultivar is adopted over a broad geographical region, its productive

life will be determined by the speed with which matching aggressive races evolve and spread to the newly expanded pastures. Improved knowledge of pathogen dissemination is needed to help contain the spread of complex races. *C. gloeosporioides* is externally seed borne but infestation can be easily treated using commonly available fungicides (Davis 1986). The dissemination of inoculum in the field is not well understood and more difficult to control.

Conidia of *C. gloeosporioides* are produced in acervuli in a mucilaginous matrix (Louis & Cook 1985), which prevents dispersal by wind alone (Gregory 1973) and requires surface wetness for splash dispersal and subsequent infection (Fitt & McCartney 1986). Weather conditions play an important role and, apart from the obvious role of moisture, wind is important in the splash dispersal of pathogens with small spores (Fitt & McCartney 1986) such as *C. gloeosporioides*. Long distance spread of many splash-dispersed pathogens occurs in the field via windblown rain droplets and other means. For instance, lesions on white beans from *C. lindemuthianum* have been detected at distances of over 4.5 m from the source (Tu 1981) and on tomato from *Septoria lycopersici*, another splash-dispersed pathogen, at 106 m (Ferrandino & Elmer 1996).

In a previous study quantitative relationships between weather, the spread of *C. gloeosporioides* conidia and the infection of *Stylosanthes scabra* were established by exposing healthy seedlings of a susceptible cultivar to natural inoculum in the field (Chakraborty & Billard 1995). Infection was generally associated with rainfall, hours of relative humidity over 95% and mean hourly relative humidity in seasons with average rainfall. In a separate field study high relative humidity at 9 am, low net evaporation and low temperature at 9 am were significantly associated with anthracnose progress (Chakraborty & Smyth 1995). Although not designed specifically to study dispersal, these studies point to the importance of weather on *C. gloeosporioides* dispersal and infection.

There have been many studies of splash dispersal under controlled conditions exploring components of the dispersal process (Fitt & McCartney 1986; Fitt et al 1989; Huber et al 1998). A recent review has summarised the complexities associated with the biophysical process of splash, characteristics of rain that generate splash and influences of plant canopy on splash dispersal (Huber et al 1998). Among *Colletotrichum* spp., half-distance (d_{50}, the distance at which the number of conidia decreases by half) (Fitt & McCartney 1986) of *C. acutatum* conidia from infected strawberry ranged from 0.6 to 1.8 cm in a rain simulator depending on ground cover (Yang et al 1990); and d_{50} for lesions caused by C*. gloeosporioides* f.sp. *aeschynomene* conidia from northern jointvetch on different surfaces was between 42 and 99 cm (Yang & Tebeest 1992). The relevance of these studies in predicting inoculum dispersal in the field remains uncertain because in the limited number of cases where results on physical aspects of splash dispersal from controlled conditions have been related to field dispersal, success has been varied (Fitt et al 1989). Further studies are needed to examine whether dispersal parameters established from controlled studies can be used to predict how far inoculum from rain splash will travel in the field. The relationship between droplet size distribution, rain intensity and the kinetic energy of incident rain offers a simple way to estimate rain splash (Huber et al 1998; Lovell et al 2002). Whether such relationships developed from controlled studies can be used to predict splash dispersal in the field is not known.

The objectives of this study were two-fold: to examine how well relationships between kinetic energy of raindrops and dispersal distance from single drop impaction and simulated rain studies explained dispersal distance of conidia under field conditions; and to develop weather-based regression models to explain dispersal in the field.

Materials and Methods

Dispersal characteristics of conidia were determined from single drop impaction and simulated rain at Unité de Recherche en Bioclimatologie, Institut National de la Recherche Agronomique (INRA), Thiverval-Grignon, France during June–September 1998. Dispersal and infection in the field was studied at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Samford Pasture Research Station (27°22'S, 152°53'E, near Brisbane, Queensland, Australia) between January and February in 1998 and 1999.

Raising plants and inoculation

S. scabra Fitzroy seedlings for single drop impaction and simulated rain studies were raised in 1:1 soil and sand mixture in growth rooms at 30/25°C day/night temperatures, 250 μE m^{-2} $second^{-1}$ light intensity and 14-hour photoperiod. Seedlings were fertilised weekly with a liquid fertiliser (SEM Engrais Universel, Germany). Cultures of *C. gloeosporioides* isolate SR24 were grown under fluorescent light for seven days on oatmeal agar (2% rolled oats and 2% agar) at the Laboratoire de Pathologie Vegetale, Institut National Agronomique – Paris Grignon, Thiverval-Grignon, France. Conidia from cultures were suspended in distilled water, standardised to 5 x 10^4 conidia/mL using a hemacytometer. Seedlings at the tenth leaf stage were inoculated by spraying with a handheld sprayer and incubated in a dew chamber for two days and in a growth room for further seven days.

For field dispersal studies batches of 6-week-old Fitzroy seedlings were raised in pots with a sandy loam soil in a naturally lit glasshouse with a 14 ± 5-hour photoperiod, 32 ± 5°C day temperature and 23 ± 3°C night temperature. Washed fine sand and peat (1:1) was added to the soil surface to conserve moisture and 'Ridomil' (Ciba-Geigy Australia Ltd., Lane Cove, Australia) was applied two days before sowing at 0.4 g/L to prevent root rot. Five scarified seeds were sown per pot and thinned to one seedling per pot after two weeks. Plants were fertilised weekly ('Thrive', Arthur Yates and Co., Homebush, New South Wales, Australia). Some seedlings from each batch were inoculated to serve as inoculum source in the field. Isolate SR24 was grown as before and seedlings were sprayed with 2 x 10^5 conidia/mL using a pressurised sprayer (Wattyl

Jet-pack, Sydney, New South Wales, Australia). Inoculated seedlings were incubated in a dew chamber for two days and in the glasshouse for further seven days, and anthracnose severity was assessed using a 10-point rating scale (Chakraborty 1990).

Single drop impaction studies

To produce single water drops of constant diameter, a drop generator was used with a flow regulator to control distilled water flow at a rate of 7.24 mL/hour from a 50-mL syringe connected by a 0.5 cm polyethylene tube to a vertically positioned hypodermic needle (Geagea et al 1999). Two drop diameters, 3.4 and 4.9 mm, and two fall heights, 25 and 100 cm, were used. For each combination of drop diameter and fall height, ten water drops were allowed to fall onto a single infected Fitzroy leaflet in each of three replicate trials. Diseased leaflets were chosen for similar size (approximately 1.5 cm^2) and severity (0.09 cm^2 lesion area) and fixed in position on a thin metal rod clamped to a laboratory stand using adhesive tape to the petiole. Splash droplets were collected on 41 microscope slides coated with a thin layer of 0.4% naphthol green B (Siegried SA, Zofingue, Switzerland) prepared as in Geagea et al (1999). The slides, placed side by side 5 cm below and centred on the leaflet, covered an area of 729 cm^2. Splashed droplets formed clear spots with a dark green margin on the naphthol green B coated slides. Conidia dispersed singly or in clumps inside the spots were counted under a microscope (250x) from five randomly selected droplets at each of 15 distances between 2.5 and 18 cm from the centre.

Rain simulator studies

The rain simulator at INRA comprised a square frame containing ten 1-m long plastic tubes in parallel rows with eight hypodermic needles inserted 11.5 cm apart along the length of each tube (Geagea et al 2000). Using 3.4 and 4.9 mm diameter drop sizes and 15 and 30 minute rain durations, the influence of each combination of drop size and duration was studied from three replicate runs of the simulator.

Distilled water was pumped from a plastic container to four rows of eight hypodermic needles using a peristaltic pump at a flow rate of 10 mL/hour for the 3.4 mm drops and 12 mL/hour for the 4.9 mm drops. Simulated rain was made to fall onto a line source of ten inoculated Fitzroy seedlings (7–12% necrotic tissue) from a 9-m height. Splash droplets were collected on double-sided adhesive tapes on microscope slides placed at distances of 15, 30, 45, 60, 75, 90 and 105 cm perpendicular to the line source, and on two rows of Fitzroy plants at distances of 15, 45, 75 and 105 cm from the line source placed at either side of the slide traps. Plants from one row were incubated in a dew chamber for two days and the number of lesions was counted after another week, while plants in the second row were used to determine the number of conidia deposited on leaves. A strip of transparent adhesive tape was pressed against the adaxial surface of each leaflet, mounted on a microscope slide and stained with lactic acid – cotton blue (5 mL 1% aqueous solution of cotton blue, 5 mL glycerol, 5 mL lactic acid and 5 mL distilled water); and conidia on ten fields were counted under the microscope. In a preliminary experiment conidial suspensions were sprayed onto double-sided adhesive tape on slides and Fitzroy leaflets. The number of deposited conidia from leaf prints compared well with the number on double-sided tape.

Dispersal in the field

A uniform grass cover was maintained at the Samford site by regular mowing. Two circular plots, 20 m in diameter, were laid out with more than 60 m between them. Nineteen diseased seedlings were placed on a round plastic tray, 32 cm in diameter and 10 cm above the ground level, in the centre of each plot, to serve as a source of inoculum (Figure 21.1a).

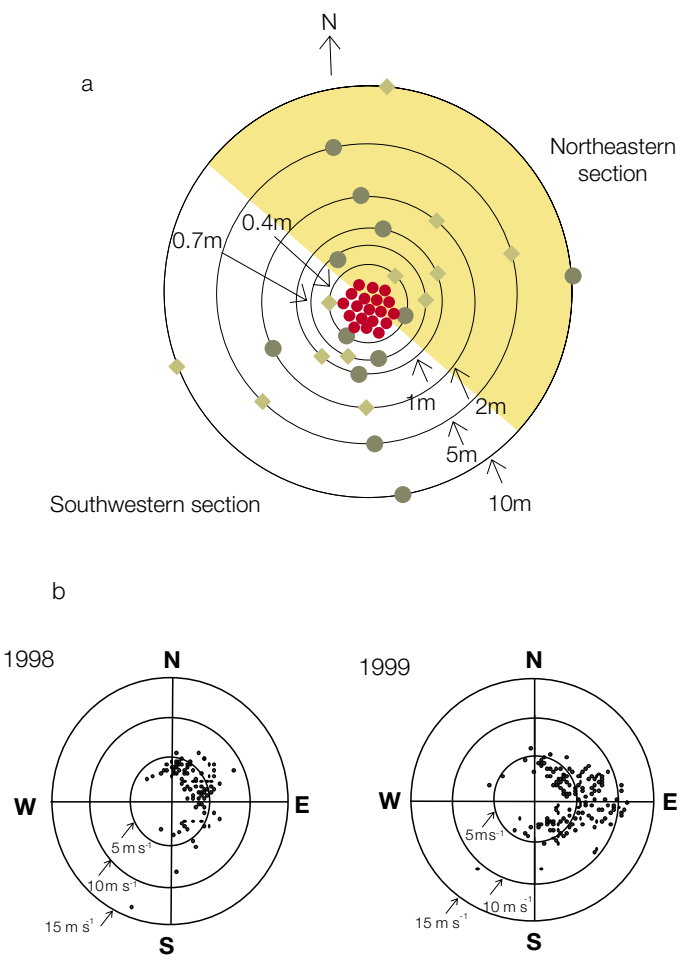


Figure 21.1 (a) Layout of a plot at CSIRO Samford Pasture Research Station used to study dispersal of *Colletotrichum gloeosporioides* conidia. The group of infected *Stylosanthes scabra* Fitzroy seedlings in the centre (●) serves as the point source of inoculum, and slide traps (◆) and disease-free Fitzroy seedlings (●) are located at each of the six distances in the northeastern (shaded) and southwestern sections; (b) Wind gusts ≥3 m $second^{-1}$ and directions of wind gust vectors recorded during periods when trap plants and slides were exposed to inoculum in 1998 and 1999.

Dispersal distance was measured by capturing conidia on disease-free Fitzroy seedlings and slide traps placed at various distances from the inoculum source. The slide trap consisted of a microscope slide with double-sided adhesive tape on one side. The slide with the adhesive side up was placed on a wooden platform (3.5 x 9.5 cm) on top of a wooden peg 20 cm above the ground and protected from direct rain with a rain shield placed 10 cm above the slide.

Six-week-old seedlings and slide traps were placed at distances of 0.4, 0.7, 1, 2, 5 and 10 m from the outermost edge of the point source in each plot. The prevailing wind direction at the field site is from the northeast (Figure 21.1b). To examine the potential influence of wind gust and direction on dispersal distance, each plot was subdivided into two sections representing northeastern and southwestern directions. The slide trap and seedlings were placed in such a way so as not to interfere with dispersal to more distant locations. Slide traps were exposed on four and seven different dates in 1998 and 1999, respectively; and seedlings were exposed on six and seven dates in 1998 and 1999, respectively. The dates for exposures were not selected to match weather forecasts. On each date, slides and healthy plants were exposed for 48 hours. Afterwards, seedlings were placed in a dew chamber for 48 hours then kept at 25°C and assessed for the number of lesions present ten days after exposure. Slides were stained with cotton blue and conidia were counted under a microscope (250x). On two dates each year, slides and trap plants were also placed at 30 m from the centre of each plot in four directions to sample *C. gloeosporioides* conidia present in the background.

Monitoring weather conditions

Environmental variables were monitored 30 m from the plots using electronic sensors connected to a data logger (Monitor Sensors, Caboolture, Queensland, Australia). Air temperature (model TA1) and relative humidity (RH, model HU1) sensors and the data logger were placed inside a Stevenson meteorological screen 1 m above the ground. A leaf wetness sensor (model LW1) was positioned 20 cm above ground level. A tipping bucket rain gauge (model RGD-02) was placed 5 m from the Stevenson screen. Sensors for radiation (model SR2), wind speed (model AN3) and wind direction (model WDD-03) were mounted on steel poles 2 m above the ground. The amount and duration of rainfall and leaf wetness were recorded as and when these events occurred. Air temperature, RH, solar radiation, wind speed and wind direction data were recorded at hourly intervals.

Data analysis

All statistical analyses were done using the SAS software (SAS Institute, Cary, NC), and conidia and lesion numbers were $ln\,(x + 0.01)$ transformed before analysis to stabilise variance. As a summary measure, the area under the dispersal gradient curve (AUDGC) was used to examine effects of drop size, fall height and rain duration on conidia and lesion numbers in the single drop impaction and simulated rain studies (Maffia & Berger 1999). AUDGC was calculated as:

$$AUDGC = \sum_{i}^{n-1}[(Y_i+Y_i+1)/2](d_{i+1}-d_i)$$

where n = the number of distances from the source, y_i = number of conidia and lesions at the ith distance, and d = distance from the source to the ith distance.

Dispersal gradients of conidia and infection gradients based on lesions were analysed for single drop impaction, rain simulation and field studies by fitting two commonly used empirical models in their linear forms: the negative exponential model (Kiyosawa & Shiyomi 1972):

$$ln(y) = ln(a) - bx$$

and the inverse power law model (Gregory 1968):

$$ln(y) = ln(a) - bln(x)$$

where y is the number of conidia or lesions, x is the distance from the source, a is related to the source strength, and b represents the rate of decrease in y with distance. Values of b were estimated by linear regression of $ln(y)$ on x and $ln(x)$, and the percentage of variance accounted for regression was determined for each data set. For the negative exponential model, the distance at which the value of y decreases by half (d_{50}) was estimated as $0.693/b$ where b is the slope parameter (Fitt & McCartney 1986); d_{50} was only computed if the linear regression analysis was significant. Model fit was evaluated based on the coefficient of determination values (R^2) and the F-test for significance of linear regression, and by an examination of residual plots.

Multiple linear regression analyses were used to determine relationships between conidia and lesion parameters with weather variables using a stepwise variable selection. Conditions for entry and retention of a variable were set at $P \leq 0.05$. In this analysis conidia and lesions were weighted by the distance from the source as follows:

$$Y_{weighted} = [\Sigma(X_i \times D_i)]/D_t$$

where $Y_{weighted}$ = weighted conidia or lesion number, X_i = conidia or lesion number at ith distance from source, D_i = ith distance from source and D_t = total distance.

Prediction of dispersal distance

Quantitative relationships between d_{50} (dependent variable) and the kinetic energy of raindrops (independent variable) were obtained from linear regression analysis and used to predict d_{50} of conidia in the field for two dates when rain was recorded and the negative exponential model fitted the dispersal data.

To calculate the kinetic energy of an incident drop, its vertical velocity was estimated by numerically solving the equation of motion for a free falling water drop of unit density (Huber et al 1997), using Runge-Kutta-Merton integration (NAG FORTRAN library routine D02BBF) (NAG 1990). The drag coefficients of drops were estimated from the equations given by Beard (1976), which take deformation of falling drops into account. The kinetic energy (*KE*) of a single incident drop was calculated from Huber et al (1998) as:

$$KE = \rho(\pi/12)(D^3V^2)$$

where ρ = density of water (1000 kg m^{-3}), D = drop diameter (m) and V = velocity (m $second^{-1}$).

For rain simulation studies the number of drops (*ND*) falling per hour is given by:

$$ND = R \div \frac{\pi D^3}{6}$$

where R = flow rate (volume of water going through the system in L $hour^{-1}$) and D = drop diameter (m). Kinetic energy flux (KE_{flux}) for the rain simulation was obtained from:

$$KE_{flux} = ND \times KE\ (D,H)$$

where D = drop diameter and H = fall height.

The kinetic energy of incident raindrops in the field was calculated assuming an exponential distribution for raindrop size (Marshall-Palmer distribution) (Marshall & Palmer 1948) and a power law for free-fall drop velocity that was calculated from Ulbrich (1983) as:

$$V(D) = 17.67D^{0.67}$$

where D = drop diameter (cm). The number of drops of a given diameter in the size interval $N(D)$ was given by Marshall and Palmer (1948) and calculated as:

$$N(D) = N_0 \exp(-\Lambda D)$$

where $N_o = 8 \times 10^5$ m^{-3} cm^{-1}, $\Lambda = 41R^{-0.21}$ (R = rain intensity in mm $hour^{-1}$), and D = drop diameter (cm). The number of incident raindrops and the kinetic energy per time interval was calculated by an integration of the complete drop size distribution (Huber et al 1998).

Results

Dispersal from single drop impaction

The number of spore-carrying droplets and *C. gloeosporioides* conidia generally decreased with distance from the inoculum source (Figure 21.2). Large droplets (>6 mm^2) contained many more conidia than smaller droplets, and these mostly spread to within 2.5 cm of the inoculum source. The negative exponential model significantly fitted the number of dispersed conidia (Table 21.1). The dispersal gradients were steep and d_{50} ranged from 1.65 to 2.57 cm. The inverse power law model was also significant but produced lower R2.

AUDGC for conidia was significantly higher at, 100 cm, than the 25 cm for both drop diameters, and a combination of 25 cm fall height and 3.4 mm drop diameter produced the lowest AUDGC values (output of analysis not given).

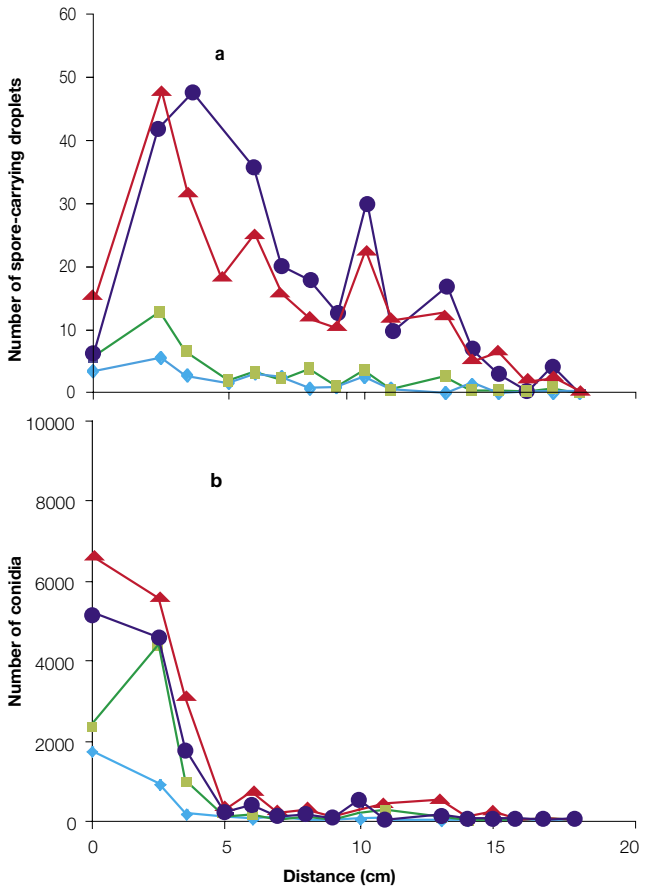


Figure 21.2 Number of spore-carrying droplets (a) and conidia of *Colletotrichum gloeosporioides* (b) dispersed at different distances from a single infected leaflet of *Stylosanthes scabra* Fitzroy under single drop impaction with drop diameter and fall height combinations, respectively, of 3.4 mm and 25 cm (◆); 3.4 mm and 100 cm (▲); 4.9 mm and 25 cm (■); and 4.9 mm and 100 cm (●).

Dispersal under simulated rain

As expected, the number of conidia collected on microscope slides and leaves and the number of lesions on trap plants decreased with increasing distance from the line source (Figure 21.3). However, more conidia were detected at greater distances from the source on leaves than on slides. This points to the efficiency of leaf surfaces in trapping conidia and is also apparent from d_{50} values on leaves (11.55–34.6 cm) and slide traps (6.3–11.55 cm) (Table 21.2). The gradients for infection were steeper than for conidia. The negative exponential model better fitted the dispersal gradients for conidia, with higher R^2 than the inverse power law model, whereas the inverse power law model better explained infection gradients.

Drop diameter significantly (P<0.001) influenced AUDGC for conidia captured on slides and leaves but rain duration was not significant (output of analysis not given). More conidia were trapped on slides after a simulated rain using 4.9-mm raindrops than with 3.4-mm drops. AUDGC for lesions did not vary significantly with drop diameter or rain duration.

Using data on conidia captured on slides from single drop impaction and rain simulation studies, the kinetic energy of 3.4-mm drops falling from heights of 0.25, 1 and 9 m was determined as 48.5, 176.6 and 760.1 μJ, respectively, and of 4.9-mm drops falling from the three heights as 147.2, 554.2 and 2949 μJ, respectively. For single drop impaction, d_{50} is significantly (R^2 = 0.89, P<0.001) explained by *ln* kinetic energy as follows:

$$d_{50} = 0.21 lnKE$$

where KE = kinetic energy (µJ) of 10 drops.

When this relationship was used to calculate d_{50} in the rain simulation, it underestimated the actual d_{50} values by 28–60%.

Instead, d_{50} in the rain simulation is significantly (R^2 = 0.96, P<0.001) explained by a power law relationship as follows:

$$d_{50} = KE^{0.14}$$

where *KE* = kinetic energy flux (µJ m^{-2} $second^{-1}$).

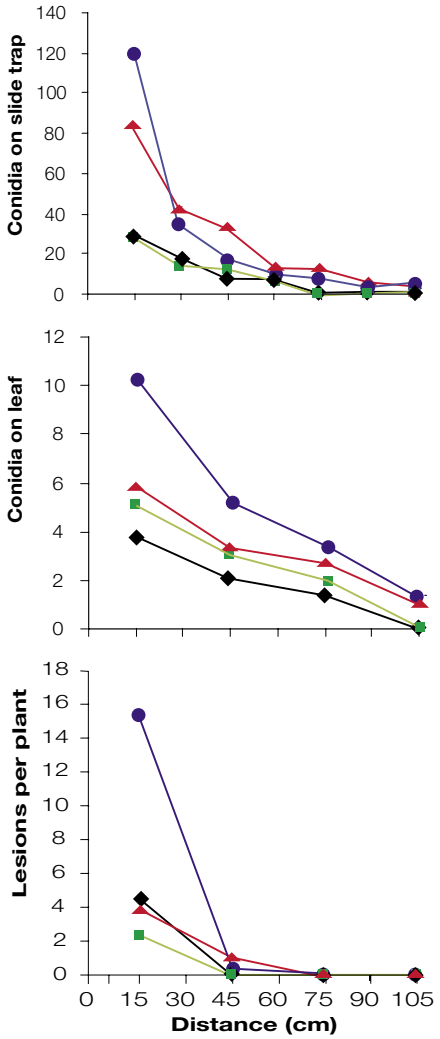


Figure 21.3 Number of *Colletotrichum gloeosporioides* conidia detected on slide traps and healthy leaves, and the number of lesions formed on *Stylosanthes scabra* Fitzroy, at different distances from an inoculum source in a rain simulator with drop diameter and rain duration combinations, respectively, of 3.4 mm and 15 minutes (◆); 3.4 mm and 30 minutes (■); 4.9 mm and 15 minutes (▲); and 4.9 mm and 30 minutes (●).

Table 21.1 Linear regression analyses using negative exponential and inverse power law models applied to the dispersal gradient of *Colletotrichum gloeosporioides* conidia captured on microscope slides when single drops fell from an infected leaflet.

Drop diameter (mm)	Fall height (cm)	Negative exponential model[a]				Inverse power law model[a]		
		Intercept	Slope	R^2	d_{50} (cm)	Intercept	Slope	R^2
3.4	25	6.91±0.50	–0.42±0.06	0.83***	1.65	4.86±0.48	–0.76±0.20	0.58*
4.9	25	7.46±0.82	–0.34±0.10	0.55**	2.04	5.81±0.58	–0.61±0.25	0.38*
3.4	100	8.22±0.60	–0.27±0.07	0.59**	2.57	6.98±0.41	–0.53±0.17	0.48**
4.9	100	8.16±0.66	–0.36±0.08	0.68***	1.93	6.41±0.51	–0.65±0.22	0.47**

[a]Parameter estimate ± standard error from three replicates * significant at P≤0.05; ** significant at P≤0.01; *** significant at P≤0.001

Table 21.2 Linear regression analyses using negative exponential and inverse power law models applied to the dispersal gradient of *Colletotrichum gloeosporioides* conidia captured on microscope slides and leaves, and lesions on *Stylosanthes scabra* Fitzroy, when simulated rain fell on a line source of infected plants.

Drop diameter (mm)	Rain duration (min)	Negative exponential model				Inverse power law model		
		Intercept	Slope	R^2	d_{50} (cm)	Intercept	Slope	R^2
Number of conidia on microscope slides[a]								
3.4	15	5.81±0.76	-0.11±0.01	0.82***	6.30	17.77±2.76	–4.68±0.69	0.71***
3.4	30	5.83±0.78	-0.11±0.01	0.82***	6.30	17.70±2.83	–4.65±0.71	0.69***
4.9	15	5.83±0.81	-0.06±0.01	0.57***	11.55	12.11±2.59	–2.52±0.65	0.44***
4.9	30	5.78±0.97	-0.07±0.01	0.54***	9.90	13.84±2.84	–3.09±0.72	0.50***
Number of conidia on leaves[a]								
3.4	15	3.06±0.77	-0.06±0.01	0.74***	11.55	8.44±2.75	–2.33±0.70	0.53**
3.4	30	3.53±0.85	-0.06±0.01	0.73***	11.55	9.14±3.00	–2.44±0.76	0.51**
4.9	15	2.01±0.20	-0.02±0.003	0.79***	34.65	3.82±0.64	–0.74±0.16	0.67***
4.9	30	2.68±0.16	-0.02±0.002	0.90***	34.65	5.07±0.58	–0.96±0.15	0.81***
Number of lesions[a]								
3.4	15	–0.95±1.25	-0.04±0.02	0.36*	__[b]	5.40±2.88	–2.31±0.73	0.50**
3.4	30	–1.49±1.09	-0.04±0.02	0.35*	__[b]	3.93±2.53	–1.97±0.84	0.48**
4.9	15	2.28±0.67	-0.07±0.01	0.84***	9.99	10.26±2.03	–3.18±0.52	0.79***
4.9	30	1.76±1.20	-0.07±0.02	0.63**	9.99	11.50±2.45	–3.63±0.62	0.77***

[a]Data from three replicates

[b] __ not provided due to low R^2 of model

* significant at P≤0.05; ** significant at P≤0.01; *** significant at P≤0.001

Dispersal in the field

Conidia of *C. gloeosporioides* were detected up to the maximum distance of 10 m on two out of four dates in 1998 and on all seven dates in 1999 (Figure 21.4). The number of conidia captured on slide traps generally declined within the first 2 m from the inoculum source on most dates, but did not decline further between 2 and 10 m. In both years between 10 and 50 conidia/cm^2 were sampled on most dates from slide traps located 10 m from the source.

Only a few lesions developed on Fitzroy seedlings except on one date in 1999. In general, fewer than four lesions per plant were found at any distance from the inoculum source. Lesions were detected up to a maximum distance of 5 m but very few were found on seedlings more than 2 m from the inoculum source (Figure 21.4). Given this, data are inadequate for a meaningful analysis and discussion is mainly restricted to the dispersal of conidia.

No conidia were detected on slide traps 30 m from the inoculum source on any of the four dates, with or without rainfall, and seedlings placed at this distance did not develop anthracnose lesions. This clearly demonstrates that there was no detectable level of background inoculum and all dispersal occurred from the point source within the two plots.

A preliminary regression analysis and tests of homogeneity of slopes between directions showed no significant difference between slopes in the northeastern and southwestern directions within each field plot, largely due to the narrow range of wind speed and gust encountered during the two years of this study. Therefore, data on conidia dispersal were pooled across directions and an analysis of variance showed that date of exposure was significant (P<0.01) in each year, and both distance from source (P<0.001) and distance x date interaction (P<0.05) were significant in 1998 but not in 1999; no other term was significant (output of analysis not given). Relatively large numbers of conidia were trapped between 2 and 10 m on all seven dates in 1999 (Figure 21.4). This suggests that conidia may be dispersed over distances greater than 10 m in the field.

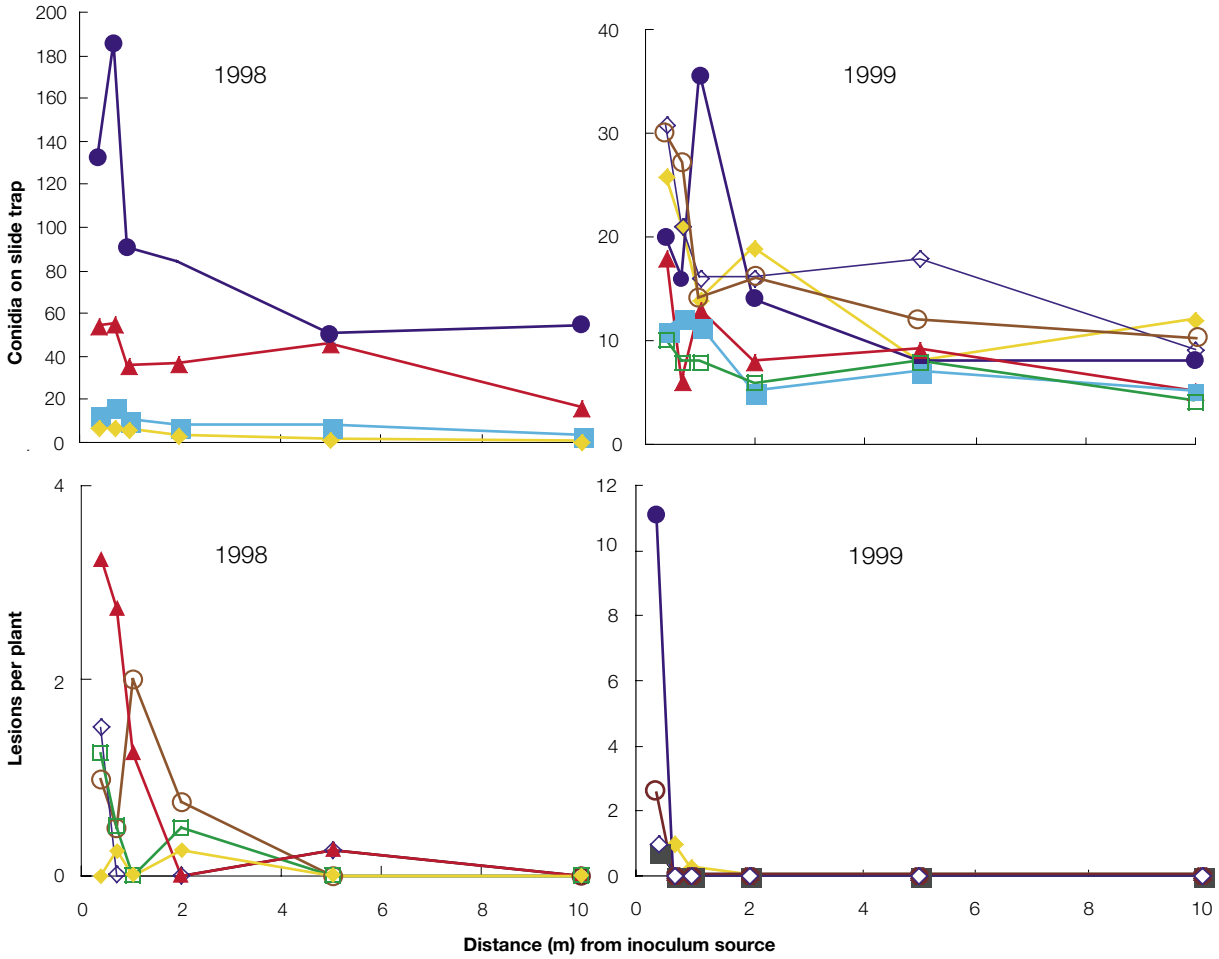


Figure 21.4 Number of *Colletotrichum gloeosporioides* conidia collected on slide traps and lesions on *Stylosanthes scabra* Fitzroy seedlings placed in two replicate field plots at different distances from a point source of inoculum on different days of the year: day 12 (●), 38 (○), 40 (◇), 43 (□), 46 (▲), 49 (■) and 51 (◆) in 1998; and day 10 (◆), 13 (●), 16 (▲), 20 (■), 25 (□), 28 (○) and 30 (◇) in 1999. Each data point is an average of four slide traps or seedlings.

The negative exponential and inverse power law models explained dispersal gradients for conidia in the two seasons with varying degree of success (Table 21.3). In 1998 both models explained dispersal on three out of four dates. Out of seven dates in 1999, the inverse power law model fitted data on four dates and the negative exponential model fitted data on two dates. When data for all dates were pooled for each year, the inverse power law model gave a higher R^2 than the negative exponential model.

Prediction of conidia dispersal using kinetic energy of raindrop

Data from day 46 in 1998 and day 30 in 1999 were used to predict d_{50} of conidia using equations developed from controlled studies. The equation from single drop impaction predicted d_{50} between 4.1 and 4.04 cm, and the equation from the rain simulation predicted 13.8 and 15.8 cm, for these two dates. The actual d_{50} values in the field were 671 cm on day 46 and 770 cm on day 30 (Table 21.3). On average, actual d_{50} values on the two dates were underpredicted by >98% using either equation.

Influence of weather on dispersal distance

According to historical weather records, 1998 and 1999 were average summer seasons, but above-average rainfall was recorded in April and May 1998 and in February 1999, and the number of rainy days for January and April 1998 and January and February 1999 were above average. The prevailing wind direction at this site is from the northeast, and for both years wind gusts ≥3 m s^{-1} were mainly concentrated in the north to east and south to east quadrants (Figure 21.1). Mean hourly wind speed was higher in 1999 than in 1998. This may have influenced dispersal in the two years, as disease severity of source plants was higher in 1998 than in 1999. In 1998 conidia were trapped in the absence of rain on days 49 and 51, and lesions were observed on day 49. In 1999 conidia were trapped in the absence of rain on days 16 and 25, but no lesions developed on either date (Figure 21.4).

Data for all dates in the two years except days 10 and 19 in 1999 (missing weather data) were pooled for correlation and regression analyses. Weather variables associated with temperature and relative humidity were among the most important factors influencing dispersal of conidia. Hours of relative humidity over 95% (RH95) were positively

Table 21.3. Linear regression analyses using negative exponential and inverse power law models applied to dispersal gradients of *Colletotrichum gloeosporioides* conidia captured on slide traps in the field during 1998 and 1999.

Day of the year	Negative exponential model				Inverse power law model		
	Intercept	Slope	R^2	d_{50} (m)	Intercept	Slope	R^2
				1998			
43	4.82±0.20	–0.11±0.04	0.61ns	–	4.69±0.12	–0.37±0.09	0.79*
46	3.94±0.15	–0.10±0.03	0.72*	6.71	3.77±0.15	–0.28±0.12	0.58ns
49	2.62±0.14	–0.16±0.03	0.88**	4.30	2.37±0.14	–0.48±0.11	0.82**
51	2.43±0.36	–0.65±0.08	0.95**	1.06	1.31±0.68	–1.74±0.54	0.72*
Mean	3.90±0.14	–0.11±0.03	0.76**	6.30	3.75±0.09	–0.35±0.07	0.85***
				1999			
10	2.97±0.20	–0.07±0.04	0.41ns	–	2.89±0.13	–0.28±0.11	0.64ns
13	3.06±0.23	–0.12±0.05	0.59ns	–	2.89±0.18	–0.37±0.15	0.61ns
16	2.44±0.23	–0.08±0.05	0.40ns	–	2.32±0.18	–0.26±0.15	0.43ns
20	2.33±0.18	–0.08±0.04	0.52ns	–	2.23±0.12	–0.28±0.10	0.66*
25	2.18±0.11	–0.07±0.02	0.70*	9.65	2.06±0.10	–0.21±0.08	0.65*
28	3.11±0.17	–0.09±0.04	0.61ns	–	2.99±0.10	–0.33±0.08	0.82**
30	3.13±0.14	–0.09±0.03	0.68*	7.70	3.00±0.11	–0.28±0.09	0.72*
Mean	2.85±0.09	–0.09±0.02	0.85**	7.70	2.72±0.03	–0.30±0.02	0.98***

ns – not significant at $P \leq 0.05$; * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$

correlated with the number of conidia ($P<0.01$) weighted by distance from the source. Mean hourly air temperature (MHTEMP) was negatively correlated ($P<0.05$) with dispersal distance and slopes of the exponential and inverse power law models.

Multiple regression equations that best describe the quantitative relationship between weather and dispersal of conidia are shown in Table 21.4. RH95 had a significant positive effect on conidia dispersal. MHTEMP was a significant negative term in the regression models for maximum dispersal distance and slopes of exponential and inverse power law models, indicating that MHTEMP reduces dispersal distance of conidia and makes the gradients steeper. Rain intensity (RINT) and hours of air temperature in the range 25–30°C (HRTEMP) were significant positive terms in the regression model for slopes of the exponential and inverse power law models. This implies that RINT and HRTEMP increase dispersal distance. Wind speed did not produce a significant effect when included in the multiple regression models. Outputs of regression models for lesions are not given due to the generally low number of lesions in both years. In general, RINT had a significant positive effect on number of lesions weighted by distance, indicating that more lesions at greater distance were recorded with increasing RINT. RH95 was a significant term in the model on the maximum distance for lesion development but this model had a R^2 of 0.56.

Discussion

Conidia of *C. gloeosporioides* travelled greater distances as the dispersal process progressed from single drop impaction to rain simulation, and to the field. The d_{50} for conidia increased from <3 cm under single drop to between 6 and 11.5 cm under simulated rain to between 106 and 965 cm in the field. Kinetic energy of drops increased with increasing drop size and fall height, as expected. However, regression equations linking kinetic energy and d_{50} developed from single drop impaction or simulated rain did not adequately explain d_{50} for conidia in the field. This indicates that factors in addition to kinetic energy of raindrops are necessary to explain conidia dispersal in the field. In the field, between 74% and 90% of the variability associated with conidia dispersal was due to weather, and RH95 was the single most important variable influencing dispersal.

Table 21.4 Regression equations that best describe quantitative relationships between dispersal of *Colletotrichum gloeosporioides* conidia in the field and weather variables during 1998 and 1999.

Dependent variable	Regression equation	R^2 adjusted
Number of conidia weighted by distance	Y = 0.14(±0.02[a])RH95	0.90***
Maximum dispersal distance	Y = 32.98(±5.33) – 1.02(±0.22)MHTEMP + 0.11(±0.04)RH95	0.74**
Slope of negative exponential model	Y = 2.62(±0.47) – 0.13(±0.02)MHTEMP + 0.03(±0.01)RINT + 0.01(±0.005)HRTEMP	0.85**
Slope of inverse power law model	Y = 6.82(±1.21) – 0.33(±0.05)MHTEMP + 0.08(±0.02)RINT + 0.03(±0.01)HRTEMP	0.85**

[a] Figure in parenthesis is the standard error of the estimated partial regression coefficient

** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$

RH95 – hours of mean hourly relative humidity over 95%; MHTEMP – mean hourly air temperature (°C); RINT – rain intensity (mm hour^{-1}); HRTEMP – hours of mean hourly air temperature in the range 25–30°C.

Our failure to predict dispersal in the field is partly due to complexities associated with the prediction of kinetic energy for natural rain episodes (Madden et al 1998). New electronic sensors can now be used to predict vertical splash dispersal in the canopy (Lovell et al 2002); nevertheless, splash variability cannot always be explained by raindrop size and impact velocity (Huber et al 1998). Dispersal in this pathogen may involve other mechanisms in addition to splash. In common with other studies on splash-dispersed pathogens (Fitt et al 1989), conidia in the field were detected at greater distances than expected for dispersal by splash alone. In general, large splash droplets follow a ballistic trajectory in short-range splash dispersal, while long-range dispersal is the result of windblown spread of small droplets (Fitt & McCartney 1986). In our study conidia travelled to the maximum distance of 10 m on two dates in the absence of rain in 1999 and on two other dates with <5 mm rain. In the splash-dispersed *Septoria lycopersici*, a proportion of waterborne conidia become airborne and travel distances up to 106 m to cause infection on tomato plants (Ferrandino & Elmer 1996). Spores of both *S. lycopersici* (Parker et al 1995) and *S. nodorum* (Jeger 1981) spread from inoculum sources in the absence of rain. This implies that other mechanisms, in addition to windblown droplets, may be involved in the dispersal of conidia of some splash-dispersed pathogens. *C. gloeosporioides* infecting *Stylosanthes* spp. produces conidia on fertile setae in acervuli (Lenné et al 1984) and on hyphal conidiophores (Cox & Irwin 1998), which can be easily dislodged by air movement and become available for aerial dispersal as dry conidia. In the related *C. graminicola* there is also evidence that conidia survive as dry spore masses that are disseminated by wind currents (Bergstrom & Nicholson 1999).

Of the two empirical models, the negative exponential model better explained gradients of conidia in single drop impaction and rain simulation experiments. Negative exponential models, in general, better explain splash dispersal than inverse power law models, as these describe gradients that are due mainly to deposition (Fitt et al 1987). In the absence of other interacting weather factors, conidia were dispersed mainly by splash deposition under controlled conditions. In contrast, the inverse power law model better explained dispersal of conidia in the field. This model is more commonly associated with wind dispersal (Fitt et al 1987; Gregory 1968), and a better fit of this model to our field dispersal data offers further indication of at least some aerial dispersal of this pathogen. External variables such as weather, source geometry and secondary disease spread are among factors that influence the fitting of empirical models (Gregory, 1968). There was no opportunity for secondary spread in the field study as the trap plants were exposed only for a short period. Also, as shown by a lack of background contamination, there were no other sources of inoculum nearby.

In the field relatively large numbers of conidia were consistently detected at 10 m and the maximum d_{50} exceeded 9 m, but none were trapped at 30 m from the inoculum source. This suggests that conidia may travel distances greater than 10 m during average summer seasons. It is likely that spread to greater distances may occur in some years when weather conditions are more favorable for dispersal. Between 74% and 90% of the variability in conidia dispersal could be explained by weather, and variables associated with relative humidity, temperature and rainfall intensity significantly influenced dispersal.

RH95 influenced both the number of conidia dispersed over a given distance and the maximum distance of dispersal. RH95 influences conidia in the same way as the duration of surface wetness (Chakraborty et al 1990; Irwin et al 1984). In *C. gloeosporioides* and other similar pathogens the mucilage surrounding conidia absorbs water, swells and dissolves when atmospheric humidity is high. This creates a suspension of spores, making them available for splash dispersal (Fitt & McCartney

1986). In the humid tropics where *Stylosanthes* spp. are naturally distributed, the amount of dew on leaves commonly exceeds their water-holding capacity, causing large water drops to fall from the top of the canopy to the lower foliage. Drops can also fall from infected leaves carrying pathogen spores as a result of light rain, fog and mist. Wind can buffet the crop and intercepted water can be shaken from leaves (Butler 1996) to aid in the dispersal of spores.

Temperatures within the optimum range for infection of *Stylosanthes* by *C. gloeosporioides* (Irwin et al 1984), as measured by HRTEMP, increased dispersal. This is evident from the positive significant relationship between HRTEMP and slopes of the negative exponential and inverse power law models: gradients become flatter with increasing duration of the optimum temperature range. In contrast, temperatures less than 25°C made the gradients steep, thus reducing dispersal distance. A similar negative relationship between mean hourly air and canopy temperature and infection has been noted earlier for this host–pathogen combination (Chakraborty & Billard 1995).

Among other variables influencing dispersal distance of conidia, RINT was a significant term in regression equations for slopes of negative exponential and inverse power law models for both conidia and lesions weighted by distance. As RINT increased, the gradient became flatter. Although RINT has previously been shown to increase the number of infections under some circumstances (Chakraborty & Billard 1995), rain intensity, in general, has a complex relationship with splash dispersal and there are inconsistent results from some studies (Huber et al 1998). For example, the infection of strawberry fruit by *Colletotrichum acutatum* increased with rain intensity up to 15–30 mm h^{-1} and then decreased with further increases in intensity, possibly due to wash off of dispersed spores (Madden et al 1996). Wind speed did not produce a significant effect when included in the multiple regression models. This is a reflection of the narrow range of wind speed (<15 m s^{-1}) encountered during the field study, and further work is necessary to elucidate the role of wind.

Of the physical characters, fall height and drop size significantly influenced dispersal in single drop impactions. Similarly, in line with findings on *Colletotrichum acutatum* (Ntahimpera et al 1997), large rain drops significantly increased d_{50} in the rain simulation. However, only a limited range of drop diameter and fall height was considered in this study and further work is needed to find important interactions between variables associated with the physical environment, the pathogen and the crop (Huber et al 1997, 1998). The possibility of more than one dispersal mechanism for *C. gloeosporioides* conidia and the difficulty of studying the complex interactions between weather and dispersal variables suggest that extrapolation of relationships from controlled conditions to the field will not be easy. In the meantime weather-based regression models can contribute to practical disease management decisions.

Acknowledgments

This work, as part of the senior author's PhD program at the University of Queensland, is funded by a scholarship from the Australian Agency for International Development (AusAID). CSIRO and the Cooperative Research Centre for Tropical Plant Protection provided infrastructure and operational support. Collaborative work was done at Institut National de la Recherche Agronomique and Laboratoire de Pathologie Vegetale in Thiverval-Grignon, France. These assistances are gratefully acknowledged.

References

Beard, K.V. 1976. Terminal velocity and shape of cloud and precipitation drops aloft. Journal of the Atmospheric Sciences 33, 851–864.

Bergstrom, G.C. & Nicholson, R.L. 1999. The biology of corn anthracnose: knowledge to exploit for improved management. Plant Disease 83, 596–608.

Butler, D.R. 1996. The presence of water on leaf surfaces and its importance for microbes and insects. Pages 267–282 In Kerstiens, G. (ed.) 'Plant cuticles: an integrated functional approach'. BIOS Scientific Publishers: Oxford.

Chakraborty, S. 1990. Expression of quantitative resistance *to Colletotrichum gloeosporioides* in *Stylosanthes scabra* at different inoculum concentration and day-night temperatures. Australian Journal of Agricultural Research 41, 89–100.

Chakraborty, S. & Billard, L. 1995. Quantitative relationships between *Colletotrichum gloeosporioides* infection of *Stylosanthes scabra* and weather under field conditions. Plant Pathology 44, 63–72.

Chakraborty, S., Fernandes, C.D., Charchar, M.J d'A. & Thomas, M.R. 2002. Pathogenic variation in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp. in a center of diversity in Brazil. Phytopathology 92, 553–562.

Chakraborty, S., Perrott, R., Ellis, N. & Thomas, M.R. 1999. New aggressive *Colletotrichum gloeosporioides* strains on *Stylosanthes scabra* detected by virulence and DNA analysis. Plant Disease 83, 333–340.

Chakraborty, S., Ratcliff, D. & McKay, F.J. 1990. Anthracnose of *Stylosanthes scabra:* effect of leaf surface wetness on disease severity. Plant Disease 74, 379–384.

Chakraborty, S. & Smyth, G.K. 1995. A stochastic model incorporating the effects of weather conditions on anthracnose development in *Stylosanthes scabra.* Journal of Phytopathology 143, 495–499.

Cox, M.L. & Irwin, J.A.G. 1998. Conidium and appressorium variation in Australian isolates of the *Colletotrichum gloeosporioides* group and closely related species. Australian Systematic Botany 1, 139–149.

Davis, R.D. 1986. *Stylosanthes* – seed treatment for control of anthracnose. QDPI Farmnote, Queensland Department of Primary Industries: Brisbane, Australia.

Davis, R.D., Irwin, J.A.G. & Cameron, D.F. 1984. Variation in virulence and pathogenic specialization of *Colletotrichum gloeosporioides* isolates from *Stylosanthes scabra* cvs. Fitzroy and Seca. Australian Journal of Agricultural Research 35, 653–662.

Ferrandino, F.J. & Elmer, W.H. 1996. *Septoria* leaf spot lesion density on trap plants exposed at varying distances from infected tomatoes. Plant Disease 80, 1059–1062.

Fitt, B.D.L., Gregory, P.H., Todd, A.D., McCartney, H.A. & McDonald, O.C. 1987. Spore dispersal and plant disease gradients: a comparison between two empirical models. Journal of Phytopathology 118, 227–242.

Fitt, B.D.L. & McCartney, H.A. 1986. Spore dispersal in splash droplets. In Ayres, P.G. & Boddy, L. (eds) 'Water, fungi, and plants', pp. 87–104. Cambridge University Press: Cambridge, UK.

Fitt, B.D.L., McCartney, H.A. & Walklate, P.J. 1989. The role of rain in dispersal of pathogen inoculum. Annual Review of Phytopathology 27, 241–270.

Geagea, L., Huber, L. & Sache, I. 1999. Dry-dispersal and rain-splash of brown (*Puccinia recondita* f.sp. *tritici*) and yellow (*P. striiformis) rust spores from infected wheat leav*es exposed to simulated raindrops. Plant Pathology 48, 472–482.

Geagea, L., Huber, L., Sache, I., Flura, D., McCartney, H.A. & Fitt, B.D.L. 2000. Influence of simulated rain on dispersal of rust spores from infected wheat seedlings. Agricultural and Forest Meteorology 101, 53–66.

Gregory, P.H. 1968. Interpreting plant disease dispersal gradients. Annual Review of Phytopathology 6, 189–212.

Gregory, P.H. 1973. The Microbiology of the Atmosphere. 2nd edition. Leonard Hill: Plymouth.

Huber, L., Madden, L.V. & Fitt, B.D.L. 1998. Rain-splash and spore dispersal: a physical perspective. In Jones, D.G. (ed.) 'The epidemiology of plant diseases', pp. 348–370. Kluwer Academic Publishing: Dordrecht.

Huber, L., McCartney, H.A. & Fitt, B.D.L. 1997. Influence of target characteristics on the amount of water splashed by impacting drops. Agricultural and Forest Meteorology 87, 201–211.

Irwin, J.A.G., Cameron, D.F. & Ratcliff, D. 1984. Influence of environmental factors on the development of the anthracnose disease of *Stylosanthes* spp. Australian Journal of Agricultural Research 35, 473–478.

Jeger, M.J. 1981. Influence of environmental conditions on spore dispersal and infection by *Septoria nodorum.* Plant Pathology 99, 29–34.

Kiyosawa, S. & Shiyomi, M. 1972. A theoretical evaluation of the effect of mixing resistant variety with susceptible variety for controlling plant disease. Annals of Phytopathological Society of Japan 38, 41–51.

Lenné, J.M., Sonoda, R.M. & Parbery, D.G. 1984. Production of conidia by setae of *Colletotrichum* species. Mycologia 76, 359–362.

Louis, I. & Cook, R.C. 1985. Conidial matrix and spore germination in some pathogens. Transactions of the British Mycological Society 84, 661–667.

Lovell, D.J., Parker, S.R., Van Peteghem, P., Webb, D.A. & Welham, S.J. 2002. Quantification of raindrop kinetic energy for improved prediction of splash-dispersed pathogens. Phytopathology 92, 497–503.

Madden, L.V., Wilson, L.L. & Ntahimpera, N. 1998. Calibration and evaluation of an electronic sensor for rainfall kinetic energy. Phytopathology 88, 950–959.

Madden, L.V., Yang, X. & Wilson, L.L. 1996. Effects of rain intensity on splash dispersal of *Colletotrichum acutatum.* Phytopathology 86, 864–874.

Maffia, L.A. & Berger, R.D. 1999. Models of plant disease epidemics. II: Gradients of bean rust. Journal of Phytopathology 147, 199–206.

Marshall, J.S. & Palmer, W.M.K. 1948. The distribution of raindrops with size. Journal of Meteorology 5, 165–166.

Miles, J.W. & Lascano, C.E. 1997. Status of *Stylosanthes* development in other countries. I. *Stylosanthes* development and utilisation in South America. Tropical Grasslands 31, 454–459.

Ntahimpera, N., Madden, L.V. & Wilson, L.L. 1997. Effect of rain distribution alteration on splash dispersal of *Colletotrichum acutatum*. Phytopathology 87, 649–655.

Numerical Algorithms Group (NAG). 1990. The NAG FORTRAN Library Manual, Mark 14. Numerical Algorithms Group Ltd.: Oxford.

Parker, S.K., Gleason, M.L. & Nutter, F.W., Jr. 1995. Influence of rain events on spatial distribution of *Septoria* leaf spot of tomato. Plant Disease 79, 148–152.

Tu, J.C. 1981. Anthracnose (*Colletotrichum lindemuthanium*) on white bean (*Phaseolus vulgaris* L.) in Southern Ontario: spread of the disease from an infection focus. Plant Disease 65, 477–480.

Ulbrich, C.W. 1983. Natural variations in the analytical form of the raindrop size distribution. Journal of Climatology and Applied Meteorology 22, 1764–1775.

Yang, X., Madden, L.V., Wilson, L.L. & Ellis, M.A. 1990. Effects of surface topography and rain intensity on splash dispersal of *Colletotrichum acutatum*. Phytopathology 80, 1115–1120.

Yang, X.B. & Tebeest, D.O. 1992. Rain dispersal of *Colletotrichum gloeosporioides* in simulated rice field conditions. Phytopathology 82, 1219–1222.