Genetic Resources Communication

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GLASSHOUSE SCREENING OF SPECIES OF STYLOSANTHES FOR RESISTANCE TO ANTHRACNOSE DISEASES

D.F. CAMERON¹, R.G. O'BRIEN² AND J.A.G. IRWIN³



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SUMMARY

The resistance to anthracnose of 278 accessions of <u>Stylosanthes</u> species was rated in glasshouse tests with Type A and Type B isolates of <u>Colletotrichum gloeosporioides</u>. With the Type A isolates <u>S</u>. <u>capitata</u>, <u>S</u>. <u>sympodialis</u> and <u>S</u>. <u>guianensis</u> were the most resistant while <u>S</u>. <u>fruticosa</u> and <u>S</u>. <u>subsericea</u> were the most susceptible species. In <u>S</u>. <u>hamata</u>, <u>S</u>. <u>humilis</u>, <u>S</u>. <u>montevidensis</u>, <u>S</u>. <u>scabra</u> and <u>S</u>. <u>viscosa</u>, between 8 and 47% of accessions were resistant to Type A isolates. Only <u>S</u>. <u>guianensis</u> and <u>S</u>. <u>montevidensis</u> were damaged by Type B isolates with 28% of <u>S</u>. <u>guianensis</u> accessions being resistant.

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Published by:

CSIRO, Division of Tropical Crops and Pastures, St. Lucia, Brisbane, Queensland 4067, Australia.

GLASSHOUSE SCREENING OF SPECIES OF STYLOSANTHES FOR

RESISTANCE TO ANTHRACNOSE DISEASES

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INTRODUCTION

Anthracnose diseases caused by <u>Colletotrichum gloeosporioides</u> have severely damaged pastures and seed crops of some <u>Stylosanthes</u> species in northern Australia (Irwin and Cameron 1978). Although chemical control measures may be justified on economic grounds in high value seed crops (Davis 1981), disease control in pastures can only be approached economically by developing resistant cultivars (Lenne <u>et al</u>. 1980), perhaps in conjunction with cheap management practices such as burning (Lenne 1982).

In glasshouse tests some lines of <u>S</u>. <u>guianensis</u>, <u>S</u>. <u>hamata</u>, <u>S</u>. <u>viscosa</u> and <u>S</u>. <u>scabra</u> showed high levels of resistance to Type A and Type B forms of <u>C</u>. <u>gloeosporioides</u> but the stability of this resistance was questioned following the detection of a second Type A race with specialization on <u>S</u>. <u>viscosa</u> CPI 33941 (Irwin and Cameron 1978). Screening of large numbers of accessions was proposed to identify highly resistant accessions which could be used in future selection programs if further pathogenic specialization was detected in Type A and Type B forms.

In this paper we report the results of glasshouse screening of accessions of <u>Stylosanthes</u> species against Type A and Type B isolates of <u>C. gloeosporioides</u>.

MATERIALS AND METHODS

A total of 278 accessions of <u>Stylosanthes</u> species was tested in two runs with 12 standard accessions included in both runs. The experimental design was a split plot in two replications with isolates as main plots and accessions as subplots.

Sixteen plants of each accession were grown in peat cups (8 cm diameter, 4 seedlings per cup) in a sand/peat/peanut-shell mix (6:3:1) fertilized with the necessary nutrients. In a preliminary test, inclusion of three isolates in the inoculum (either for Type A or Type B) did not obscure the disease reaction of the most pathogenic isolate for each isolate/host combination so composite inocula were used to screen for resistance to Type A and Type B respectively. Isolates 21257^{\dagger} , 21613 (both race 1, R.D. Davis, J.A.G. Irwin, D.F. Cameron unpublished data) and 21365 (race 2) were used for Type A and 21718, 21719 and 21722 (all race 1, D.F. Cameron, J.A.G. Irwin, R.G. O'Brien unpublished data) for Type B. Individual isolates were prepared by streaking conidia onto Ca²⁺ V-8 agar plates and incubating at 25°C under near ultraviolet light for 10 days. Individual spore suspensions were prepared (c. 1 x 10⁶ spores

[†] Queensland Department of Primary Industries, Plant Pathology Branch accession numbers.

 ml^{-1}) and mixed to give the composite inocula (<u>c</u>. 1 x 10⁶ spores ml^{-1}) for Types A and B respectively. Plants six to eight weeks old were sprayed with a spore suspension until run-off and then boxes of plants were enclosed for 48 h in plastic sheets within a polythene humidity chamber (relative humidity > 85%) in a glasshouse. After removal of the plastic sheets the plants were kept in the chamber for a further eight days. The leaves of each plant were rated for disease severity, 10 days after inoculation, on a 1-10 scale (1 = no visible symptoms, 5 = 10-25% leaf necrosis, 10 = plant death).

RESULTS AND DISCUSSION

A brief summary of the results for seven of the species was published previously (Cameron, O'Brien and Irwin 1980). Type A inoculum produced moderate to severe damage on all species except <u>S</u>. <u>capitata</u> and <u>S</u>. <u>sympodialis</u>, but Type B was damaging to only two species, <u>S</u>. <u>guianensis</u> and <u>S</u>. <u>montevidensis</u>. Comparisons between the two runs for those standard accession/inoculum type combinations causing disease showed that leaf disease ratings were similar (r = 0.82, P < 0.01) so the results for the two runs were combined. Results for all species for the Type A inoculation are summarised in Table 1, and details of disease reactions for individual accessions are shown in Table 2.

All accessions of S. fruticosa, S. subsericea, S sp. 'aff. hamata', S. angustifolia, S. erecta, S. calcicola and S. sp. were classed as susceptible or highly susceptible to the Type A inoculum. S. fruticosa and S. subsericea would be unlikely to be useful in regions where regular disease epiphytotics are experienced, but larger numbers of accessions of the other species need to be tested to see if resistance can be found. Although some accessions of S. hamata, S. humilis, S. montevidensis, S. scabra and S. viscosa were classed as susceptible or highly susceptible, there is scope for selection against susceptibility to the Type A inoculum among the accessions of these five species classed as resistant. Almost all of the <u>S</u>. <u>guianensis</u> (97%) and all the <u>S</u>. <u>capitata</u> and <u>S</u>. <u>sympodialis</u> accessions were either resistant or only slightly susceptible to the Type A inoculum.

Results for the inoculation of <u>S</u>. <u>guianensis</u> and <u>S</u>. <u>montevidensis</u> with Type B inoculum are shown in Table 2. Cultivars Schofield and Endeavour were included in the 31% of <u>S</u>. <u>guianensis</u> accessions classed as highly susceptible. Cultivars Graham, Cook and Oxley were in the resistant, slightly susceptible and susceptible groups respectively. There was again reasonable scope for selection for resistance within <u>S</u>. <u>guianensis</u> with 24% of the accessions classed as resistant. None of the four accessions of <u>S</u>. <u>montevidensis</u> was resistant to the Type B inoculum.

Screening of the 278 accessions of <u>Stylosanthes</u> species has confirmed the marked specificity of Type B disease on <u>S. guianensis</u> and the related species <u>S. montevidensis</u>. Lenne and Sonoda (1979) tested 96 accessions of <u>Stylosanthes</u> species against two isolates of <u>C</u>. <u>gloeosporioides</u>, one each from Florida and Australia. These authors considered that their isolates could probably be classified as Type A and the reactions across the 13 species tested are generally consistent with expectation for Type A isolates. Fifteen of the accessions tested by Lenne and Sonoda (1979) were also tested by us and the disease scores of our Type A inoculum on these common accessions were more similar to those for their Australian isolate (r = 0.54, P < 0.05) than to those for their Florida isolate (r = 0.32, P > 0.05).

Glasshouse tests for disease resistance provide a rapid means of screening large numbers of accessions against defined isolates of a disease organism. With organisms such as <u>C</u>. <u>gloeosporioides</u>, which show considerable pathogenic variation, repeated screening is necessary as new variants of the organism are recognised. Further variation in both Type A (Davis <u>et al</u>. 1984 and unpublished data) and Type B (D.F. Cameron, J.A.G. Irwin, R.G. O'Brien unpublished data) isolates of <u>C</u>. <u>gloeosporioides</u> has already been observed in Queensland and much additional variation is already apparent in South America (Lenne <u>et al</u>. 1982, D.F. Cameron and M. Charchar unpublished data). This challenge is being met by further screening of selected resistant accessions against new Australian races of Type A and Type B disease and through introduction of resistant accessions from major screening programs based in Colombia (C.I.A.T.) and Brazil (EMBRAPA/C.I.A.T.).

ACKNOWLEDGEMENTS

We thank Mr J. Zwierzchaczewski and Mr P. Langdon for capable technical assistance.

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Species .	No. accessions screened	No. rea HS	in ctio S	dise on ¹ c SS	% resistant	
S. angustifolia	2	1	1	0	0	0
S. calcicola	. · · 1	1	0	0	0	0
S. capitata	8	0	0	1	7	88
S. erecta	3	3	0	0	0	0
S. fruticosa	22	18	4	0	0	0
S. guianensis	61	0	2	17	42	69
S. hamata	55	0	10	19	26	47
S. sp.	2	0	2	0	0	0
S. sp. aff. hamata	3	1	2	0	0	0
S. humilis	41	1	11	21	8	
S. montevidensis	4	0	3	0	1	25
S. scabra	39	1	17	18	3	8
S. subsericea	16	9	7	0	0	0
S. sympodialis	5	0	0	1	4	80
S. viscosa	16	2	4	- 4	6	38

Table 1: Summary of disease reactions of <u>Stylosanthes</u> species to Type A inoculum of <u>Colletotrichum</u> gloeosporioides.

¹ R (resistant) - Disease rating (DR) ≤ 2 ; SS (slightly susceptible) - 2<DR<3; S (susceptible) - 3<DR \leq 5; HS (highly susceptible) - DR>5.

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Species	Accession No. ¹ or Cultivar	Disease reaction ²	Species	Accession No. or Cultivar	Disease reaction	Species	Accession No. or Cultivar	Disease reaction
. angustifolia	33433	S S	6. fruticosa	47068	HS	S. guianensis	36257	R
et	40236	IIS		48386	S		37204A	R
. calcicola	36045	HS	· • •	48387	S		37205B	R
. capitata	40238	R	- 11	60354	HS	H	37688	R
	40240A	R	48	60356	HS	11	38222	R
H 1997	40241	R	tt	60357	lis	0	38385	R
 A state of the sta	49809A	. R	••	75151	IIS		38391	R.
*	55838	SS	и,	75152	[÷] IIS	м., н	39112	R-
H (1997)	55840	R	5. guianensis	cv. Cook	R	11	39114	R
	55842	R	11	cv. Endeavour	SS	11	39115	SS
H	55843	R	tt .	cv. Oxlev	R	11	40246	R
. crecta	34118	HS	11	cv. Schofield	S	al -	40255	SS
· =	35015	HS	ta :	Q8442 ³	R	11	40256	R
e [1, 1]	35020	IIS	** _	9215	R		40257	R
. fruticosa	25368	IIS		11491	SS		40258	R
14	32717	S	11	11492	R	11	40259	R
•	32871	HS	14	11846	R	11	40261	SS
• •	40615	HS	48	11849	SS	49	40263	R
j 🗰	40764 A	115	11.	33034	SS	11	40293	SS
* *	41116	115	11	33479	R	11	40294	R
•	41117	S	11 .	33501A	R		40295	R
•	41219	415	4	33706B	Ŕ	P .	40297	SS
	41220	IIS	**	33978	SS	11	40567	R
H	43349	us	**	34000	S	11	41209	R
H .	45174	115	11	34440	SS	17	41218	SS
••	45175	llS	**	34906	R	11	43206	R
	45249	us	*1	34912	SS.	12	46589A	R
54	47067	85	47	34915	SS	1f	49819	R
		•••	**	34478	12		448428	R

Table 2: Disease reaction of accessions of Stylosanthes spp. to Type A inoculum of Colletotrichum gloeosporioides.

S. guianensis	55806	R	•	61672B	SS	S. humilis	49826	55
	55845	R		62160 2	B	<u> </u>	47020	
	55846	SS	•	65361	R	, 김 지난 14 - 2012 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 16 2013 - 1 - 16 2013 - 16 201	430278	55
ti.	55847	R	0	65363	p	이는 방법 유민 관계에서 있는	49020A 520//	D
	55848	R		65365	n C		52047	R
	65358	SS	같은 것은 것은 # 집 것으로	• 65360	J D	에서 동네가 동네는 것이 가지 않는다. 같은 아이들은 바람이 바람이 가지 않는다.	52047	n D
H	65360	20 R		65370	л р		22003	A CC
	65957	R		65771	N CC		52819	29
•	67652	22		70350	ა <u>ა</u> ი		55849	5
FE	67653	DD P	: : : : : : : : : : : : : : : : : : :	70358	7		5585V	22
H	68836	к D		70,359	K R		55851	55
S. hamata	CV Verano	N 92		70300	R		55852	55
<u> </u>	33205	ມມ D		70301	R		55853	55
	33203	n e		70320	ĸ		55854	S
.	35251	Э р		70521	S		57246	S
	27/127	K CC		70522	R		58729	R
u	37037	55		70523	R		61667	SS
	37038	22		70524	R		61668	S
	38843	ĸ		70525	SS		61672A	SS
	40268	ĸ		70526	SS		61674	R
	40275B	R		70529	R		62159	SS
	46587	SS	an an Alexandra an 🗰 an an an an Alexandra an Alexandra an Alexandra an Alexandra an Alexandra an Alexandra an	72850	SS		63463	SS
	46588	S		72859	SS: S		63464	SS
	49080	R	S. sp.	34148	S		65373	R
	55802	SS	1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 -	55797	S	1.1	65374	: SS
	55812	SS	S. sp. aff. h	amat.a 55804	lis	S. montevidens	is 11494	R
	55820	S		55813	S		11496	S
**	55821	S	1	55871	S	•	11847	S
n e	55822	SS	S. humilis	cv. Paterson	S		53962	S
	55823	SS		tv. Lawson	S	S. scabra ·	cy. Fitzroy	S
11	55824	SS	•	cv. Gordon	S		cv. Seca	R
	55825	SS	•	DC29 ⁴	IIS		08240	55
#	55826	SS	1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 -	33830	S		34750	55
	55827	S	a ta ang tao an 🗚 🖓 a sa t	33979	SS	•	34907	55
•	55828	S	•	34116	- 115 - C	•	34707	55 50
•	55830	SS	•	34757	22	•	20112	29 29
	55831	S	•	387538	00 CC	•	10282	
•	56211			60760	00		40202	
•	57968			40209	29		40201	2
en (* 1	61624	n C		40270	22		40284	22
•	61624	•••	ti i i i i i i i i i i i i i i i i i i	40473	Π.		40280	2
•	61670	Ni in		40278	55		40287	55
•	010/0	ĸ		46884	S		40288	S
•	010/IA	R		48385	SS		40289	S
한 1975년 - 프 랑 알 양 양	616718	55	,	4982.4A	55		40290	K
•	61672B	• R	· · · · · · · · · · · · · · · · · · ·	49825	R		40291	R

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Species	Accession No. or Cultivar	Disease reaction	Species	Accession No. or Cultivar	Disease reaction	Species	Accession No. or Cultívar	Disease reaction
S. scabra	40301	S	S. subsericea	08435	115	S. viscosa	33941	IIS
D. <u>Scubra</u>	40301	5		08441	S	11 11 11	36906	SS
e 1977 - 1	49300	22	**	33940	Š,	**	38611	ŝ
11	49833	SS	11	13942	Š	u j	40264B	R
ti	49834	HS	H	33943	HS	11	40296	SS
$\{1,\dots,n_{n}\} \in \{1,\dots,n_{n}\}$	52056	SS	**	37274	S	11	40302	R
$(\mathbf{u}_{i}) \in [\mathbf{u}_{i}]$	52060	SS	11	38604	HS	H	41212	ŝ
H	55799	Š	°U	38605	HS	U.	50223	Š
11	55803	Š	11	38609	HS		51575	HS
tt.	55805	S		38610	HS		55859A	R
11	55811	S	2 (1	41214	HS	tt in the second	55859B	.R
11	55817	SS	11	41217	HS	$H_{\rm eff} = \int_{-\infty}^{\infty} dx dx dx$	55862	R
. 11	55818	SS		46586	IIS	n 1	55863	R
11	55857	S ·	1 2 H	50153	S	11	61675	SS
	55858	S	11	67657	S			
11	55860	SS	ŧ	67658	S			
18 July 11 July 1	55866	S	S. sympodialis	65958	R			
	55867	. · S		65959	SS ·			
0	55868	SS	11	65960	R			
a presidenti H uan Magalag	55870	SS	1	65961	R	la su contra la contra de		
11 Jan 19	55872	S	u u	67703	R			
11	55874	S	S. viscosa	33436	SS			
11	55875	SS		33831	S			
		•				a state of the sta		

1 Commonwealth Plant Introduction number. 2 Commonwealth Plant Introduction number. R (resistant)- Disease rating (DR) ≤ 2; SS (slightly susceptible)- 2 < DR ≤ 3; S (susceptible)- 3 < DR ≤ 5; 3 IIS (highly susceptible)- DR > 5. 4 Queensland Department of Primary Industries number. Local collection number.

Species	Accession No. ¹ or Cultivar	Disease reaction ²	Species	Accession No. or Cultivar	Disease reaction	Species	Accession No. or Cultivar	Diseas c reaction
S. guianensis	cv. Cook	SS	S. guianensis	37688	SS	S. guianensis	49842B	R
1	cv. Endeavour	IIS	11	38222	SS	1	55806	R
	cv. Graham	R		38385	IIS		55845	S
H	cv. Oxley	S	н	38391	HS		55846	SS
	cv. Schofield	HS	Ħ	39112	R		55847	HS
	Q8442 ³	S		39114	SS	•	55848	HS
	9215	SS	11	39115	S	· · · · · · · · · · · · · · · ·	65358	S
"	11491	SS		40246	SS		65360	R
	11492	SS	11	40255	R	이 같은 것이 같이 바람이 같아요. 것	65957	S
•	11846	R	11	40256	HS		67652	HS
11	11849	R	#	40257	R		67653	HS
	33034	HS		40258	S		68836	SS
	33479	KS	•	40259	S	S. montevidensis	11494	HS
11	33501A	R	•	40261	S		11496	SS
	33706B	HS	•	40263	SS	•	11847	۰S
	33978	S	••	40293	SS	an an tha 🗰 a la sainte	53962	SS
•	34000	R	•	40294	R			
•	34440	HS	••	40295	SS			
Ħ	34906	SS		40297	S			
11	34912	R	•	40567	Š			
en e	34915	Š	•	41209	HS			
•	34928	SS	•	41218	HS			
••	36257	R	•	43206	HS			
Ħ	37204A	HS		46589A	HS			
•	37205B	HS	•	49819	2			

Table 3: Disease reaction of accessions of Stylosanthes guianensis and S. montevidensis to Type B inoculum of Colletotrichum gloeosporioides.

¹ Commonwealth Plant Introduction number. ² R (resistant)- Disease rating (DR) ≤ 2 ; SS (slightly susceptible)- 2 \leq DR ≤ 3 ; S (susceptible)- 3 \leq DR ≤ 5 ; ³ HS (highly susceptible)- DR > 5. ³ Queensland Department of Primary Industries accession.