VARIATION IN *DESMODIUM INTORTUM*: A PRELIMINARY STUDY

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ABSTRACT

Variation in growth, persistence, flowering time, seed yield and seed size, leafiness, legume little leaf disease reaction, nodulation, and salt tolerance is reported for a group of Desmodium intortum *introductions grown at two field sites and in glasshouse experiments in south-east Queensland. No single accession was superior to cv. Greenleaf and available variation provides a restricted source from which improved cultivars can be bred. Greater variation is available from interspecific hybridization, particularly with* D. sandwicense.

INTRODUCTION

Greenleaf is high in tannins (Hutton and Coote, 1966) but their importance mended for use in sown pastures in south-east Queensland (Ostrowski, 1969; Rees, 1972). Problems in ease of establishment, persistence, disease and insect resistance, nodulating ability, and seed yield of cv. Greenleaf have been noted (Hutton, 1969; Bryan, 1969). Saline soils may also pose a problem since these occur in some areas to which *Desmodium* is climatically adapted (Evans, 1967).

Of the problems listed above, ease of establishment may be improved by increasing seed size which is correlated with seedling vigour (Imrie, 1972). Slow establishment has also been attributed to poor nodulation by Hutton and Coote (1972) who found variation in nodulating ability among lines selected from cv. Greenleaf, and a response to selection in the progeny of those lines.

Frosts frequently prevent seed maturation of the late flowering Greenleaf. Selection for earlier flowering could improve seed yields and thereby aid persistence in grazed pastures (Hutton, 1964) where increases in stocking rate can reduce the proportion of Greenleaf (Bryan and Evans, 1973). In experimental plots persistence may also be reduced by legume little leaf disease but this appears less of a problem in grazed pastures (R. J. Jones, personal communication).

Greenleaf is high in tannins (Hutton and Coote, 1966) but their importance remains in doubt. While tannins have been shown to reduce protein digestibility (Donnelly and Anthony, 1969; Cope and Burns, 1971) there is no evidence that they have any effect on the grazing ruminant (McLeod, 1973). Because of chemical bonding between tannin and protein, mineralization of nitrogen in leaf litter from *D. intortum* was less than in leaves from *Macroptilium atropurpureum*. Reduced mineralization could affect spring growth of an associate pasture grass and nitrogen balance of the pasture (Vallis and Jones, 1973). Lower levels of tannin appear desirable.

In this paper variation in agronomic characters of Greenleaf and 33 other accessions of *D. intortum* is reported.

EXPERIMENTAL METHODS

Field Experiments

Inoculated seed was sown in peat pellets in a glasshouse at the Samford Pasture Research Station, 25 km north-west of Brisbane. Seedlings were transferred to the field at both Samford and Beerwah (80 km north of Brisbane) when eight to ten weeks old.

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† There is some confusion in *Desmodium* nomenclature with regard to what should be classified as *D. intortum* and *D. aparines*. In this paper all accessions are assigned to *D. intortum* pending clarification of the taxonomy of this group.

At Samford there were two replicate rows of each accession with five plants per row. Rows were 2 m apart and plants 2 m apart within rows. The nursery was kept relatively free of weeds during the first summer by cultivation and herbicide sprays. Grass and weed growth were allowed in the following two years so that *Desmodium* growth could be assessed in a competitive situation. Growth between rows was checked by regular mowing. Plants were cut to a height of 15 cm during winter each year. The nursery, of about 1 ha, was grazed during the third season with approximately 25 cattle/ha from October 15 to 25, December 21 to 24, January 18 to 19, and March 20 to 28. Growth was subjectively rated in spring and summer each year. A '0' to '5' scale was used with '0' indicating absence due to death and '5' indicating vigorous growth and complete cover of a single plant plot. Scores of '1' to '4' were allocated to plants having less spread and vigour of growth.

At Beerwah a single row of each accession was planted, rows being 2.5 m apart and plants 1 m apart within rows. Results are available for one year only. Rows were rated for growth during summer, flowering dates recorded, and seed yield and seed size measured.

Glasshouse Experiments

(a) Legume Little Leaf Disease Resistance. Two plants of each accession were grown in pots until stems were 30 to 50 cm long when stems 10 to 15 cm long from diseased plants were grafted onto them. Cut ends of diseased stems were immersed in water in specimen tubes attached to supporting stakes. Plants were examined weekly for the expression of symptoms of little leaf disease.

(b) Salt Tolerance. Three replicated experiments were conducted to test for salt (NaCl) tolerance. Plants were grown in washed river sand in 15 cm square plastic pots and irrigated with nutrient solution using equipment and methods described by Hutton (1971). Chloride levels in the control and salt solutions were 2 and 17 milliequivalents/litre respectively. In two of the experiments seedlings were inoculated with *Rhizobium* culture CB627 at the rate of 2 ml broth per pot of four plants. In the third experiment plants received mineral nitrogen as NH_4NO_3. The effect of salt stress was determined by comparing the yield of plants grown in salt solution with the yields of paired controls. Plants were harvested when eight weeks old.

(c) Nodulation. Nodulation was assessed on plants grown on nutrient agar in test tubes and inoculated with *Rhizobium* culture CB627. In each trial there was a control (CPI 17916) and five other accessions. Equipment, methods and rating system were as described by Hutton and Coote (1972). Plants were rated 28 days after sowing.

Laboratory Analyses

(a) Leaf: stem ratios. Ratios were determined on a dry matter basis on samples consisting of the terminal portions of five randomly selected stems from each row. Each stem had five expanded leaves.

(b) Tannin content. The Folin-Dennis method (Burns, 1963) which provides a measure of total polyphenols was used. Assessments were made on leaf samples collected in January 1971, dried at 80°C, and ground to pass a 1 mm sieve. Results are expressed as percent tannin equivalent on a plant dry matter basis.

RESULTS

Growth and Persistence

Accessions were classified according to whether they made good, moderate or poor growth during two seasons at Samford and one season at Beerwah (Table 1). Not all accessions were grown at both sites but of those that were CPI nos. 18155, 38716 and 43201 rated highly at both sites while CPI 40128, a Bolivian introduction rated poorly at both sites. Bolivian introductions generally did not perform well.

TABLE 1

Classification of D. intortum *accessions according to bulk of forage produced over two seasons at Samford and one season at Beerwah.*

Forage Bulk	Samford	Beerwah
High: mean score > 3.5	17916,* 18007, 18155, 38716, 38719, 43201, Q8382, Greenleaf	18383, 23189, 38720, 43201, 46549, 46558, 18155, 38716
Medium: mean score 2.0 ± 3.5	18009, 28447, 38713, 38715, 40136, 45259	17916, 18009, 40123, 40126, 40152, 40153, 46551, 46553, 46554, 46563, 46564, 28447, 38715, Q8382, P1481
Low: mean score < 2.0	38720, 40124, 40126, 40128, 40156, 43201, 33814	40121, 40128, 45259, 46550, 46552, 33814

* Plant introduction number.

Accessions were rated satisfactory or unsatisfactory for persistence at the end of the third season at Samford. Those rated unsatisfactory were CPI 40126 and CPI 40128. These accessions had 20 and 40% plant death respectively. Growth habit, which varied from prostrate trailing types, through spreading semi-erect, to semi-erect to erect shrubs did not appear to be associated with either yield or persistence.

Flowering Time

All except the earliest accessions (CPI nos. 40128, 40156 and 45259) failed to flower at Samford in the first season. In the second season all accessions except Q8382 flowered during May and set some seed before being frosted. In the more favourable climate at Beerwah flowering extended over a longer period. All accessions flowered and set seed. Dates of commencement of flowering are presented in Table 2. Flowering later than mid-May would be too late for satisfactory seed production in south-east Queensland in most years.

Seed Size and Seed Yield

Seed size varied from 1.20 to 3.24 g/1000 with the majority of accessions having seed of size less than 2 g/1000. Yield varied from none to 350 g/row (Table 2). The CPI 18009 seed sample was lost but no seed was harvested from CPI 40128 and CPI 46550. Highest seed yields were obtained from accessions which produced a large number of inflorescences and which readily self tripped.

Forage Quality

A high leaf: stem ratio is desirable for high feed value and accessibility of leaves. There was considerable variation in leafiness of accessions (Table 2). Those with low ratios were generally coarser types with thick stems, large leaves and long internodes.

Most accessions had tannin levels within the range 5 to 7.5% tannin equivalent (Table 2). The extremes were 3.43 and 9.31% suggesting sufficient variability for selection.

TABLE 2

Origin of and variation among accessions of D. intortum.

C.P.I. No.[1]	Country of Origin	Flowering Date at Beerwah	Seed[2] Size	Seed[3] Yield	Leaf: stem Ratio	% Tannin Equivalent	Nodulation[4] Score
			Grown at Samford only				
Greenleaf	Australia[5]	8-14 May[6]			0.99	6.51	90
18007	Hawaii				1.09	5.35	
38713	Hawaii				1.15	7.28	
38719	Hawaii				1.35	7.05	
40156	Bolivia				0.78	3.82	61*
			Grown at Samford and Beerwah				
17916	El Salvador	16 May	1.33	110	1.19	5.55	100
18009	Hawaii	17 April			1.39	5.47	134*
18155	Hawaii	14 May	1.82	119	1.02	6.55	
28447	Costa Rica	30 May	1.94	29	1.05	7.42	92
38715	Hawaii	11 June	1.42	149	0.90	6.32	
38716	Hawaii	20 May	1.75	242	1.05	7.28	
38720	Hawaii	28 May	1.35	3	0.86	8.58	95
40126	Bolivia	25 April	1.41	142	1.43	9.31	116
40128	Bolivia	10 April			1.41	3.43	106
43201	Bolivia	13 May	2.86	350	1.14	8.04	91
45259	Venezuela	27 April	2.77	68	0.80	6.18	125*
Q8382	Guatemala	30 May	1.72	75	1.17	5.06	121
			Grown at Beerwah only				
18383	Hawaii	15 May	1.40	231			
23189	Philippines	14 May	1.59	123			114
40121	Brazil	1 April	2.22	19			
40123	Brazil	14 April	1.99	6			
40152	Bolivia	27 April	3.24	31			
40153	Bolivia	1 May	2.17	60			
33814	Mexico	1 May	1.92	13			7*
46549	Guatemala	17 May	1.44	166			79*
46550	Guatemala	26 May					120
46551	Guatemala	16 May	1.49	73			56*
46552	Guatemala	7 June	2.12	156			122*
46553	Guatemala	20 May	1.20	58			72*
46554	Guatemala	9 June	2.09	60			100
46558	Guatemala	20 May	2.25	121			
46563	Guatemala	2[illegible] May	1.85	77			53*
46564	Guatemala	21 May	1.26	67			92

[1] Plant introduction number.
[2] g/1000 seeds.
[3] g/row of 6 plants.
[4] As percent of score of CPI 17916.
[5] Greenleaf was released in 1963 under the name Beerwah and consisted of a mixture of the introductions CPI 17916, CPI 18009, and CPI 23189.
[6] Flowering date of Greenleaf grown in plots adjacent to introduction nursery.
* Significantly different ($P = 0.05$) from CPI 17916.

Legume Little Leaf Disease

All accessions tested for resistance to legume little leaf disease were susceptible. Symptoms developed in six to eight weeks in CPI nos. 17916, 18007, 18009, 38713, 38715, 38716, 38719, 38720, 40122, 40125, 40126, 40128, 40156, 43201, and Q8382. However, symptoms did not develop until about 15 weeks after grafting in CPI nos. 18155, 28447, and 45259. A repeat test on CPI 18155 and CPI 45259 gave similar results. No legume little leaf occurred in either field nursery.

Nodulation

Mean nodulation scores, each of 54 plants, are presented (Table 2) as a percentage of the score of the CPI 17916 control. Of nineteen accessions four were significantly better, six significantly worse, and nine no different from CPI 17916. CPI 33814 gave an extremely poor result and was obviously not effectively nodulated by the commercial inoculum CB627.

Salt Tolerance

Fifteen accessions and cv. Greenleaf were tested for growth response to salt stress (Table 3). Stressed plants grown with mineral nitrogen in the nutrient solution averaged 78.4% of the yield of non-stressed plants. There was no correlation ($r = 0.16$) between seedling vigour as measured by the yield of non-stressed plants, and yield depression due to salt stress.

TABLE 3

Yield of plants subjected to salt stress as a percentage of the yield of paired controls.

C.P.I. No.	Yield of controls grown with mineral N (g)	Yield of stressed as % of non-stressed	
		Mineral N	Nodulated
Greenleaf	1.22	46.9	39.9
17916	3.88	86.4	88.6
40126	0.79	99.0	35.5
23189	0.30	12.1	36.3
28447	1.07	59.3	35.9
33814	0.73	119.2	49.3
40128	0.53	91.6	42.2
40136	1.11	80.6	62.3
43201	2.08	99.7	69.6
45259	1.96	82.6	55.6
18009	1.50	97.3	97.9
Q8382	2.89	65.5	45.9
40156			29.5
18007			37.0
18155			63.6
38720			50.8
L.S.D. (5%)	0.46		

When plants were inoculated with *Rhizobium* the yield of stressed plants averaged only 52.2% of the yield of non-stressed plants. The regression of stressed plant yield on nodule mass was calculated and found to be highly significant ($P < 0.001$) with 83% of the variation in plant yield being explained by the regression. This suggested that yield reduction was largely due to an effect of salt on nodulation.

DISCUSSION AND CONCLUSIONS

Within *D. intortum* there was variation for all characters studied. The earliest flowering material was mostly of Bolivian origin but these accessions generally lacked vigour. Seed yields varied considerably. Although the data is from unreplicated plots the figures presented are considered to provide a good guide to the relative yielding ability of accessions. Observations suggest that seed yield is closely associated with flowering behaviour. In accessions on which flowers are self tripping seed set is good, but others lack this ability and set very little seed in the absence of a large bee population. Yield is also affected by flower density and seed size.

Until the effects of high tannin levels on feed utilization, nitrogen transfer, and grass/legume balance are better defined the importance of tannins cannot be properly assessed. The variation required to produce cultivars lower in tannin than cv. Greenleaf is available but the accessions with the lowest levels of tannin were of Bolivian origin and agronomically poor.

The study by Hutton and Coote (1972) of early nodulation in selections from cv. Greenleaf showed that improvement of nodulation is possible in this cultivar. The experiments reported here show that there is also significant variation among accessions for nodulating ability and possibly an additional source of genes for improved nodulation. The failure of CPI 33814 to effectively nodulate with *Rhizobium* strain CB627 indicates the importance of testing new introductions against the commercial strain of inoculum, and the collection of nodules together with seed of legumes for introduction. The poor yield performance of CPI 33814 (Table 1) may be largely due to its failure to nodulate satisfactorily.

The finding that high salinity had an effect on nodulation is in agreement with the conclusion of Wilson (1970) who studied response to salinity in *Glycine*. There were accessions which nodulated and yielded better than cv. Greenleaf under saline conditions indicating variation for this character and the possibility of obtaining cultivars suitable for saline soils.

No single accession was superior to cv. Greenleaf in all characters and available variation in *D. intortum* provides a limited source from which improved cultivars can be bred. A more useful source of variability, particularly for earliness and large seed size is the species *D. sandwicense*. Several early flowering, large seeded, and vigorous segregants from hybrids between *D. intortum* and *D. sandwicense* have been obtained (Imrie, unpublished data).

ACKNOWLEDGEMENTS

The interest and advice of Dr. E. M. Hutton is appreciated. Mr. M. N. McLeod supervised the tannin analyses. *Rhizobium* cultures were supplied by Dr. D. O. Norris and Dr. R. A. Date. Technical assistance was provided by Mr. J. J. Barrett.

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[Accepted for publication June 10th, 1973]