Bot. Bull. Academia Sinica (1990) **31:** 143-147

Histochemical localization of phenolic deposits in the leaf blades of three grass species from southern Africa.

J. D. Theunissen
Grassland Research Centre, Private Bag X05, Lynn East 0039, Republic of South Africa

(Received November 17, 1989; Accepted January 31, 1990)

Abstract. The distribution of phenolic deposits in the leaves of *Themeda triandra, Digitaria eriantha* and *Setaria sphacelata* var. *sphacelata* from different habitats was investigated during cultivation under uniform environmental conditions. The plants retained their leaf phenolic distribution pattern over a period of 10 months. No phenolic deposits were found in the leaf blades of *D. eriantha* and *S. sphacelata* var. *sphacelata*. However, individuals of *T. triandra* collected from habitats in the lower rainfall regions had phenolic deposits in both epidermal layers, whereas phenolic deposits were absent or present only in the adaxial epidermal layer of plants collected from habitats in the higher rainfall regions.

Key words: Anatomy; *Digitaria eriantha;* Epidermis; Histochemistry; Phenolic deposits; Poaceae; *Setaria sphacelata* var. *sphacelata*; *Themeda triandra.*

Introduction

Plant phenolics of which the precise physiological function is unknown, constitute a group of natural products of structural diversity and wide phylogenetic distribution (Zucker, 1983). Phenolic substances can be found in different cellular sites, for example, within the cell wall, in cytoplasmic vacuoles, or may be dispersed in the cytoplasm (Chaffe and Durzan, 1973). Salatino *et al.* (1988) mentions that phenolic deposits are more often found in external tissue of plant organs, such as the epidermal layer, and has also been detected in relatively young and undifferentiated tissue. With regard to plant parts that normally bear cells with phenolic deposits, Esau (1977) comments that no tissue lacks polyphenols entirely. Foliar phenolics have been shown to fluctuate as a result of varying light intensities (Waring *et al.*, 1985), water (Tempel, 1981) and nutrient supply (Brown *et al.*, 1984). McKey *et al.* (1978) has shown that plants inhabiting unfavourable sites have relatively high concentrations of phenolics.

Polyphenolic deposits are unknown in algae, mosses and most monocotyledons (McLeod, 1974). Although reports of the presence of phenolic deposits could not be found in the extensive comparative anatomical literature of the Poaceae, the occurence of phenolic deposits in this family has recently been investigated by Ellis (1987). *Digitaria eriantha* Steud., *Setaria sphacelata* (Schumach.) Moss var. *sphacelata* and *Themeda triandra* Forssk., which inhabit a wide range of vegetation and soil types over large geographical areas, are important pasture grasses in southern Africa. These species were used in a preliminary survey aimed at determining whether the presence and distribution of phenolic deposits are influenced by environmental factors.

Materials and Methods

Five plants of *D. eriantha*, *S. sphacelata* var.

[1] Present address: Department Plant Sciences, Potchefstroom University for CHE, Potchefstroom, 2520, South Africa.

sphacelata and *T. triandra* were collected in each of four different rainfall regions (400-500 mm, 500-600 mm, 600-700 mm and 700-800 mm) in two habitats, namely, shallow soil (dry habitat) and deep soil or water course (moist habitat), in the grassland biome of southern Africa. The plants were grown in polyethylene bags (200 mm diameter), in a glasshouse maintained at 27/22°C day/night temperature and watered every third day. During cultivation, each tuft was defoliated every sixty days for a period of 10 months to enhance vegetative growth among all the plants simultaneously. The presence of phenolic deposits was determined by the histochemical localization of phenolics before and after cultivation. The middle section of 15 leaf blades per plant was used in this study and prepared as follows: Material was fixed in 4% paraformaldehyde at pH 7.3. After desilification (Breakwell, 1914) leaf segments were dehydrated in a graded alcohol series, embedded in 2-hydroxyethyl metacrylate according to the GMA-method of O'Brien and McCully (1981) and sectioned at 3 μm. The presence of leaf phenolic deposits was indicated by staining sections with $FeCl_3$ (Johansen, 1940) and confirmed with the nitroso-reaction as suggested by Reeve (1951). For general histology, sections were stained with 0.5% aqueous toluidine blue O. Background staining caused by toluidine blue O was removed by placing the sections in cellusolve for a few seconds. Micrographs were taken with a Zeiss photomicroscope.

Results

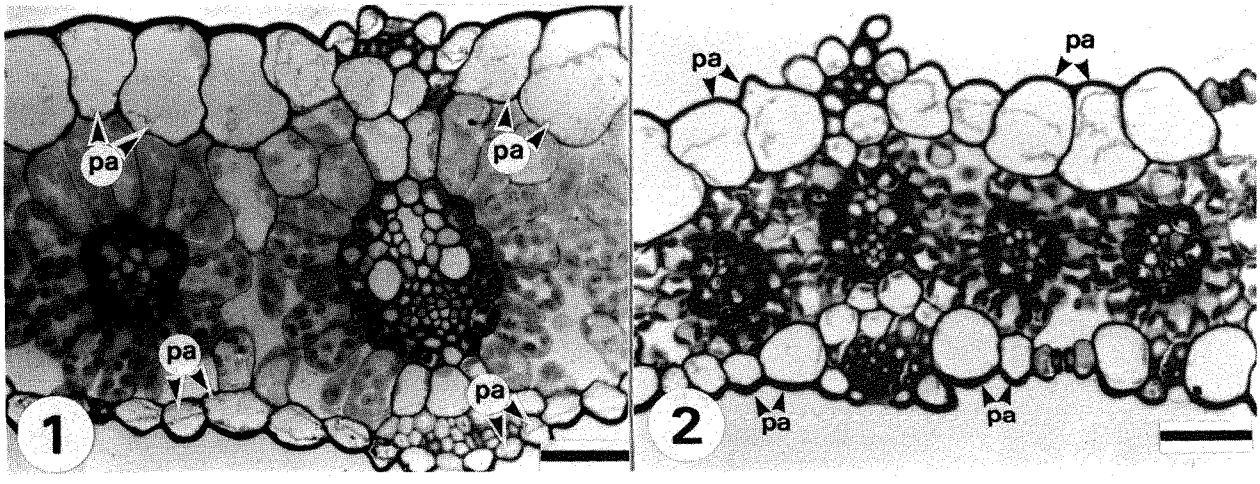
No phenolic deposits were found in the leaves of any of the individuals of *D. eriantha* (Fig. 1) and *S. sphacelata* var. *sphacelata* (Fig. 2) investigated. However, there is a remarkable difference in the distribution of leaf phenolic deposits between plants of *T. triandra* collected from the different rainfall regions and habitats (Table 1).

Furthermore, each plant of the latter mentioned species studied retained its leaf phenolic distribution

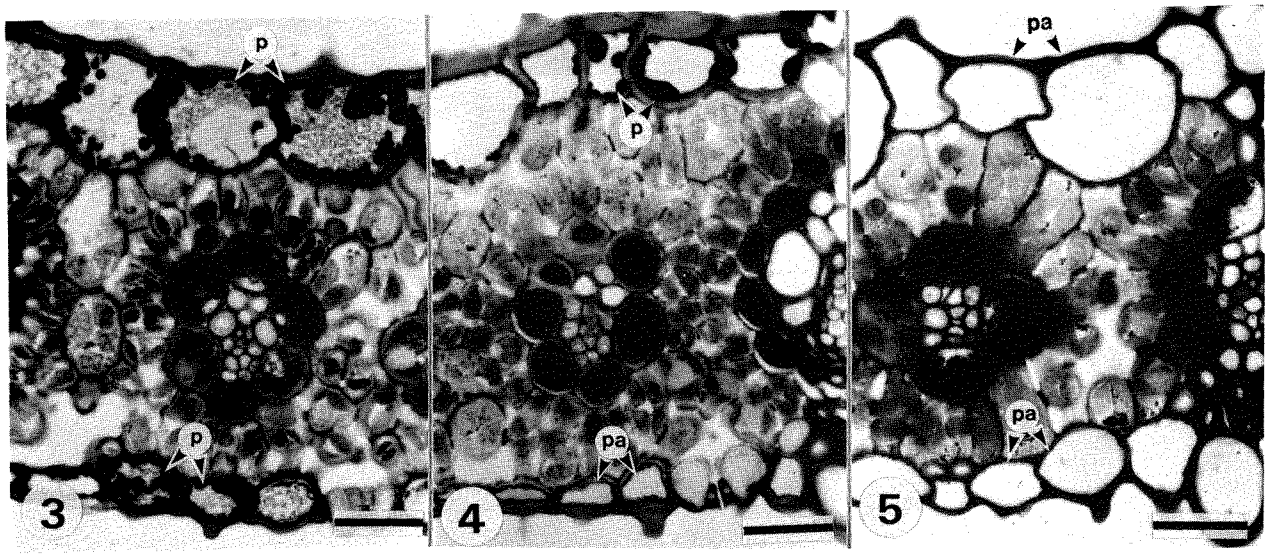
Table 1. *Distribution of phenolic deposits in the leaf epidermal layers of* ***T. triandra*** *from different rainfall regions and habitats.*

Rainfall region (mm)	Habitat	Distribution* of phenolics
400 to 500	moist	++
	dry	++
500 to 600	moist	++
	dry	++
600 to 700	moist	+
	dry	+
700 to 800	moist	+
	dry	-

* -, absent; +, present in adaxial epidermis; ++, present in both abaxial and adaxial epidermis.



Figs. 1-2. Micrographs of transverse sections of the leaf blades of *D. eriantha* and *S. sphacelata* var. *sphacelata*, stained with toluidine blue O showing no phenolic deposits in the epidermal layers. 1, *D. eriantha*; 2, *S. sphacelata* var. *sphacelata*. pa-cells with no phenolic deposits. Bars=10 μm.



Figs. 3-5. Micrographs of transverse sections of the leaf blades of *T. triandra*, stained with toluidine blue O showing the distribution of phenolic deposits in the epidermal layers. 3, *T. triandra* growing in low rainfall regions showing phenolic deposits in both the abaxial and adaxial epidermal layers; 4, *T. triandra* growing in high rainfall regions showing phenolic deposits in the adaxial epidermal layer; 5, *T. triandra* growing in high rainfall regions with phenolic deposits absent. p-phenolic deposits; pa -cells with no phenolic deposits. Bars=5 μm.

pattern during cultivation under uniform environmental conditions. The nitroso-reaction and $FeCl_3$-test indicated that the distribution of phenolic deposits in the leaves of *T. triandra*, which appear as dark stained granular material when stained with toluidine blue O, is restricted to the epidermal layers and situated peripherally in the epidermal cells (Figs 3 and 4). Individuals of *T. triandra* collected from habitats in the lower rainfall regions have phenolic deposits in both epidermal layers (Fig. 3), whereas phenolic deposits are completely absent or present only in the adaxial layer of tufts collected from habitats in the higher rainfall regions (Figs 4 and 5).

Discussion

Although representatives of only eight habitats were investigated, this study indicates the fluctuation in the distribution pattern of leaf phenolic deposits within *T. triandra* grown under uniform environmental conditions, whereas no phenolic deposits were formed in the leaves of *D. eriantha* and *S. sphacelata* var. *sphacelata* before or after cultivation. It therefore seems that the formation and distribution of phenolics in the species studied are genetically controlled, as the individuals retained their original distribution pattern, irrespective of change in environmental conditions, over a period of 10 months. It has previously been stated that Australian sclerophyllous plants have a high phenolic content when growing in dry conditions (Grieve, 1953). The present study indicates that the leaf epidermal cells of representatives of *T. triandra* from low rainfall regions also exhibit more phenolic deposits than individuals occupying moist areas.

With regard to the possible function of phenolics in leaf tissue, Salatino *et al.* (1988) ascribes to phenolics the function of protecting inner tissues against excess visible and/or ultraviolet irradiation when these substances occur in peripheral cells. It appears that this function cannot be assigned to *T. triandra*, as these substances were absent in both the ab- and adaxial layers of several plants grown under uniform environmental conditions. Another possibility seems to be the assignment of an antiherbivory function to polyphenols (Swain, 1979; Robbins *et al.*, 1987). Various authors (Theron and Booysen, 1966; Tainton *et al.*, 1985; Danckwerts, 1987) have indicated that *T. triandra* is a highly palatable species and since palatability is measured by determining animal preference (Tribe and Gordon, 1950), an antiherbivory function can probably not be assigned to phenolic deposits in this species. Comparative ecological studies (Bosch & Janse Van Rensburg, 1987) have shown that *T. triandra*, although having phenolic deposits and occuring sympatrically

with various other palatable species, was found to be utilized to such an extent by herbivores that it decreased in abundance. From a pasture ecological point of view this study emphasizes the necessity for further investigations especially with regard to the presence, distribution and function of phenolic compounds in the Poaceae.

Acknowledgments. The author would like to thank Prof. G. H. J. Krüger and Mr. G. F. Smith for their helpful suggestions on the manuscript. Financial assistance granted by the Department of Agricultural Development, South Africa is gratefully acknowledged.

Literature Cited

Bosch, O. J. H. and F. P. Janse Van Rensburg. 1987. Ecological status of species on grazing gradients on shallow soils in the western grassland biome of South Africa. J. Grassld. Soc. Sth. Afr. **4**: 143-147.

Breakwell, E. 1914. A study of the leaf anatomy of some native species of the genus *Andropogon* (Gramineae). Proc. Linn. Soc. N. S. W. **39**: 385-394.

Brown, P. H., R. D. Graham, and D. J. D. Nicholas. 1984. The effects of manganese and nitrate supply on the levels of phenolics and lignin in young wheat plants. Pl. Soil **81**: 437-440.

Chaffe, S. C. and D. J. Durzan. 1973. Tannin inclusions in cell suspension cultures of White Spruce. Planta **113**: 251-262.

Danckwerts, J. E. 1987. Towards improved livestock production off semi-arid grassveld and savannas in the Eastern Cape. S. Afr. J. Sci. **83:** 346.

Ellis, R. P. 1987. Tanniniferous epidermal cells in the Poaceae. Proceedings of the 13th South African Association of Botanists Congress (Abstracts), University of Natal, Durban.

Esau, K. 1977. Anatomy of Seed Plants, Wiley, New York.

Grieve B. J. 1953. The physiology of sclerophyll plants. J. Proc. R. Soc. West. Aust. **39**: 31-45.

Johansen, D. A. 1940. Plant Microtechnique, McGraw-Hill, New York.

McKey, D., P. G. Waterman, C. N. Mbi, J. S. Gartlan, and T. T. Struhsaker. 1978. Phenolic content of vegetation in two African rain forests: Ecological implications. Science **202**: 61-64.

McLeod, M. N. 1974. Plant tannins-their role in forage quality. Nutr. Abstr. Rev. **44**: 804-815.

O'Brien, T. P. and M. E. McCully. 1981. The study of plant structure. Principles and Selected Methods, Termarcarphi, Melbourne.

Reeve, R. M. 1951. Histochemical tests for polyphenols in plant tissues. Stain Technol. **26**: 91-96.

Robbins, C. T., T. A. Hanley, A. E. Hagerman, O. Hjeljord, D. L. Baker, C. C. Schwartz, and W. W. Mautz. 1987. Role of tannins in defending plants against ruminants: Reduction in protein availability. Ecology **68**: 98-107.

Salatino, A., W. R. Monteiro, and N. Bomtempi. 1988. Histochemical localization of phenolic deposits in shoot apices of common species of Asteraceae. Ann. Bot. **61**: 557-559.

Swain, T. 1979. Tannins and lignans. *In* G. A. Rosenthal and D. H. Janzen (eds.), Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York, pp. 657-682.

Tainton, N. M., D. I. Bransby, and P. De V. Booysen. 1985. Common Veld and Pasture Grasses of Natal, Shuter and Shooter, Pietermaritzburg.

Tempel, A. S. 1981. Field studies of the relationship between herbivore damage and tannin concentration in bracken *Pteridium aquilinum* Kuhn.). Oecologia **51**: 97-106.

Theron, E. P. and P. De V. Booysen. 1966. Palatability of grasses. Proc. Grassl. Soc. south. Afr. **1**: 111-120.

Tribe, D. E. and J. G. Gordon. 1950. An experimented study of palatability. Agric. Prog. **25**: 94-101.

Waring, R. H., A. J. S. McDonald, S. E. T. Larsson, A. Wiren, A. Arwidsson, A. Ericsson, and T. Lohammar. 1985. Differences in chemical composition of plants grown at constant growth rates with stable mineral nutrition. Oecologia **66**: 157-160.

Zucker, W. V. 1983. Tannins: Does structure determine function? Am. Nat. **121**: 335-365.

以組織化學方法研究南非三種草本植物葉片之酚類化合物的堆積情形

J. D. Theunissen

Grassland Research Centre, Private Bag X05,
Lynn East 0039, Republic of South Africa.

作者對栽培於相同生育地之 *Themeda triandra, Digitaria eriantha* 和 *Setaria sphacelata* var. *sphacelata* 等草本植物葉片之酚類的堆積情形加以研究，其中在 *D. eriantha* 和 *S. sphacelata* var. *sphacelata* 的葉片中無酚類化合物堆積的現象。然而，採自較低雨量棲地之 *T. triandra*，其葉片之上下表皮均有酚類化合物之堆積，而採自較高雨量地區之同種植物則缺乏、或僅存在於上表皮。經十月之觀察研究，其結果仍然不變。