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Interspecies and location variation in oxalic acid concentrations in certain *Atriplex* species and *Cassia sturtii*

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Abstract

The aim of this study was to evaluate the interspecies variation in oxalic acid (OA) concentrations of leaves between *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii*. Significant differences in the oxalic acid concentration were noted between the three *Atriplex* spp. and *C. sturtii* at Hatfield. *Cassia sturtii* at Lovedale also had a significantly lower OA concentration than the *Atriplex* spp. No significant differences were noted between the *Atriplex* spp. at Lovedale, although the species at Lovedale contained significantly higher OA concentrations than at Hatfield. The OA concentrations recorded in this study were not considered to be toxic to grazing livestock.

Keywords: *Atriplex, Cassia sturtii,* oxalic acid, cation balance
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Introduction

According to Marais (1997) the intake of excessive amounts of oxalate by mammals leads to clinical conditions such as chronic renal failure, calcium oxalate urolithiasis, hypocalcaemia, nutritional secondary hyperparathyroidism and death. Chronic oxalate poisoning in herbivores has a depressing effect on animal production. Acute toxicity usually affects small numbers of animals, but can occasionally cause the death of hundreds of head of livestock in a single incidence. Poisoning is most common when animals are not adapted and / or hungry. Acute toxicity has been recorded in cattle consuming pasture that contained oxalate at a concentration of 6.9%, while the lethal oxalic acid (OA) dose for sheep is approximately 1.1 g/kg body weight. According to Marais (1997), sheep which were not adapted, died over a period of five days after exposure to young buffalo grass (*Cenchrus ciliaris*) with a soluble oxalate content of about 2.5%. Watson *et al*. (1987) also noted young *Atriplex* material containing high OA concentrations of up to 9.07%, and levels decreasing to 2.81% as the plants grew older. No information is available on the OA concentrations in young *Cassia sturtii* plants.

The objective of this study was to evaluate the interspecies variation in OA concentrations in leaves between *Atriplex canescens, A. halimus, A. nummularia* and *C. sturtii.*

Materials and Methods

Leaves were collected from two experimental sites different in ecological conditions. Site one was at the Experimental Farm of the University of Pretoria, Gauteng, South Africa at an altitude of 1360 m (coordinates 025°15'28.9"E, 25°45'03.6" S). It is a summer rainfall area with an average precipitation of 650 mm per annum. The soil type is a Hutton form (MacVicar *et al*., 1977), well drained, slightly acidic and consists of a good nutrient status and an effective depth of 600 mm+. According to soil analysis, the soil pH$_{(H_2O)}$ was 5.7, P status 25 mg/kg, K status 200 mg/kg while the Ca, Mg and Na status were 800, 400 and 440 mg/kg respectively.

Site two was at the farm Lovedale in the Kenhardt district, Northern Cape province, South Africa at an altitude of 1015 m (coordinates 19°44'0.57" E, 29°18'58.8" S). It is a summer rainfall area with an average annual rainfall of approximately 130mm. The soil is also a Hutton form, slightly alkaline and consists of a good nutrient status. According to soil analysis, the soil pH$_{(H_2O)}$ was 8.4, P status 14 mg/kg, K status 337 mg/kg, while Ca, Mg and Na status were 3445, 136 and 179 mg/kg respectively. This type is a shallow calcareous sandy soil with less than 10% clay and an effective depth of not more than 300 mm.

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Leaves were collected from *Atriplex canescens* (Pursch.) cv. Santa Rita (Fourwing Saltbush) Origin: North America), *Atriplex halimus* L. (Origin: Asia, Mediterranean), *Atriplex nummularia* L. (Oldman Saltbush) (Origin: Australia) and *Cassia sturtii* (Origin: Australia).

Sample material randomly collected for each species on both sites was from approximately five year old plants. Leaves (mostly mature) were dried in a force draught oven for 24 hours at 60 °C and milled through a 1 mm screen. Oxalic acid concentration was measured through colorimetric determination of OA as oxalyldihyrazidee, as described by Figenschou & Marais (2000) (personal communication, Cedara Agricultural Research Institute, KwaZulu-Natal, South Africa). This method is quite different from the titration method of Moir (1953) for determining total oxalates. Oxalic acid was extracted from 0.5 g of the milled plant material and measured against 10 mL of a standard pre-prepared OA solution. A series of steps were followed until a mixture with a blue colour was formed. A darker blue colour represented a higher oxalate concentration. The absorption of the mixture was read at 600 nm on a COALAB (model dds CP500) colorimetric spectrophotometer. Calcium and Mg concentrations were determined by atomic absorption spectrophotometry and Na and K concentrations by flame photometry (AOAC, 2000).

Three samples per species per location were analysed. An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between species, locations and first order interactions for the dependant variables. The level of significance between least square means was tested with the help of the Bonferroni's test according to Samuels (1989).

Results and Discussion

The OA concentrations of the three *Atriplex* spp. at Hatfield and Lovedale were higher ($P < 0.05$) than those of *C. sturtii* (Table 1). All the species except *A. canescens* had lower ($P < 0.05$) OA concentrations at Hatfield than at Lovedale.

Oxalic acid concentrations of 5.8% recorded by Wilson (1966) for *A. nummularia* were higher than values recorded in the present study. It could be that the author used younger plants during the experiment. According to Watson *et al*. (1987) the OA concentrations recorded in this experiment were not considered to be toxic to grazing livestock.

Table 1 Oxalic acid concentration (%) of leaf material for *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii* at two different locations (DM basis)

Location	Species			
	A. canescens	*A. halimus*	*A. nummularia*	*C. sturtii*
Hatfield	3.30_1^c (± 0.12)*	2.66_1^b (± 0.08)	3.26_1^c (± 0.07)	0.85_1^a (± 0.25)
Lovedale	3.50_1^b (± 0.01)	3.44_2^b (± 0.03)	3.51_2^b (± 0.03)	1.11_2^a (± 0.11)

Row (a,b,c) and column (1,2) means with common scripts do not differ ($P > 0.05$)
* Standard deviation

To explain the above results, it is important to be able to determine whether plants are calciophobes or calciotrophes (Wollenweber, 2002 – personal communication, Department of Plant Biology, The Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, Denmark). Calciophobes are enriched in oxalate as the dominant carboxylate binding Ca^{2+} contents and resulting in low free vascualar Ca^{2+}, whereas calciotrophes show high total and free Ca^{2+} contents (Ca^{2+} : K > 1) and less oxalate. These physiotypes – defining plants according to their eco-physiological properties – are quite constant and reveal information on how species adapt to their environment. According to Wollenweber (2002) – (personal communication), many eco-physiological studies have emphasised site-specific properties of the investigated plants (xero- *vs*. hygrophytes; glyco- *vs*. halophytes; acidophile *vs*. basiphile plants; calcifuge *vs*. calcicole plants). There is, however, a relationship between ecological and physiological aspects of plant metabolism, and certain physiological properties of plants (i.e. enzyme activities, ion ratios and ion balance) may be used for the interpretation of results from ecological studies. These considerations resulted in the formulation of the physiotype concept. A physiotype being a taxonomic unit with defined physiological properties (i.e. the ratio Ca^{2+} : K^+ or organic / inorganic ions – assessable *via* biochemical analysis of plant matter).

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To determine the physiotypes of the species used for analysis, the percentages as noted in Table 1 and values determined for Ca, Mg, Na and K, in the study, were converted to micro-equivalents per g dry mass (not taking into account differences in biomass) and are represented in Table 2 and graphically in Figures 1 to 3.

From the cation concentration (Fig. 1) it can be seen that the highest cation concentration was in *A. halimus* at both sites, while it was the lowest in *C. sturtii*. Assuming that Ca is mainly bound by oxalate, the following was noted (Fig. 2): The Ca concentration was the highest in *A. canescens* at both sites. Lower values were observed at Lovedale. However, higher oxalate concentrations were noted which lead to low free Ca. *Cassia sturtii* in this case had a high value of free Ca, indicating that this is calciotrophic species.

A Ca:K ratio of almost equal to 1, was noted at Hatfield (Fig. 3), while the ratio was < 1 at Lovedale for the *Atriplex* spp. For *C. sturtii* a higher Ca than K concentration was noted, thus a further indication of the calciotrophic nature of *C. sturtii*.

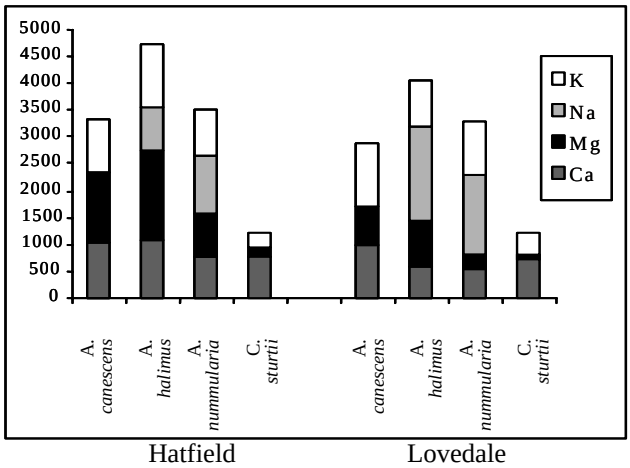

Fig 1 Comparison of cation balances at the two sites (Units: micro equivalents per g dry mass)

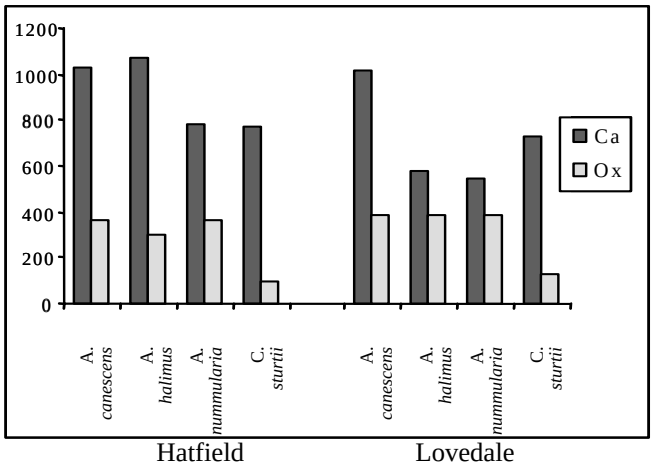

Fig 2 Comparison between Ca and oxalate concentrations at the two sites (Units: micro equivalents per g dry mass)

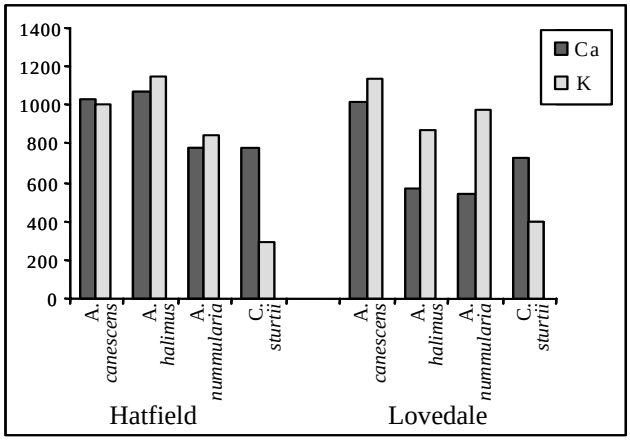

Fig 3 Comparison between Ca and K ratios at the two sites (Units: micro-equivalents per g dry mass)

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Table 2 Percentages of Ca, Mg, Na, K and oxalate converted to micro-equivalents per g dry mass

Location	Species	µeq /g DM					
		Ca	Mg	Na	K	Oxalate	K / Ca
Hatfield	*A. canescens*	1 028	1 325	4	997	367	0.97
	A. halimus	1 073	1 670	814	1 154	295	1.08
	A. nummularia	778	798	1 103	844	362	1.08
	C. sturtii	773	165	1	289	94	0.37
Lovedale	*A. canescens*	1 013	683	35	1 133	389	1.12
	A. halimus	574	856	1 755	875	382	1.52
	A. nummularia	544	247	1 513	972	390	1.79
	C. sturtii	724	99	4	402	123	0.55

Conclusion

The high total free Ca^{2+} and less oxalate concentration in *C. sturtii* indicates an unlikely possibility of oxalate poisoning to occur in animals grazing this plant. A possibility of poisoning is present in the *Atriplex* spp., especially in young plants where the oxalate levels may play a role irrespective of the Ca:K ratio (Watson *et al*., 1987).

Further work must be done on *C. sturtii* to determine the OA status at various stages of growth for this species, since the status of younger plants is unknown and not found in any literature.

Acknowledgement

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