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PRELIMINARY OBSERVATIONS ON THE CYANOGENIC PROPERTIES OF TWO WESTERN AUSTRALIAN *ACACIA* SPECIES

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ABSTRACT

Populations of two known cyanogenic Western Australian Acacia *species were sampled to examine intra-population variations in HCN production and to determine whether the production of cyanogenic glucosides was related to time of year or age of plant. All 20 plants of* A. signata *and 9 of the 40 plants of* A. resinomarginea *examined gave positive reactions for HCN. These reactions were usually strongest in spring-summer, corresponding with the period of flowering, fruiting and new shoot production. Weaker reactions were recorded for autumn-winter when plants were dormant. The production of cyanogenic glucosides seems to be unrelated to plant age.*

INTRODUCTION

Forty four Australian species of *Acacia* are known to be cyanogenic (Conn et al. 1985), but little is known of variability in cyanogenic glucoside production within populations of these species. The present study was undertaken to examine intra-population variations in two known cyanogenic species and to see whether the production of cyanogenic glucosides was related to time of year or age of plant.

METHODS

Species studied. *Acacia resinomarginea* and *A. signata* were selected for this study because both have been shown previously to contain cyanogenic glucosides (Conn et al. 1985) and because both were common and grew together in a readily accessible area. They belong to *Acacia* section *Juliflorae*, the group containing most cyanogenic Australian acacias (Conn et al. 1985). These two arborescent species are reasonably widespread in the wheatbelt region of south-west Western Australia (Maslin and Pedley 1982). *Acacia resinomarginea* flowers from September to November (spring) at which time new shoots are usually developing on most plants. *Acacia signata* flowers from

August to October and although new shoots may also be present during this period they are less frequent than on plants of *A. resinomarginea*. Both species possess mature seeds in the December-January (summer) period during which time some actively growing new shoots may also be present. These phenological data were taken from herbarium sheets and supplemented with a few observations on tagged plants at the study sites.

Study sites. Two sites were selected in the north-central wheatbelt region of south-west Western Australia. The first site was located 15 km west of Mukinbudin, on the road to Bencubbin, and comprised a climax stand of mixed *Acacia* shrubland in which both *A. resinomarginea* and *A. signata* were common. The second site, located on the Mukinbudin-Bencubbin road about 10 km west of site 1 (near Welbungin siding), comprised a regenerating, monotypic stand of *A. resinomarginea*. The soils at both sites were nutrient-deficient yellow sands.

Sampling procedure. This experiment was conducted during 1983. Sixty plants were labelled with numbered tags at the two sites - 20 each of *A. resinomarginea* and *A. signata* at site 1 (sample numbers 1-20 for each species) and 20 of *A. resinomarginea* at site 2 (sample numbers 21-40). About 5-10 grams of foliage was removed from each plant at 2-3 monthly intervals viz. February (summer), April (autumn), July (winter) and October (spring). Samples were gathered by stripping all phyllodes from one randomly selected branchlet. As a result each sample consisted of phyllodes ranging in age up to about 2-3 years old. We did not attempt to standardize our sampling by taking phyllodes of the same age. Plants of varying ages were selected and these were broadly categorized as young (to 2.5 m tall) or mature (above 2.5 m tall). This height class parameter was selected because at about 2-2.5 m the plants reach biological maturity as evidenced by the presence of inflorescences. For *A. resinomarginea* we tested 24 young and 16 mature plants and for *A. signata* 13 young and 7 mature plants.

Test for cyanogenesis. About 20 mg of phyllode material was randomly selected from each sample and tested for its ability to release HCN using the Feigl-Anger method described in Conn et al. (1985). Briefly, this procedure involved the homogenization of foliage in the presence of a β-glucosidase and the subsequent detection of liberated HCN by specially treated filter papers. The degree to which these Feigl-Anger filter papers turned blue was taken as a crude quantitative measure of the amount of HCN liberated and thus the level of cyanogenic glucoside present in the tissue (see caption to Table 1). Although the Feigl-Anger responses were recorded at 1 hour, 4 hour, 12 hour, 24 hour and 48 hour intervals (Bennett 1984) only the 12 hour results are presented here because these best illustrate the relative differences between the plants tested.

RESULTS AND DISCUSSION

The results are presented in Table 1.

Twenty nine of the 60 plants tested released HCN, however, the two species differed greatly in their response to our test procedure. All 20 *A. signata* plants released HCN but in *A. resinomarginea* only nine of the 40 plants were HCN positive. The intra-population variation in cyanogenesis exhibited by *A. resinomarginea* differed between the two sites. At site 1 two plants (10%) were cyanogenic whereas at site 2 seven plants (35%) were cyanogenic. There are no obvious edaphic differences between the two sites

(soil analyses were not conducted) and the ratios of young to mature plants in both populations were approximately the same. Stand structure, as stated above, did vary between the two sites.

Although our method of measuring the quantity of HCN produced was rather crude, the results do show a trend in both species for high levels of HCN production in February (summer) and low levels in April (autumn) and July (winter). However, the two species differed in their October (spring) responses to the HCN test - *A. signata* showed strong reactions (equivalent to the summer value) while *A. resinomarginea* showed relatively weak reactions (but often slightly above the autumn and winter values). These seasonal variations occurred in both young (biologically immature) and mature individuals. As cyanogenic glucosides provide plants with a chemical defence against herbivory (Harborne 1982) it is not surprising that we found highest HCN levels at the time when they were most required, i.e. during the period of new shoot and seed production. It is possible that a glucosidase was responsible for hydrolyzing the cyanogenic glucosides during the dormancy period (i.e. autumn-winter) but we did not test for the presence of such enzymes.

The production of cyanogenic glucosides in both *A. resinomarginea* and *A. signata* seems to be unrelated to plant age. Cyanogenic and acyanogenic individuals occurred in the young and mature age classes of both species. A total of 37 young plants were tested of which 20 (54%) were cyanogenic while of the 23 mature plants tested 9 (39%) were cyanogenic. These differences are not statistically significant. Foulds (1982) showed that in *Lotus australis* cyanogenic individuals can lose the ability to produce glucosides with age. Although we did detect seasonal variation in the amount of HCN liberated in both species examined, no cyanogenic plants completely lost their ability to liberate HCN nor did acyanogenic ones acquire this ability. It is not known whether this would have applied had our trial run for a period longer than one year.

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Table 1. Results of tests on 40 plants of *Acacia resinomarginea* and 20 plants of *A. signata* for their ability to release HCN in the presence of a hydrolyzing enzyme. Plants were categorized as young (to 2.5 m tall) or mature (above 2.5 m).

Species	Sample number	Age class (and height of plant)	Class of Feigl-Anger reaction after 12 hours*			
			Spring (Oct.)	Summer (Feb.)	Autumn (April)	Winter (July)
A. resinomarginea	21	Young (1 m)	0.5	2	1	0+
	30	Young (1 m)	1.5	3	2	0+
	26	Young (1.5 m)	1.5	2.5	1	0.5
	29	Young (1.5 m)	1	3	1	0+
	19	Young (1.8 m)	1.5	3	1	0.5
	9	Young (2 m)	1.5	1.5	0+	0+
	35	Young (2.5 m)	1.5	0.5	1	0+
	2,3,5, 10-14, 20,22-25,27, 28,34, 36	Young (1-2.5 m)	0	0	0	0
	39	Mature (5 m)	1.5	2	1	0.5
	40	Mature (7 m)	1	3	1.5	0.5
	1,4,6-8,15-18, 31-33,37, 38	Mature (3.5-7 m)	0	0	0	0
A. signata	1	Young (0.8 m)	3	3	1.5	1.5
	3	Young (0.8 m)	3	3	2	1
	4	Young (0.8 m)	3	3	1	1
	7	Young (1.2 m)	3	3	1	1
	8	Young (1.2 m)	3	3	1	1.5
	15	Young (1.3 m)	3	3	1	1.5
	9	Young (1.5 m)	1	3	1.5	1
	10	Young (1.5 m)	3	3	1	0.5
	13	Young (1.5 m)	3	3	0.5	1.5
	5	Young (1.8 m)	3	3	1	1
	14	Young (2 m)	3	3	0.5	1
	2	Young (2.3 m)	3	3	1.5	0.5
	16	Young (2.5 m)	3	3	1.5	1.5
	20	Mature (3 m)	3	3	1.5	1
	12	Mature (3.5 m)	3	3	1	1.5
	6	Mature (4 m)	3	3	1	1.5
	11	Mature (4 m)	3	3	1	1.5
	17	Mature (4 m)	3	3	1.5	0.5
	18	Mature (4 m)	3	3	1.5	0.5
	19	Mature (4 m)	3	3	Not recorded	1.5

+ After 24 hours samples 9 and 30 were recorded in class 1 and sample 29 in class 0.5; after 48 hours samples 21 and 35 were recorded in class 1.

* A crude quantitative estimate of the amount of glucoside present is obtained by recording the degree to which the Feigl-Anger paper turned blue. The following scale of classes was adopted: 0 - no reaction, paper remained white; 1 - slight reaction (lower 1/3 of paper light blue, remainder white); 2 - medium reaction (lower 1/3 of paper dark blue and/or whole paper light to medium blue); 3 - strong reaction (whole paper dark blue).

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