Cyanogenic Glycosides in Plants

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ABSTRACT

The presence of cyanogenic glycosides was determined in 70 plant species from the campus of the State University of Londrina, PR, Brazil, and a further 45 plant species from the Forestry Reserve on the Doralice Farm in Ibiporã, PR, Brazil. Of the vegetative species from the State University of Londrina, 7.1% showed cyanogenic glycosides: Manihot esculenta *(Euphorbiaceae)*, Passiflora edulis *(Passifloraceae)*, Macadamia ternifolia *(Proteaceae)*, Prunus persica *(Rosaceae) and* Beloperone sp *(Acanthaceae).The first four species were considered to be potentially cyanogenic in the field. From the Forestry Reserve on the Doralice Farm, the plant species with cyanogenic glycosides were:* Holocalix balanseae *(Caesalpinaceae)*, Nectranda megapotamica *(Lauraceae)*, Trichilia casareti *(Meliaceae)*, Trichilia elegans *(Meliaceae) and* Rapanea umbellata *(Myrsinaceae), making 11.1% of the total species analyzed. Only* Holocalix balanseae *was considered to be potentially cyanogenic in the field.*

Key words: cyanogenic glycosides; cyanogenic plants; cyanogenesis

INTRODUCTION

Cyanogenesis is the ability of some plants to synthesize cyanogenic glycosides, which when enzymically hydrolyzed, release cyanohydric acid (HCN), known as prussic acid (Harborne, 1972, 1986, 1993). In most cases, hydrolysis is accomplished by the β-glucosidase, producing sugars and a cyanohydrin that spontaneously decomposes to HCN and a ketone or aldehyde (Figure 1). The second step can also be catalyzed by the hydroxynitrile lyase, which is widespread in cyanogenic plants (Harborne, 1993; Gruhnert et al, 1994). In the intact plant, the enzyme and the cyanogenic glycoside remain separated, but if the plant tissue is damaged both are put in contact and cyanohydric acid is released (Bell, 1981; Gruhnert et al, 1994). Cyanohydric acid is extremely toxic to a wide spectrum of organisms, due to its ability of linking with metals (Fe++, Mn++ and Cu++) that are functional groups of many enzymes, inhibiting processes like the reduction of oxygen in the cytochrome respiratory chain, electron transport in the photosynthesis, and the activity of enzymes like catalase, oxidase (Cheeke, 1995; McMahon et al, 1995).

There is strong evidence that cyanogenesis is one of the mechanisms that can serve to the plant as a protective device against predators such as the herbivores. The level of cyanogenic glycosides produced is dependent upon the age and variety of the plant, as well as environmental factors (Cooper-Driver & Swain, 1976; Woodhead & Bernays, 1977).

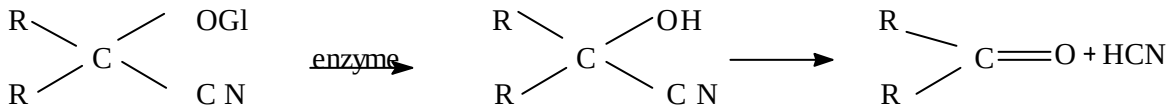

Figure 1 – Pathway of release of HCN by cyanogenic plants.

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It is usual to find cyanogenic and acyanogenic plants within the same species, where the function of cyanogenesis is revealed through their phenotypic characteristics. Cyanogenesis may not necessarily be used for plant survival; it may take part in metabolic and excretory processes but there certainly is a characteristic of value for these species (Harborne, 1972; Cooper-Driver & Swain, 1976; Woodhead & Bernays, 1977; Tokarnia et al, 1994).

Cyanogenic glycosides are widely distributed among 100 families of flowering plants. They are also found in some species of ferns, fungi and bacteria (Harborne, 1972, 1993). There are many economical important plants highly cyanogenic, including white clover, linum, almond, sorghum, the rubber tree and cassava (Tokarnia et al, 1994; Cheeke, 1995).

The aim of this work was to detect cyanogenic glycosides in vegetative species from the Forestry Reserve on the Doralice Farm, Ibiporã, PR, Brazil, and from the campus of the State University of Londrina, Londrina, PR, Brazil.

MATERIAL AND METHODS

Samples: The plant samples were harvested from March to September in 1996, at two localities: a) a Forestry Reserve on the Doralice Farm in Ibiporã, PR, Brazil (23º 16' S and 51º 01' W, altitude 484m). This farm has a 100 ha spread covered by a continuous forest around which there are areas of cultivation, limited to the east by the Tibagi River and bounded by pieces of ciliar forest (Carmo, 1995); b) the campus of the State University of Londrina in Paraná state that occupies an area of 230 ha east of the city of Londrina (Ornelas, 1991).

Identification of the plants: The plant specimens were processed according to Fidalgo & Bonomi (1984), and deposited in the Herbarium of the State University of Londrina as reference material. The identification of the plants was performed from published data and comparison with specimens held at the herbarium. Classification of the species was accomplished according to Cronquist (1988).

Detection of cyanogenic glycosides: Cyanogenic glycosides were detected using the technique of the picrate-impregnated paper according to Harbone (1972). The assay was performed in triplicate. Fresh plant material was cut into small pieces and placed in a test tube with 1.5mL of distilled water, and 6 drops of chloroform, followed by briefly crushing the material with a glass rod. The tube was stoppered with a cork containing a strip of picrate-impregnated paper hanging down from the stopper, and incubated at ambient temperature for 2 h. A colour change of the paper, from yellow to brown-red, indicated the release of HCN by the plant. If there was no release of HCN within 2 h, indicating a negative test, the tube was left at ambient temperature for 24 and 48 h, so that it could be re-examined. A brown-red coloration within 2 h indicated the presence of cyanogenic glycoside and the respective hydrolytic enzyme, and the plants were considered cyanogenic in the field. A brown-red color appearing within 48 h indicated that the cyanogenic glycoside spontaneously released HCN without the action of enzyme. No colour change after 48 h indicated that the test was negative for cyanogenic glycoside.

Picrate paper preparation: Strips of filter paper (5.0 X 1.5cm) were soaked in an aqueous solution of 0.05M picric acid, previously neutralized with sodium bicarbonate, and filtered. The impregnated paper was left to dry at ambient temperature.

RESULTS AND DISCUSSION

Forty five plant species were analyzed from the Forestry Reserve on the Doralice Farm. The results are shown in Table 1.

Table 1 - List of plant species from the Forestry Reserve on the Doralice Farm, Ibiporã, PR, Brazil assayed for the presence of cyanogenic glycosides

FAMILY	SPECIES	COMMOM NAME	VEGETATIVE PART
Acanthaceae	*Justicia brasiliana*	Junta-de-cobra-vermelha	Leaf
Alismataceae	*Echinodorus grandiflorus*	Chapéu-de-couro	Leaf
Apocynaceae	*Aspidosperma polyneuron*	Peroba-rosa	Leaf
Bombacaceae	*Chorisia speciosa*	Paineira	Leaf
Caesalpinaceae	*Bauhinia forticata*	Pata-de-vaca	Leaf
Caesalpinaceae	*Holocalyx balansae*	Alecrim	Leaf
Capparidaceae	*Capparidastrum sp*		Leaf
Cecropiaceae	*Cecropia glazioui*	Embaúba	Leaf
Cecropiaceae	*Cecropia pachystachya*	Embaúba	Leaf
Euphorbiaceae	*Croton floribundus*	Capixingui	Leaf
Fabaceae	*Lonchocarpus guilleminianus*	Embirá-branca	Leaf
Fabaceae	*Machaerium hatschbachii*	Caviúna	Leaf
Lauraceae	*Endlicheria paniculata*	Canela-de-frade	Leaf
Lauraceae	*Nectandra megapotamica*	Canela-preta	Leaf
Lauraceae	*Ocotea indecora*	Canela	Fruit
Malvaceae	*Bastardiopsis densiflora*		Peduncle
Melastomataceae	*Miconia discolor*	Pixirica	Leaf
Meliaceae	*Cabralea canjerana*	Canjerana, canjaran	Fruit
Meliaceae	*Guarea kunthiana*	Figo-do-mato	Fruit/seed/leaf
Meliaceae	*Guarea macrophylla*	Ataúba	Fruit
Meliaceae	*Trichia casaretti*	Catiguá-vermelho	Fruit
Meliaceae	*Trichilia catigua*	Catiguá	Leaf
Meliaceae	*Trichilia elegans*	Pau-ervilha	Leaf
Meliaceae	*Trichilia pallida*	Baga-de-morcego	Leaf
Mimosaceae	*Acacia polyphylla*	Monjoleiro	Leaf
Mimosaceae	*Anadenanthera colubrina*	Angico	Leaf
Mimosaceae	*Inga marginata*	Ingá-mirim	Leaf
Mimosaceae	*Inga striata*	Ingá-banana	Leaf
Mimosaceae	*Piptadenia gonoacantha*	Pau-jacaré	Leaf
Moraceae	*Ficus guaranitica*	Figo-do-mato	Fruit
Moraceae	*Sorocea bomplandi*	Falsa espinheira-santa	Leaf
Myrsinaceae	*Rapanea umbellata*	Capororoca	Leaf
Nyctaginaceae	*Bougainvillea spectabilis*	Primavera	Leaf
Nyctaginaceae	*Pisonia aculeata*	Pega-pinto	Fruit
Phytolaccaceae	*Galesia intergrifolia*	Pau-d'alho	Leaf
Piperaceae	*Piper sp*		Leaf
Rubiaceae	*Palicourea sp*		Leaf
Rutaceae	*Baufourodendron Riedelianum*	Pau-marfin	Leaf
Rutaceae	*Pilocarpus pennatifolius*	Cutia-branca, jaborandi	Leaf
Rutaceae	*Zanthoxylum riedelianum*	Mamica-de-porca	Leaf
Sapotaceae	*Crysophyllum gonocarpum*	Guatambú-de-leite	Leaf
Simaroubaceae	*Picramnia ramiflora*	Cedrilho, cedrinho	Leaf
Verbenaceae	*Aegiphila sp*		Leaf
Verbenaceae	*Vitex megapotamica*	Tarumã	Leaf
Violaceae	*Hybanthus biggibosus*		Leaf

Of the species examined only *Holocalix balanseae* (*Caesaepinaseae*) released HCN within 2 h, showing that this plant species has cyanogenic glycoside and the specific enzyme for its hydrolysis. The plants, *Nectandra megapotamica* (*Lauraceae*), *Trichilia casareti* and *Trichilia elegans* (*Meliaceae*), released HCN slowly, within 24 h, while *Rapanea umbellata* (Myrsinaceae) released cyanide after 24 h. The HCN of the cyanogenic glycoside in these cases was not released enzymatically.

Table 2 - List of plant species from the Campus of the State University of Londrina, Londrina, PR, Brazil, assayed for the presence of cyanogenic glycosides

FAMILY	SPECIES	COMMON NAME	VEGETATIVE PART
Acanthaceae	*Beloperone sp*	Camarãozinho-de-jardim	Flower
Agavaceae	*Agave sp*	Lírio-de-nossa senhora	Leaf
Agavaceae	*Cordyline sp*	Cordiline	Flower
Agavaceae	*Sancevieria sp*	Espada-de-são jorge	Leaf
Agavaceae	*Yucca aloefolia*	Vela-da-pureza	Leaf
Anacardiaceae	*Mangifera indica*	Mangueira	Fruit/inflorescence
Annonaceae	*Annona cearensis*	Fruta-do-conde	Fruit
Apocynaceae	*Plumeria rubra*		Flower/Stalk
Araucariaceae	*Araucaria angustifolia*	Pinheiro-do-Paraná	Fruit
Asclepiadaceae	*Asclepia curassavica*		Flower
Asteraceae	*Bidens pilosa*	Picão	Leaf
Asteraceae	*Eupatorium maximilianii*		Flower
Asteraceae	*Taraxacum officinale*	Dente-de-leão	Flower
Asteraceae	*Vernonia polyanthes*		Flower
Asteraceae	*Wedellia paludosa*		Flower
Bignoniaceae	*Jacaranda micrantha*	Caroba	Fruit
Bignoniaceae	*Tabebuia chrysotricha*	Ipê-amarelo	Flower
Bignoniaceae	*Tabebuia heptaphylla*	Ipê-roxo	Fruit
Bignoniaceae	*Tabebuia roseo-alba*	Ipê-branco	Fruit
Bixaceae	*Bicha olerana*	Urucum	Flower
Bombacaceae	*Chorisia speciosa*	Paineira	Fruit
Caesalpinaceae	*Bauhinia forticata*	Pata-de-vaca	Seed
Caesalpinaceae	*Caesalpinia peltophoroides*	Sibipiruna	Flower
Caesalpinaceae	*Cassia grandis*	Canafístula	Leaf
Caesalpinaceae	*Delonix regia*	Falmboyant	Fruit
Cecropiaceae	*Cecropia adenopus*	Embaúba	Leaf
Chrysobalanceae	*Correpia grandiflora*	Oiticica	Leaf
Commelinaceae	*Zebrina sp*	Zebrinha	Leaf
Convolvulaceae	*Catharantus roseus*	Boa-noite	Flower
Convolvulaceae	*Ipomea geramoelit*	Bom-dia	Flower
Crassulaceae	*Bryophyllum sp*	Folha-da-fortuna	Leaf
Ericaceae	*Rhododendron indicum*	Azaléia	Flower
Euphorbiaceae	*Euphorbia heterophylla*	Leiteiro	Leaf
Euphorbiaceae	*Euphorbia tirucalli*	Coroa-de-cristo	Leaf
Euphorbiaceae	*Manihot esculenta*	Mandioca, cassava	Leaf
Euphorbiaceae	*Ricinus comunis*	Mamona	Seed
Fabaceae	*Cajanus cajanus*	Feijão-andu	Seed
Fabaceae	*Erythrina speciosa*	Eritrina, suinã	Flower
Fabaceae	*Leucena glauca*		Leaf
Fabaceae	*Machaerium stiptatum*		Leaf
Fabaceae	*Phaseolus vulgaris*	Feijão	Fruit
Geraniaceae	*Geranium sp*	Gerânio	Flower
Lamiaceae	*Melissa offinalis*	Erva cidreira	Leaf
Lamiaceae	*Origanum majorana*	Orégano	Leaf
Lauraceae	*Persea gratissima*	Abacateiro	Fruit
Liliaceae	*Aloe vera*	Babosa	Leaf
Liliaceae	*Lilium sp*	Lírio	Bulb
Malvaceae	*Hibiscus rosa sinensis*	Hibisco	Flower
Melastomataceae	*Tibouchina granulosa*	Quaresmeira	Flower
Meliaceae	*Cedrela fissilis*	Cedro	Fruit
Meliaceae	*Melia azedarach*	Santa-bárbara	Seed/leaf/fruit
Mimosaceae	*Calliandra selloi*	Esponjinha,cabelo-de-anjo	Leaf
Moraceae	*Artocarpus incisa*	Fruta-pão	Fruit
Moraceae	*Ficus auriculata*	Figo-bravo	Fruit
Moraceae	*Ficus elastica*	Seringueira-falsa	Leaf
Myrtaceae	*Psidium guajava*	Goiabeira	Leaf
Nyctaginaceae	*Bougainvillea spectabilis*	Primavera	Flower
Oleaceae	*Jasminum sp*	Jasmin	Flower
Passifloraceae	*Passiflora edulis*	Maracujá	Floral button/leaf
Plantaginaceae	*Plantago major*	Tansagem	Leaf

(Cont.)

Table 2 - (cont.) List of plant species from the Campus of the State University of Londrina, Londrina, PR, Brazil, assayed for the presence of cyanogenic glycosides

FAMILY	SPECIES	COMMON NAME	VEGETATIVE PART
Poaceae	*Brachiaria sp*		Leaf
Proteaceae	*Grevillea robusta*	Grevilia	Stem
Proteaceae	*Macadamia ternifolia*	Noz, macadamia	Leaf/flower
Rosaceae	*Prunus persica*	Pessegueiro	Flower/leaf
Rubiaceae	*Coffea arabica*	Café	Leaf
Rutaceae	*Citrus sinensi*	Laranjeira	Leaf/flower
Tiliaceae	*Corchorus capsularis*		Leaf
Tropaeolaceae	*Tropaeolum brasilienses*	Capuchinha	Flower
Urticaceae	*Boehmeria caudata*	Assa-peixe	Leaf
Verbenaceae	*Lantana camara*		Flower

Cyanogenesis could be revealed comparing plants through their phenotypic characteristics. As confirmed by Harborne (1972, 1992), chemical polymorphism in clover was derived by geneticists through breeding experiments, which showed that two genes were responsible for cyanogenesis: Ac, controlling the synthesis of cyanogenic glycoside, and Li that controls the synthesis of the enzyme necessary for its breakdown. Four genotypes (Ac Li, Ac Li, Ac Li and ac li) in natural populations were identified phenotypically by the chemical test employing picrate paper. Only type Ac Li was registered as cyanogenic in the field.

Following analyses it was concluded that of the 45 species of the Forestry Reserve examined, only 11,1% released HCN, and could be described as producers of cyanogenic glycoside. Only one plant species (*Holocalix balansae*) could released cyanide within 2 h, and can be considered cyanogenic in the field.

Seventy one plant species from the campus of the State University of Londrina were analysed. The results are shown in Table 2.

Of the 70 species examined, 7.1% released HCN within 2 h, and were considered cyanogenic in the field. They included *Manihot esculenta* (Euphorbiaceae), *Passiflora edulis* (Passifloraceae), *Macadamia ternifolia* (Proteaceae) and *Prunus persica* (Rosaceae). *Beleperone sp* (Acanthaceae) released HCN within 24 h; although it was cyanogenic it is not cyanogenic in the field because the evolution of HCN is very slow; most likely non-enzymic.

In general, wild plant species are more resistant to predators due to the presence of toxic factors acting as defense mechanism against them. Sotelo et al (1995), comparing the chemical composition of cultivated and wild beans (*Phaseolus vulgaris*), showed that although the cultivated beans had better profiles of amino acids than the wild beans, the content of anti-nutritional factors was less. At the campus of University of Londrina, the percentage of plants having cyanogenic glycosides was lower (7.1%) than those examined from the Forestry Reserve at the Doralice Farm (11.1%), but four species were cyanogenic in the field against one species from the Forestry Reserve.

The plants cultivated in the university campus were mainly exotic introduced species, coming from places totally different from which they were introduced, and certainly suffered evolutionary pressure before their adaptation to the new habitat. By comparison, plants in the Forestry Reserve were native species having evolved together with same adaptative conditions in specific ecosystem. The plant species containing cyanogenic glycosides in the University campus, and those in the Forestry Reserve were different, with both sources having suffered diverse types of predation. It was, however, difficult to reach some conclusion with respect to these differences.

The importance of this work relied at the large number of plant species that were analysed. The study could serve as reference to new studies about cyanogenic glycosides in these plants.

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RESUMO

A presença de glicosídeos cianogênicos foi testada em 70 espécies de plantas do Campus da Universidade Estadual de Londrina, PR, Brasil e em 45 espécies de plantas do Remanescente Florestal da Fazenda Doralice, Ibiporã, PR, Brasil. Das espécies vegetais da Universidade Estadual de Londrina, 7,1% apresentaram glicosídeos cianogênicos: *Manihot esculenta* (Euphorbiaceae), *Passiflora edulis* (Passifloraceae), *Macadamia ternifolia* (Proteaceae), *Prunus persica* (Rosaceae) e *Beloperone sp* (Acanthaceae). As primeiras quatro espécies foram consideradas potencialmente cianogênicas no campo. Do Remanescente Florestal da Fazenda Doralice, as espécies vegetais com glicosídeos cianogênicos foram: *Holocalix balanseae* (Caesalpinaceae), *Nectandra megapotamica* (Lauraceae), *Trichilia casareti* (Meliaceae), *Trichilia elegans* (Meliaceae) e *Rapanea umbellata* (Myrsinaceae), perfazendo 11,1% das espécies totais analisadas. Somente *Holocalix balanseae* foi considerada ser potencialmente cianogênica no campo.

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