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# 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).

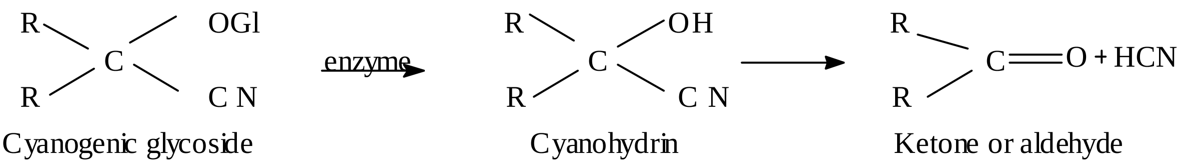


**Figure 1 –** Pathway of release of HCN by cyanogenic plants.

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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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