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# A survey of cyanogenesis in plants of Santa Rosa National Park, Costa Rica

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Resumen; Se comunica los resultados de una investigación de la producción de cianuro por partes aéreos de 103 especies de plantas en el Parque Nacional Santa Rosa, Costa Rica. Tres especies produjeron cianuro: *Paullinia cururu* (Sapindaceae), *Manihot aesculifolia* (Euphorbiaceae), y *Acacia farnesiana* (Mimosaceae). Es el primer informe de un cianógeno en las hojas de *P. cururu*. Se hace un resumen sobre los conocimientos corrientes de cianogénesis en el género *Acacia*.

The secondary chemical constituents of tropical plants are of interest for a variety of reasons, including their importance in mediating plant-herbivore interactions and their potential as sources of chemicals for medicine and agriculture. As part of a survey of the secondary chemical constituents of plants in Santa Rosa National Park (Guanacaste Province, Costa Rica), the aerial tissues of 103 species were tested for cyanogenic activity during the 1983 wet season.*

Plant tissues were tested for cyanogenic activity using the Feigl-Anger test (Feigl and Anger, 1966; Tantisewie *et al.*, 1969) Indicator paper was prepared by soaking filter paper (Whatman No. 1) in a solution of copper ethylacetoacetate and 4,4' -tetramethyldiaminodiphenylmethane dissolved in chloroform (Feigl and Anger, 1966). The paper was air dried, cut unto strips, and sealed in airtight bottles until used. Indicator paper prepared in this way yielded strong positive reactions to the presence of HCN even after being stored for four months in the field.

All plant tissues were tested in the field immediately after collection. In brief, plant parts were macerated in screw cap vials using a small wooden dowel. A piece of the indicator paper was then inserted and held in place by the cap of the vial so that it did not directly contact the macerated tissues. Vials were then tightly sealed and allowed to stand for at least 4-6 hours at ambient temperature. In the presence of HCN, the indicator paper turned blue after 1-2 hours. This color intensified with time, becoming very dark overnight. In this study, no exogeneous $\beta$-glucosidases were added to promote HCN release from macerated plant tissues.

Individual plants were marked and tested between May 16 and June 4 (the onset of the rainy season at Santa Rosa), when many species of plants were flushing young leaves. Whenever possible, these plants were retested between July 25 and August 7, at which time most species bore mature leaves. Although the goal of the survey was to test leaf material, other tissues were tested as time and availability permitted. Leaves were classified as "young" from the time of initial emergence from buds until they attained full size and normal coloration. Thereafter they were classified as "mature". Fruits were tested for activity by thoroughly grinding pulp and immature seeds together. Mature seeds were removed from other fruiting structures and tested separately.

Of the 103 species tested for cyanogenic activity, 3 showed positive results: *Paullinia cururu* (Sapindaceae), *Manihot aesculifolia* (Euphorbiaceae), and *Acacia farnesiana* (Mimosaceae). Both young and mature leaves of all 3 species showed cyanogenic activity. Strong activity was detected during both the early- and

*A list of species tested may be obtained by writing to the author.



Cianuro, plantas Paullinia cururu Manihot aesculifolia Acacia

mid-rainy season test periods in each species. Under the conditions of the survey, cyanogenic activity is exhibited only by plant species which contain both cyanogenic glycosides and appropriate β-glucosidases. Some plants are known to contain cyanogens but lack the enzymes required to liberate HCN (Jones, 1966; Conn and Maslin, 1983). It is possible that some species found to be non-cyanogenic in this study might liberate HCN if exogeneous β-glucosidase were added to crushed leaf tissues.

From available information, this appears to be the first record of cyanogenic activity in the vegetative parts of *Paullinia cururu.* The seeds of *P. cururu* have been reported to contain cyanolipids, as well as nonlipid cyanogens (Seigler and Kawahara, 1976). The latter may prove to be the same as those present in the vegetative parts. The isolation and characterization of the cyanogen in *P. cururu* leaves are being undertaken by Dr. J.E. Poulton, of the Department of Botany, University of Iowa.

The genus *Manihot* is widely known for its cyanogenic activity because of its importance as a staple food crop throughout the tropics. Although this author can find no previous references to cyanogenic activity in *Manihot aesculifolia,* this behavior is not at all unexpected.

The amount and seasonality of cyanide content of *Acacia farnesiana* leaves have previously been investigated at Santa Rosa National Park (Seigler *et al.,* 1979; Janzen *et al.,* 1980). Many *Acacia* species have been shown to be cyanogenic (Gibbs, 1974; Tjon Sie Fat, 1979), but the cyanogens have rarely been identified. It has been suggested that *Acacia* species which maintain an association with ants of the genus *Pseudomyrmex* lack cyanogens because they are defended from herbivore damage by the ants (Rehr *et al.,* 1973). The observed absence of cyanogenesis in *A. collinsii* (an ant-acacia) tested in this study supports this idea. However, dried herbarium specimens of *A. collinsii* have been reported as cyanogenic (Seigler *et al.,* 1978) and 3 other ant-acacias have been found to liberate HCN in the field (Seigler *et al.,* 1978; Seigler *et al.,* 1983). In addition, many *Acacia* species are known to produce cyanogens but not the β-glucosidases required for the release of HCN; thus, more ant-acacias may prove to be cyanogenic when exogenous β-glucosidases are used in Feigl-Anger tests. While the assertion that ant-acacias totally lack HCN appears to be incorrect, it is not yet known whether ant-acacias in general lack significant amounts of β-glucosidases or maintain lower concentrations of cyanogenic glycosides than do non-ant-acacias. The idea that ant-acacias invest less heavily in cyanadiae as an anti-herbivore defense than non-ant-acacias may be correct, but remains to be critically tested.

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