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Cyanogenic Constituents in Plants from the Galápagos Islands

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Key Word Index—Galápagos Islands; cyanogenic compounds; cyanide; hydrogen cyanide; secondary metabolites; endemic; screening; grazing; herbivory.

Abstract—A screening for cyanogenic constituents in leaves of 475 specimens of plants from the Galápagos Islands, both fresh material tested on the Islands and herbarium material tested in Copenhagen show that of the 97 species tested on fresh material, 24 proved to contain cyanogenic constituents at a level corresponding to 10 mg of HCN per kg material, while 27 species were slightly positive, i.e. released between 2.5 and 10 mg HCN per kg. In total for analyses of fresh and dried material, 33 genera are reported for the first time to contain cyanogenic species, and 92 species hitherto not reported cyanogenic showed positive reaction to some extent. The importance of these findings in relation to herbivory is discussed.

Introduction

Secondary compounds in plants have gained growing interest during the last three decades. This has been partly due to the introduction of new sophisticated methods of analysis, and to an increase in general knowledge concerning plant constituents following the greater availability of fresh or herbarium material.

The evolutionary processes behind the ability of plants to produce secondary compounds constitute the common background for the interest shown by chemotaxonomists and ecologists—and may be the basis for applied biotechnological studies. Taxonomists seek support for their proposed relationship by means of chemical similarities [1], and ecologists try to explain the role of secondary compounds in plant–animal or plant–plant interactions [2]. The contributions used by taxonomists are usually based on screening of the systematic groups of interest for a number of different chemical constituents, whereas ecologists have tended to approach their problems from specific types of interaction: i.e. whether the presence of a given compound in a plant species acts as protection against grazing from certain animals. This difference in approach may be the reason why relatively few papers combine the aim of taxonomists to understand the evolutionary process leading to the present day taxa with those of ecologists to give explanations for the same processes.

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In the present project, we attempt to apply the screening approach of the taxonomist with the ecologist's search for causality. We have carried out our studies using cyanogenic compounds on the unique flora of the Galápagos Islands.

Cyanogenic compounds are defined by their ability to release hydrogen cyanide (HCN) either spontanously (labile compounds, α-hydroxynitriles=cyanohydrins) or after enzymic (or acid) hydrolysis. The hydrolysis may be catalysed by different enzymes as for cyanogenic glycosides, cyanogenic lipids and epoxides. At present this group of plant constituents comprise about 50 characterized compounds [3]. The semiquantitative method of ref. [4] can be carried out even under difficult field conditions. Hegnauer [5] suggested the term cyanophoric for plants which after injury, release more than 10 mg of HCN/kg fresh material, an ability which is dependent on the presence of both cyanogenic constituents and (in most cases) hydrolytic enzymes.

The Galápagos Islands lie on the equator around 1000–1200 km west of the South American continent. Their flora is relatively undisturbed by cultivation, and even if introduced animals and plants seriously affect some areas, it is reasonable to believe that few endemic plant species have gone extinct [6] and that the impact by the alien organisms is too short term to have affected evolutionary processes. The indigenous flora comprises roughly 550 species of which 200 evolved in the islands (endemics) and 350 also occur in other areas of the world (mainly in Andean South America). Because of this limited number of species, and due to the fact that a majority of the species are rather common or well localized in the archipelago [6], it has been possible to test a fair amount of the total flora. The high percentage of endemics makes it possible to analyse endemic and other indigenous plants (termed natives) as two units of comparable size. The occurrence of several endemic groups with well understood phylogeny makes it furthermore possible to trace patterns back in evolutionary history.

The cyanogenic compounds have often been interpreted as herbivore repellants, and some experimental evidence have been produced by means of food preference assays [7]. The Galápagos Islands have a very odd herbivorous fauna—the only large herbivores are giant tortoises and iguanas, so defence against mammiferous herbivores is superfluous. The invertebrate herbivorous fauna lacks important tropical groups such as leaf cutting ants and termites, and even if snails, grasshoppers and lepidopterans occur, they seem not to exhibit a hard grazing pressure except in some specific cases.

A summary of the aim of the project and some results from preliminary investigations have been published earlier [8]. In this, it was pointed out that the grouping of the material into endemics and natives leads to the conclusion that cyanogenesis is more frequent among plant species which also occur on the South American mainland, than in those which evolved in the archipelago. This conclusion is further supported by the results based on herbarium material. As the vast majority of the plants surveyed has never been examined for cyanogenic compounds before (none of the endemics), we find it appropriate to confine the present paper to phytochemical aspects, i.e. analytical procedures, novel records of the presence of cyanogenic compounds and comparisons with published analyses of cyanophoric plants.

Results and Discussion

Table 1 shows the results from the screening of fresh leaf material on the Galápagos Island from November 1984 till May 1985. The period was extremely dry. Consequently, many annuals died or never germinated. This caused a somewhat biased composition of the screened material in that perennials (mostly arboreals) are over-represented. Only monocotyledones and dicotyledones have been screened. The positive species have been divided into two groups: Species of which fresh material yielded more than 10 mg HCN kg^{-1} fresh weight are reported as 'cyanogenic', in accordance with the limits mentioned above, see [5]. Species of which fresh material yielded 2.5–10 mg HCN kg^{-1} fresh weight are reported as 'slightly cyanogenic'. In the Table, results are expressed as nmol HCN released g^{-1} fresh weight. Since, at the present time, only the presence or absence of cyanogenic compounds have been tested, no knowledge is available about the presence of enzymes which make the plants with cyanogenic glycosides, lipids or epoxides cyanophoric.

The results from Table 1 show that 23 species are reported 'cyanogenic' for the first time. One of these (*Cissus sicyoides*) has previously been reported negative with respect to cyanogenic glycosides [9]. *Prosopis juliflora* (mesquite) has previously been reported positive [9, 10] as well as negative [11]. We found no evidence of positive reaction. 25 of the 'slightly cyanogenic' species have not previously been reported positive.

The novel positive reports at the species level infer that six genera for the first time are reported 'cyanogenic', viz. *Scalesia, Commicarpus, Zanthoxylum, Mecardonia, Acnistus,* and *Phoradendron.* If we accept the reaction in the 'slightly cyanogenic' plants as evidence, the number of new records of cyanogenesis at the generic level increase to 19.

Table 2 presents the results from the screening on herbarium material of leaves from plants belonging to the monocotyledons and dicotyledons. The screened material was selected among species of which fresh material had not been examined. In Table 2, no distinction is made between 'cyanogenic' and 'slightly cyanogenic' species due to the great variation in dry matter contents and possible loss of constituents during

TABLE 1. THE DISTRIBUTION OF CYANOGENIC SPECIES IN THE GALÁPAGOS ISLANDS: FRESH MATERIAL

Taxon (Endemic, E)	Cyanogenic ≥370 nmol HCN g^{-1} fr. wt	Slightly cyanogenic 93–369 nmol HCN g^{-1} fr. wt	Literature reports on cyanogenesis* Species	Genus
Monocotyledones				
Commelinaceae				
Commelina diffusa				9
Gramineae				
Aristida subspicata E				10
Panicum dichotomiflorum				10
P. glutinosum	>1000			10
Paspalum galapageium E				10
Pennisetum pauperum E				9
Sporobulus virginicus		96, 167		10
Trisetum howellii E				
Dicotyledones				
Acantaceae				
Justicia galapagana E				
Aizoaceae				
Sesuvium portulacastrum				
Amaranthaceae				
Alternanthera echinocephala	>1000			10
A. filifolia ssp. nov. E				10
A. filifolia ssp. *filifolia* E				10
A. filifolia ssp. *glaucescens* E	545			10
A. halimifolia	>1000, >750	99		10
Froelichia nudicaulis E				
Apocynaceae				
Vallesia glabra				
Asteraceae				
Ageratum conyzoides		239		9
Baccharis gnidiifolia		145		
Darwiniothamnus lancifolius E genus		188, (42,61)		
D. tenuifolius E genus		153, (56)		
Encelia hispida E		217		
Jaegeria gracilis E				
Lecocarpus lecocarpoides E genus		111		
Macraea laricifolia E		120		
Pectis subsquarrosa E		125		
Scalesia affinis E genus		109, 172		
S. cordata E	498			
S. helleri E				
S. incisa E		128, 100		
S. microcephala E				
S. pedunculata E	575			
S. snodgrasii E	614			
Boraginaceae				
Cordia leucophlyctis E				25−, 26+
C. lutea				25−, 26+
C. revoluta E				25−, 26+
C. scouleri E	667			25−, 26+
Tiquilia fusca E				
Tournefortia psilostachya		319		
T. pubescens E		185, 197		
T. rufo-sericea E		231, (74)		
Cactaceae				
Brachycereus nesioticus E				
Jasminocereus thouarsii var. *delicatus* E				
J. thouarsii var. *sclerocarpus* E				
Opuntia echios var. *gigantea* E		163		10
O. helleri E				10
O. insularis E		105, (37)		10
O. megaspermum var. *megaspermum* E		128		10
Caryophyllaceae				
Drymaria monticola E				

TABLE 1.—*CONTINUED*

Taxon (Endemic, E)	Cyanogenic ≧370 nmol HCN g^{-1} fr. wt	Slightly cyanogenic 93–369 nmol HCN g^{-1} fr. wt	Literature reports on cyanogenesis* Species	Genus
Celastraceae				
Maytenus octogona		167		
Convolvulaceae				
Ipomoea alba	>750			10
I. habeliana E				10
I. pes-caprae		100	9±	10
I. triloba	413			10
Ericaceae				
Pernettya howellii E				
Euphorbiaceae				
Acalypha parvula E				10, 19
A. wigginsii E				10, 19
Chamaesyce punctulata E				10†
Croton scouleri E		301		
Phyllanthus carolinensis				27
Leguminosae				
Acacia insulae-iacobi E	>1000			3,10
A. macracantha	>1500,		3, 30	3, 10
	>1500		31	28, 29
	>1000			
A. rorudiana E	>1000			3, 10
				28, 29
Cassia bicapsularis				
Desmodium canum		128		9
Parkinsonia aculeata		133		
Prosopis juliflora			9, 10	
Tephrosia decumbens	>750			9
Vigna luteola				
Malvaceae				
Hibiscus tiliaceus				
Melastomaceae				
Miconia robinsoniana E				
Menispermaceae				
Cissampelos pareira				
Molluginaceae				
Mollugo snodgrasii E	>1000			10
Myrtaceae				
Psidium galapageium E				9
Nyctaginaceae				
Commicarpus tuberosus	389			
Cryptocarpus pyriformis		301		
Pisonia floribunda E				
Passifloraceae				
Passiflora colinvauxii E	>1500			32–34
P. foetida var. *galapagensis* E	>1500		13	32–34
Plumbaginaceae				
Plumbago scandens		323		9
Polygonaceae				
Polygonum galapagense E				9
Rhamnaceae				
Scutia pauciflora				
Rhizophoraceae				
Rhizophora mangle				
Rubiaceae				
Borreria ericaefolia E		135, (46)		
Chiococca alba				
Psychotria rufipes E				
Rutaceae				
Zanthoxylum fagara	867			
Scrophulariaceae				
Mecardonia dianthera	>750			
Simarubaceae				
Castela galapageia E				

TABLE 1.—*CONTINUED*

Taxon (Endemic, E)	Cyanogenic ≥370 nmol HCN g^{-1} fr. wt	Slightly cyanogenic 93–369 nmol HCN g^{-1} fr. wt	Literature reports on cyanogenesis* Species	Genus
Solanaceae				
Acnistus ellipticus E	>750			
Sterculiaceae				
Waltheria ovata		103		
Verbenaceae				
Clerodendron molle	>750			10
Lantana peduncularis E				
Lippia rosmarinifolius E				
Verbena stewartii E				
Viscaceae				
Phoradendron henslowii E	767			
Vitaceae				
Cissus sicyoides	>750		9–	

*Compounds identified are: acalyphin from *Acalypha* spp.; taxiphyllin from *Phyllanthus* spp.; proacacipetalin from *Acacia macracantha*; gynocardin and passicoccin from *Passiflora* spp.

†Several species of *Euphorbia* are known to be cyanogenic. The genus *Chamaesyce* is separated from the genus *Euphorbia*.

‡One specimen of each was tested in the field except for *Sporobulus virginicus. Darwiniothamnus tenuifolius, Scalesia affinis, S. Incisa, Tournefortia pubescens, T. rufo-sericea, Opuntia insularis, Croton scouleri, Psidium galapageium, Pisonia floribunda, Borreria ericafolia* (2 specimens each); *Alternanthera halimifolia, Darwiniothamnus lancifolius, Acacia macracantha* (3 specimens each).

TABLE 2. THE DISTRIBUTION OF CYANOGENIC SPECIES IN THE GALÁPAGOS ISLANDS: HERBARIUM MATERIAL

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
Monocotyledones			
Bromeliaceae			
Tillandsia insularis var. *latilamina* E			
T. insularis var. *insularis* E			
Cyperaceae			
Bulbostylis hirtella	31		
Cyperus anderssonii E			9
C. brevifolius			9
C. confertus			9
C. distans			9
C. elegans	18		9
C. grandifolius E			9
C. laevigatus	43		9
C. ligularis	63		9
C. rivularis	86		9
C. virens			9
Eleocharis fistulosa			
E. maculosa			
E. mutata			
E. nodulosa			
E. sellowiana			
Fimbristylis dichotoma	48		9
Rhynchospora corymbosa			
R. nervosa			
R. rugosa			
Scleria nutans			
S. pterota			
Gramineae			
Anthephora hermaphrodita			10, 35‡
Aristida divulsa E	40		10
A. repens E	31		10

TABLE 2.—CONTINUED

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
Bouteloua disticha			10, 36
Cenchrus echinatus			
C. platyacanthus E (2)			
Chloris mollis			10, 36
C. virgata (2)			10, 36
Distichlis spicata			
Eragrostis cilianensis	20		
E. ciliaris	15		
E. mexicana	43		
E. pilosa	24		
Eriochloa pacifica			
Ichnanthus nemorosus	45		
Mühlenbergia microsperma	40		
Oplismemus setarius			
Setaria setosa			
Sporobulus pyrimidatus			10, 35‡
S. virginicus			10, 35
Trichoneura lindleyana E (2)*			
Hypoxidaceae			
Hypoxis decumbens			
Orchidaceae			
Epidendrum spicatum E			
Erythrodes sp.			
Govenia utriculata			
Habenaria alata			
H. monorrhiza			
Ionopsis utricularioides			
Liparis nervosa	17		
Ponthieva maculata	16		
Prescottia oligantha	28		
Dicotyledones			
Acanthaceae			
Blechum brownei		9	
Justicia galapagana E*			
Ruellia floribunda	35		
Tetramerium nervosum			
Aizoaceae			
Sesuvium edmonstonei E			
Trianthema portulacastrum			
Amaranthaceae			
Amaranthus anderssonii E			9
A. dubius	45		9
A. sclerantoides E			9
Lithophila radicata E			
L. subscaposa E			
Pleuropetalum darwinii E			
Apocynaceae			
*Vallesia glabra**			
Asteraceae			
Acanthospermum microcarpum			10
*Ageratum conyzoides**			9
*Baccharis gnidiifolia**			
B. steetzii E			
Blainvillea dichotoma			
Brickellia diffusa			
Chrysanthellum pusillum E			
Eclipta alba			
Elvira repens E			
Encelia hispida E*			
Enydra maritima			
Gnaphalium vira-vira			
Jaegeria crassa E			

TABLE 2.—*CONTINUED*

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
J. gracilis E*			
Macraea laricifolia E*			
Pectis subsquarrosa E*			
P. tenuifolia E			
Sonchus oleraceus E			
Spilanthes diffusa E			
Avicenniaceae			
Avicennia germinans			
Basellaceae			
Anredera ramosa			
Batidaceae			
Batis maritima			
Boraginaceae			
Cordia anderssonii E			26
C. leucophlyctis E*			26
*C. lutea**			26
C. revoluta E*	875		26
Heliotropium anderssonii E	363		26, 37
H. angiospermum			26, 37
H. curassavicum			26, 37
Tiquilia darwinii E			
T. fusca E*			
T. nesiotica E			
*Tournefortia psilostachya**			
T. pubescens E*			
T. rufo-Sericea E*			
Burseraceae			
Bursera graveolens			
Campanulaceae			
Lobelia xalapensis			
Caryophyllaceae			
Drymaria rotundifolia E			
D. cordata			
Celastraceae			
*Maytenus octogona**			
Chenopodiaceae			
Atriplex peruvianum			35
Chenopodium murale			35
Salicornia fruticosa			
Combretaceae			
Conocarpus erecta			
Laguncularia racemosa			
Convolvulaceae			
Dicondra repens			
Evolvulus glaber			
E. simplex			
*Ipomoea alba**			10
I. habeliana E*	45		10
I. linearifolia			10
I. triloba (2)*	43, 0		10
Merremia aegyptica			35
Cuburbitaceae			
Elaterium carthagenense			
Euphorbiaceae			
Acalypha abingdonii E			9, 10
A. baurii E			9, 10
A. baurii × parvula E			9, 10
A. wiggensii E*			9, 10
Chamaesyce abdita E	313		10
C. amplexicaulis E			10
C. galapageia E	35		10
C. nummularia E			10
C. punctulata E*			10
C. recurva E	413		10

TABLE 2.—*CONTINUED*

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
C. viminea E	103		10
Euphorbia equisetiformis E			10
Goodeniaceae			
Scaevola plumieri			
Hypericaceae			
Hypericum uliginosum			
Labiatae			
Hyptis spicigera			
Salvia insularum E			
S. occidentalis			
S. prostrata E			
Teucrium vesicarium			
Leguminosae			
*Acacia macracantha**	34100	30, 31	3, 10, 28, 29
A. rorudiana E*	30000		3, 10, 28, 29
Canavalia maritima			
*Cassia bicapsularis**			
C. occidentalis			
Crotalaria incana			
C. pumila			
Dalea tenuicaulis E			
Desmanthus virgatus	338		
Desmodium glabrum			9
D. limense			9
D. procumbens			9
Galactia striata			
G. tenuiflora			
Neptunia plena	69		
Phaseolus mollis E	>1000		9
Rhynchosia minima	83		
Stylosanthes sympodialis			
Tephrosia decumbens E*			9
*Vigna luteola**			
Zornia curvata			
Loasaceae			
Mentzelia aspera			
Sclerothrix fasciculata			
Malvaceae			
Abutilon depauperatum E			
Bastardia viscosa	50		
Gossypium barbadense			10
Herrisantia crispa			
Sida hederifolia			9
S. salvifolia			9
S. spinosa			9
Urocarpidium insulare E			
Molluginaceae			
Mollugo flavescens ssp. *Anderssonii* E			10
M. flavescens ssp. *gracillima* E			10
M. flavescens ssp. *insularis* E			10
M. flavescens ssp. *striata* E	10		10
M. floriana ssp. *floriana* E			10
Myrtaceae			
Psidium galapageium E*	72		9
Nyctaginaceae			
Boerhaavia erecta			
*Commicarpus tuberosus**			
*Coryptocarpus pyriformis**			
Pisonia floribunda E*			
Onagraceae			
Ludwigia peploides			
Oxalidaceae			
Oxalis cornellii			

TABLE 2.—*CONTINUED*

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
Passifloraceae			
Passiflora colinvauxii E (3)*	>1000, 240, 440		32, 33, 34
P. foetida var. *galapagensis* E (5)*	>1000, >1000, 570, >1000, 0	10	32, 33, 34
P. suberosa (7)	836, 122, 482, 56, 547, 2 negative	10	32, 33, 34
Piperaceae			
Peperomia galapagensis E			
P. petiolata E			
Plumbaginaceae			
*Plumbago scandens**			9
Polygalaceae			
Polygala galapageia E			
P. sancti-georgii E			
Polygonaceae			
Polygonum opelausanum			9
Portulacaceae			
Calandrinia galapagosa E			
Ranunculaceae			
Ranunculus flagelliformis			
Rubiaceae			
Borreria ericaefolia E*			
Galium galapagoense E			
Psychotria rufipes E*			
Spermacoce confusa			
Rutaceae			
*Zanthoxylum fagara**	6		
Sapindaceae			
Cardiospermum galapageium E (3)	>1000, 472, 16000		10, 38, 39, 40, 41
Dodonae viscosa	45	10	
Scrophulariaceae			
Calceolaria meistantha			
Capraria biflora			
C. peruviana			
Castilleja arvensis			
Galvezia leucantha E			
Lindernia anagallidea			
*Mecardonia dianthera**			
Scoparia dulcis			
Simaroubaceae			
Castela galapageia E*			
Solanaceae			
Acnistus ellipticus E*			
Cacabus miersii			
Capsicum galapagoense E			
Grabowskia boerhaaviaefolia			
Lycium minimum E			35
Lycopersicon cheesmanii E			
Nicotinana glutinosa			
Physalis angulata (2)			
P. galapagöensis E (2)			
Solanum erianthum			
Sterculiaceae			
*Waltheria ovata**			
Tiliaceae			
Corchorus orinocensis			
Ulmaceae			
Trema micrantha			10
Umbelliferae			
Apium laciniatum			
A. leptophyllum			
Hydrocotyle umbellata			
Urticaceae			
Fleurya aestuans			

TABLE 2.—CONTINUED

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
Parietaria debilis (2)			
Pilea baurii E			
P. microphylla			
Urera caracasana			
Verbenaceae			
Clerodendrum molle var. *glabrescens*			10
C. molle var. molle*	40		10
Duranta donbeyana			9
D. repens var. repens			9
Lantana peduncularis E*			
Lippia rosmarinifolia E*			
Phyla nodiflora	20		
Phyla strigulosa	>1000		
Verbena litoralis			
V. sedula E			
V. stewartii E*			
V. townsendii E			
Viscaceae			
Phoradendron henslowii E*			
Vitaceae			
*Cissus sicyoides**		9–	
Zygophyllaceae			
Kallstroemia adscendens E			
Tribulus terrestris			

*Indicates that fresh material has also been examined: see Table 1.
†Compounds identified are: triglochinin from *Bouteloua* spp. and *Chloris* spp.; prussic acid from *Cordia* spp. and *Heliotropium* spp.; acalyphin from all *Acalypha* spp.; proacacipetalin from *Acacia macracantha*; gynocardin and passicoccin from *Passiflora* spp.; cardiospermin from *Cardiospermum* spp. In addition cyanolipids are reported in the genera *Cardispermum* and *Heliotropium*.
‡Only wilted specimens are cyanophoric.

drying and storing. The drying conditions (initial water content, drying temperature and duration) have not been uniform, and the herbarium specimens have been stored for different periods.

Some of the species have been tested on fresh material as well as on herbarium material and are marked with an asterisk in Table 2. Many of the species which are considered 'cyanogenic' based on fresh material are negative or only slightly positive when tested on herbarium material. This may be due to the different drying and storage conditions, but it cannot be excluded that the discrepances are caused by phenotypic or genotypic variation. It must be noted, however, that both seasonal and developmental (ontogenetic) variations with regard to cyanogenesis have been described in literature [12–14], as have differences between and within organs of the same plant [15].

The assays on herbarium material yielded positive reaction from 42 species of spermatophytes which have not previously been reported cyanogenic. 14 genera from which no species has previously been reported positive, proved to comprise cyanogenic plants: *Bulbostylis, Eragrostis, Ichnanthus, Mühlenbergia, Liparis, Ponthieva, Prescottia, Ruellia, Desmanthus, Neptunia, Rhynchosia, Bastardia, Zanthoxylum,* and *Phyla*.

Table 3 shows the result from the screening of herbarium material of leaves from ferns and allies, these were only assayed on herbarium material. Of the positive species, eight represent new records. The genus concept within the ferns is somewhat unsettled, so we refrain from comments on the generic level.

Some of the ferns release remarkably high amounts of HCN. We analysed six specimens of *Cheilanthes microphylla*, a small epilithic fern. From five specimens, where 1 mg was analysed, more HCN was produced than the maximum calibration value. The calculated release was 138 000 nmol HCN/g dry weight. *Polypodium angustifolium* and *Grammitis delitescens*, which

TABLE 3. THE DISTRIBUTION OF CYANOGENIC SPECIES IN THE GALÁPAGOS ISLANDS: FERNS AND ALLIES, HERBARIUM MATERIAL

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
Cyatheaceae			
Cyathea weatherbyana E			
Equisetaceae			
Equisetum bogetense (2)			
Hymenophyllaceae			
Hymenophyllum sp.			
Trichomanes reptans			
Lycopodiaceae			
Lycopodium cernuum			
L. clavatum			
L. dichotomum			
L. passerinoides			
L. reflexum			
L. thyoides			
Ophioglossaceae			
Ophioglossum palmatum			
Polypodiaceae			
Adiantum concinnum			
A. henslovianum	>1000		
A. macrophyllum			
A. patens			
A. villosum			
Anogramma chaerophylla			
Asplenium auritum			5, 35, 42
A. cristatum			5, 35, 42
A. feei			5, 35, 42
A. formosum var. *carolinum*	35		5, 35, 42
A. otites			5, 35, 42
A. praemorsum			5, 35, 42
A. pumilum			5, 35, 42
A. serra var. *imrayanum*			5, 35, 42
Blechnum falciforme			5, 35
B. lehmannii			5, 35
B. occidentale			5, 35
B. polypodioides			5, 35
Cheilanthes microphylla (6)	138 000*		5, 43, 44
Ctenitis pleiosoros (2) E			
C. sloanei			
C. sp.			
Dennstaedtia cicutaria			
D. globulifera			
Diplazium subobtusum			
Doryopteris pedata			
Dryopteris patula			5
Elaphoglossum engelii			
E. firmum			
E. glossophyllum			
E. minutum			
E. tenuiculum			
E. yarumalense			
Grammitis delitescens (2)	57 800		
G. serrulata			
Hemionitis palmata			
Histiopteris incisa			
Hypolepis hostilis			
Mildella intramarginalis (2)			
Nephrolepis biserrata (2)			
N. cordifolia	72		
N. pectinata			
Notholaena galapagensis E			
Pityrogramma calomelanos			
P. calomelanos var. *calomelanos*			
P. tartarea			

TABLE 3.—*CONTINUED*

Taxon (Endemic E) (No. in parentheses is specimens examined if more than 1)	nmol HCN g^{-1} dry wt	Literature reports on cyanogenesis† Species	Genus
Polypodium angustifolium (2)	37 200, >1000		5, 45
P. aureum	34		5, 45
P. dispersum			5, 45
P. insularum			5, 45
P. lanceolatum			5, 45
P. phyllitidis	479		5, 45
P. polypodioides			5, 45
P. steirolepis			5, 45
P. tridens E			5, 45
Polystichum gelidum			
P. muricatum			
Pteridium aquilinum	68	5, 46	
Pteris quadriaurita			
Rumohra adiantiformis (2)			
Tectaria aequatoriensis (2)			
Thelypteris balbisii			5
T. conspersa			5
T. gardneriana			5
T. grandis (2)			5
T. linkiana			5
T. patens			5
Trachypteris pinnata			
Woodsia montevidensis			

*All specimens are strongly 'cyanogenic'.
†Compounds identified are: vicianin from *Polypodium* sp.; and prunasin from *Pteridium aquilinum*.

are both epiphytic, released amounts of 37 200 and 57 800 nmol HCN g^{-1}, respectively. It is remarkable that *Grammitis*, a minute plant (fronds 2–8 cm long) which is difficult to discern from the mosses it grows among, has been found only on *Acnistus* branches. *Acnistus ellipticus* is an endemic Solanaceous shrub which released more than 750 nmol HCN/g fresh weight when tested on fresh material.

Experimental

A total of 475 specimens (376 specific or subspecific taxa) were analysed for the presence of cyanogenic constituents. Of these, 114—representing 97 different taxa—were screened as fresh material. The specimens were tested for the presence of cyanogenic compounds by the quantitative field method of Brimer and Mølgaard [4], a modified Guignard test [16].

In order to conduct this test, 100–200 mg of leaf material is crushed in a microtest tube, placed in a rigid PVC block. The sample is moisted with about 500 μl of an aqueous solution containing a crude mixture of different β-glycosidases [5% of β-glucuronidase from *Helix pomatia* (Sigma G-0876)]. The tube is closed by a reagent strip containing sodium picrate. The release of HCN from the crushed material is indicated by the development of orange to dark red coloration. All analyses were conducted over a period of 24 h, (at ambient temperature on Galápagos, at 40° in laboratory) after which the reagent strip was removed and stored for densitometry measurements.

The quantitative evaluation was carried out on a Vitatron TLD 100 densitometer, employing amygdalin (Fluka AG) for the calibration graph. A positive reaction indicates the presence of hydrogen cyanide, α-hydroxynitriles (cyanohydrins) or cyanogenic glycosides in the plant material. It also allows the detection of cyanogenic lipids and epoxides, provided that enzymes (lipases, epoxidases) able to hydrolyze these compounds are present in the material tested. On the other hand, this version of the test gives no information concerning the presence or absence of such enzymes or of β-glycosidases, nor does it indicate the type of cyanogenic constituents (if any). The analyses on fresh material—conducted in the field—must be considered semiquantitative due to problems of sample weighing (a small hand balance) and to the fact that only one analysis was performed on most species.

Nomenclature and taxonomic concepts follow ref. [17] except for the following taxa where revisions on Galápagos material or new records have been published: *Scalesia* [18], *Tiquilia* [19], *Hydrocotyle*, *Verbena* and *Thelypteris* [20], *Grammitis* and *Woodsia* [21] *Lecocarpus* [22], *Chamaesyca* [23], and *Darwiniothamnus* [24].

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Resumen Español—Se reporte una examinación a largo plaza (un 'screening') para mostrar presencia de compuestos cianogenicos en hojas de plantas indigenas de Galápagos. Hemos examinado 475 especimenes, unas en estado verde y algunas tomadas de material seca de herbario. Los resultados muestran que 24 de los 97 especies analisadas como verdes contienen compuestos cianogenicos correspondiente a mas que 10 mg HCN kg^{-1} peso verde, mientras 26 especies estuviron ligeramente positivas (2.5–10 mg HCN kg^{-1}). 33 generos son por primera vez reportados como contiendo especies cianogenicas, y 92 especies previomente no conocido cianogenicos se han mostrada positivas. La importancia de estos resultados en relación de herbivoría se discute.