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A SURVEY FOR CYANOGENESIS IN FERNS AND GYMNOSPERMS

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Abstract—298 species of ferns and 48 species of gymnosperms were tested for cyanogenic activity. 5% of the fern species examined gave positive results, but these showed no correlation with taxonomic groupings. None of the gymnosperms surveyed gave a positive result. The limitations of a survey of this type for detecting cyanogenesis in plants is discussed.

INTRODUCTION

Compounds liberating hydrogen cyanide upon hydrolysis with either acids or appropriate hydrolases are extremely widespread in nature. Cyanogenic glycosides are known to occur in at least 800 species of plants representing 70–80 families [1]. They have been found in bacteria and fungi, ferns, a few gymnosperms and in both monocotyledonous and dicotyledonous angiosperms. This cyanogenic ability of plants has importance as a human poison, one of the best known examples being cassava, the prime constituent of the diet in many parts of Africa [2]. It is also being increasingly shown to be important to the plant as a defence mechanism against animal [3,4] and insect [4–6] predation. The isolation and characterization of naturally occurring cyanogenic compounds has been recently reviewed [7] and the release of hydrogen cyanide can be readily detected by simple and reasonably specific colour tests [8,9].

As a continuation of our work on the biochemical systematics of ferns [10] and the role of secondary plant compounds as feeding deterrents [4,6], it was decided to expand the work of Eyjolfsson [11] and to see whether cyanogenesis was common among fern species. Gymnosperm and fern species were chosen at random from the living collection at Kew and were screened for their ability to produce HCN. This paper discusses the results of screening 298 fern species and 48 species of gymnosperms for cyanogenic activity.

RESULTS AND DISCUSSION

The results of the cyanogenic tests on ferns are listed in Table 1, and those for gymnosperms in Table 2. Out of the 298 fern species tested only sixteen gave a positive result, approximately 5%, suggesting that cyanogenesis is not a common phenomenon among fern species. There was no particular correlation between cyanogenesis and fern taxonomy, positive species being present in the Davalliaceae (5 positive species in the 10 examined, *Davallia* and *Humata*), Polypodiaceae (2 out of 40, *Phlebodium* and *Campyloneuron*), Pteridaceae (2 out of 19, *Actiniopteris* and *Pteris*), Sinopteridaceae (2 out of 16, *Cheilanthes*), Dennstaedtiaceae (3 out of 16, *Microlepia* and *Pteridium*) and isolated examples in the Vittariaceae (1 out of 1, *Pteridanetium*), Aspidiaceae (1 out of 48, *Pteridrys*) and Blechnaceae (1 out of 13, *Stenochlaena*). Of the 48 species of gymnosperms tested, all gave negative results. These results reflect the low frequency of cyanogenic plants in the gymnosperms [11,13,14].

It must be emphasized, however, that when screening for cyanogenic plants positive results are much more important than negative ones. Many factors are involved in the production of cyanogenic compounds, amongst which are the age of the plant, young plants usually being more cyanogenic than old ones [4], the season of the year, parts of the plant examined, as there are often differences between cyanogenesis in the stem, root or leaves, ecological factors [3], including temperature changes, stress conditions and genetic variation. Most if not all plants examined have been shown to be polymorphic for cyanogenesis [15] including *Pteridium aquilinum* [16] and to possess several phenotypes. Random

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Table 1. Cyanogenesis in ferns

Order and family ¹	No. examined/ No. positive	Species tested (* means positive)
Ophioglossales; Ophioglossaceae	1:0	<i>Ophioglossum reticulatum</i> L.
Marattiales; Angiopteridaceae	1:0	<i>Angiopteris hypoleuca</i> de Vriese
Osmundales; Osmundaceae	4:0	<i>Osmunda banksiifolia</i> (Pr.) Kuhn, <i>O. japonica</i> Thbg., <i>O. regalis</i> L., <i>Todea barbara</i> (L.) Moore
Schizaeales; Schizaeaceae	4:0	<i>Anemia simii</i> Tardieu, <i>Lygodium circinatum</i> (Burm.) Sw., <i>Schizaea confusa</i> Selling, <i>S. pectinata</i> (L.) Sw.
Pteridales; Pteridaceae	19:1	<i>Acrostichum aureum</i> L., <i>A. daneaefolium</i> Langsd. et Fisch., <i>Actiniopteris semiflabelata</i> Pich. Sem.*, <i>Pteris altissima</i> Poir., <i>P. arguta</i> Ait., <i>P. biaurita</i> L., <i>P. catoptera</i> Kze., <i>P. cretica</i> L., <i>P. falcata</i> Sod., <i>P. fauriei</i> Hieron., <i>P. longifolia</i> L., <i>P. longipes</i> Don., <i>P. mutilata</i> L., <i>P. pacifica</i> Hieron., <i>P. podophylla</i> Sw., <i>P. straminea</i> Mett., <i>P. tremula</i> R.Br., <i>P. tripartita</i> Sw., <i>P. wallichiana</i> Ag.
Sinopteridaceae	16:2	<i>Cheilanthes argentea</i> (Gmel.) Kze., <i>C. chusana</i> Hk., <i>C. farinosa</i> (Forsk.) Klf., <i>C. kuhnii</i> Milde, <i>C. myriophylla</i> Desv., <i>C. notholaenoides</i> (Desv.) Maxon ex Weath*, <i>C. pulchella</i> Bory*, <i>C. sieberi</i> Kze., <i>C. sinuata</i> (Sw.) Domin., <i>C. tenuifolia</i> (Burm.) Sw., <i>Doryopteris palmata</i> J. Sm., <i>D. pedata</i> (L.) Fée, <i>Pellaea angulosa</i> (Bory.) Bak., <i>P. bridgesii</i> Hk., <i>P. falcata</i> (R.Br.) Fée, <i>P. viridis</i> (Forsk.) Prantl
Cryptogrammeae	1:0	<i>Onychium japonicum</i> (Thbg.) Kze.
Parkeriaceae	1:0	<i>Ceratopteris cornuta</i> Le Prieur
Hemionitidaceae	6:0	<i>Coniogramme</i> Fée sp., <i>Hemionitis arifolia</i> (Burm.) Moore, <i>Ptyrogramma calomelanos</i> (L.) Link, <i>P. chrysophylla</i> (Sw.) Link, <i>Syngamma pinnata</i> J. Sm. Lond., <i>Taenitis</i> Willd. sp.
Adiantaceae	16:0	<i>Adiantum capillus-veneris</i> L., <i>A. concinnum</i> Willd., <i>A. diaphanum</i> Bl., <i>A. fulvum</i> Raoul, <i>A. henslovianum</i> Hk., <i>A. hispidulum</i> Sw., <i>A. lorentzii</i> Hier., <i>A. macrophyllum</i> Sw., <i>A. peruvianum</i> Kl., <i>A. polyphyllum</i> Willd., <i>A. pulverulentum</i> L., <i>A. raddianum</i> Pr., <i>A. soboliferum</i> Wall., <i>A. tenerum</i> Sw., <i>A. tetraphyllum</i> Willd., <i>A. trapeziforme</i> L.
Vittariaceae	1:1	<i>Pteridanetium citrifolium</i> (L.) Copel.*
Dicksoniales; Dicksoniaceae	7:0	<i>Cibotium barometz</i> (L.) J. Sm., <i>C. glaucum</i> (Sm.) Hk., <i>C. hawaiiense</i> Nakai et Ogura, <i>Dicksonia antarctica</i> Lab., <i>D. sellowiana</i> (Pr.) Hk., <i>D. squarrosa</i> (Forst.) Sw., <i>D. youngiae</i> C. Moore
Dennstaedtiaceae	16:3	<i>Blotiella pubescens</i> R. Tryon, <i>Dennstaedtia bipinnata</i> (Cav.) Maxon, <i>D. flaccida</i> (Forst.) Bernh., <i>D. globulifera</i> (Poir.) Hier., <i>D. samoensis</i> (Brack.) Moore, <i>Histiopteris incisa</i> (Thbg.) J. Sm., <i>Hypolepis aspidioides</i> Christ, <i>H. distans</i> (Col.) Hk., <i>H. punctata</i> (Thbg.) Mett., <i>H. tenuifolia</i> (Forst.) Bernh., <i>Microlepia hirta</i> (Klf.) Pr., <i>M. marginata</i> (Houtt.) C.Ch., <i>M. platyphylla</i> (Don) J. Sm. <i>M. setosa</i> (Sm.) Alston*, <i>M. strigosa</i> (Thbg.) Pr.*, <i>Pteridium aquilinum</i> (L.) Kuhn*
Lindsaeaceae	1:0	<i>Lindsaea kirkii</i> Hk.
Davalliales; Davalliaceae	10:5	<i>Davallia campylura</i> Kze., <i>D. canariensis</i> (L.) Sm., <i>D. epiphylla</i> Spr.*, <i>D. solida</i> (Forst.) Sw.*, <i>D. trichomanoides</i> Bl.* <i>Davallodes novoguineense</i> (Ros.) Cop., <i>Humata griffithiana</i> (Hk.) C.Chr., <i>H. pusilla</i> (Mett.) Carr., <i>Leucostegia immersa</i> (Wall.) Pr., <i>Rumohra adiantiformis</i> (Forst.) Ching
Oleandraceae	14:0	<i>Arthropteris orientalis</i> (Gmel.) C. Chr., <i>Oleandra articulata</i> (Sw.) Pr., <i>O. colubrina</i> (Blanco) C., <i>O. dimorpha</i> Copel., <i>Nephrolepis acutifolia</i> (Desv.) Christ, <i>N. biserrata</i> (Sw.) Schott, <i>N. cordifolia</i> (L.) Pr., <i>N. duffii</i> Moore, <i>N. exaltata</i> (L.) Schott, <i>N. falcata</i> (Cav.) C. Chr., <i>N. hirsutula</i> (Forst.) Pr., <i>N. lauterbachii</i> Christ, <i>N. multiflora</i> (Roxb.) Jarrett, <i>N. pectinata</i> (Willd.) Schott
Hymenophyllales Hymenophyllaceae	3:0	<i>Cephalomanes</i> Presl. sp., <i>Mecodium sanguinolentum</i> (G. Forst.) Presl ex Copel., <i>Trichomanes</i> L. sp.
Gleicheniales Gleicheniaceae	2:0	<i>Gleichenia glauca</i> (Tbg.) Hk., <i>G. linearis</i> (Burm.) Clarke
Cyatheales Cyatheaceae	5:0	<i>Cyathea contaminans</i> (Wall. ex Hook.) Copel., <i>C. dealbata</i> (Forst.) Sw., <i>C. medullaris</i> (Forst.) Sw., <i>C. sangirensis</i> (Christ) Copel., <i>C. smithii</i> Hk.
Aspidiales Thelypteridaceae	31:0	<i>Christella harveyi</i> Lev., <i>C. prolifera</i> Pr., <i>Cyclosorus appendiculatus</i> (Blume) Ching, <i>C. atropinosus</i> (C.Chr. ex Kjellb. et C.Chr.) Ching, <i>C. contingus</i> (Rosenst.) Ching, <i>C. dentatus</i> (Forsk.) Ching, <i>C. diversilobus</i> (Presl.) Ching, <i>C. gongyloides</i> Link, <i>C. halconensis</i> Copel., <i>C. invisus</i> (G. Forst.) Copel., <i>C. jaculosus</i> (Christ) H.Ito, <i>C. lanceola</i> (Christ) Copel., <i>C. latipinnus</i> (Hook. ex Bak. in Hook. et Bak.) Tartieu ex Tartieu et C.Chr., <i>C. nymphalis</i> (G.Forst.) Ching, <i>C. pennigerus</i> (G.Forst.) Ching, <i>C. philippinensis</i> (Bak.) Copel., <i>C. piloso-squamatus</i> (v.Ald. v.Ros) Ching, <i>C. procurrens</i> (Mett.) Copel., <i>C. productus</i> (Kaulf.) Ching, <i>C. sumatranus</i> (v.Ald. v.Ros) Ching, <i>C. terrestris</i> (Copel.) Copel., <i>Goniopteris poiteana</i> (Bory.) Ching, <i>Macrothelypteris polypodioides</i> (Hook.) Holttum, <i>Pneumatopteris michaelis</i> (Nakai) Holt., <i>Sphaerostephanos polycarpa</i> (Bl.) Cop., <i>Thelypteris asplenoides</i> (Swartz) Proctor, <i>T. asterothrix</i> (Fée) Proctor, <i>T. dentata</i> (Forssk.) E.St.John, <i>T. erbescent</i> (Wall ex Hook.) Ching, <i>T. uliginosa</i> (Kunze) Ching, <i>T. viridifrons</i> Tagawa

Table 1. cont'd.

Order and family*	No. examined/ No. positive	Species tested (* means positive)
Aspleniaceae	15:0	<i>Asplenium aethiopicum</i> (N.L.Burm.) Becherq., <i>A. auriculatum</i> (Thbg.) Kuhn, <i>A. brachycarpum</i> (Mett.) Kuhn, <i>A. currori</i> Hk., <i>A. lastii</i> C.Chr., <i>A. lucidum</i> Forst., <i>A. marinum</i> L., <i>A. nidus</i> L., <i>A. oceanicum</i> C. Chr., <i>A. schweinfurthii</i> Bak., <i>A. serra</i> Langsd. et Fisch., <i>A. thunbergii</i> Kze., <i>A. unilaterale</i> Lam., <i>A. viviparum</i> (L.fil.) Pr., <i>Neottopteris phyllitidis</i> (D.Don) H.Ito
Athyriaceae	11:0	<i>Athyrium filix femina</i> (L.) Roth, <i>Callipteris</i> Bory. sp., <i>Diplazium atratum</i> Christ, <i>D. bulbiferum</i> Brack., <i>D. caudatum</i> J. Sm., <i>D. dilatatum</i> Bl., <i>D. griffithiana</i> Hk., <i>D. maxima</i> (Fourn.) Bak., <i>D. proliferum</i> (Lam.) Kaulf., <i>Lunathyrium boryanum</i> (Willd.) H.Ohba, <i>L. japonicum</i> (Thunb.) Kurata
Aspidiaceae	48:1	<i>Ctenitis ampla</i> (Humb. et Bonpl. ex Willd.) Ching, <i>C. currori</i> (Mett. ex Kuhn) Tardieu, <i>C. protensa</i> (Afz. ex Swartz) Ching, <i>C. subincisa</i> (Willd.) Ching, <i>Cyclodium meniscioides</i> (Willd.) Pr., <i>Cyclopeltis novoguineensis</i> Ros., <i>Cyrtomium caryotideum</i> Pr., <i>C. fortunei</i> J. Sm., <i>Dryopteris aquilinoides</i> (Desv.) C.Chr., <i>D. carthusiana</i> (Vill.) H. P. Fuchs, <i>D. decomposita</i> (R.Br.) O.Ktze., <i>D. erythrosora</i> (Eat.) O.Ktze., <i>D. filixmas</i> (L.) Schott, <i>D. lacera</i> (Thbg.) O.Ktze., <i>D. lepida</i> (Moore) C.Chr., <i>D. nipponensis</i> Koidzumi in Mayebar, <i>D. paleacea</i> (Moore) Fomin., <i>D. squamiseta</i> (Hk.) O.Ktze., <i>D. subarborescens</i> (Bak.) C.Chr., <i>Gymnocarpium jessoense</i> (Koidz.) Koidz., <i>Heterogonium profereoides</i> (Christ) Cop., <i>H. wigmanii</i> (Racib.) Holttum, <i>Hypodematum crenatum</i> Kuhn, <i>Lastreopsis hispida</i> (Swartz) Tindale, <i>Pleocnemia conjugata</i> Pr., <i>P. tripinnata</i> Holttum, <i>Polystichum aculeatum</i> (L.) Schott, <i>P. kurokawae</i> Tagawa, <i>Psomiocarpia apiifolia</i> Pr., <i>Pteridrys australis</i> Ching ex C.Chr. et Ching, <i>P. olivacea</i> (Rosenst.) Copel*, <i>Tectaria apiifolia</i> Cop., <i>T. bamleriana</i> (Ross) C.Chr., <i>T. cesatiana</i> (C.Chr.) Cop., <i>T. christovalensis</i> (C.Chr.) Alston, <i>T. cicutaria</i> (L.) Cop., <i>T. coadunata</i> C.Chr., <i>T. crenata</i> Cav., <i>T. fernandensis</i> (Bak.) C.Chr., <i>T. fuscipes</i> (Wall.) C.Chr., <i>T. gymnocarpa</i> Cop., <i>T. incisa</i> Cav., <i>T. irregularis</i> (Pr.) Cop., <i>T. macrodonta</i> (Fée) C.Chr., <i>T. menyanthidis</i> (Pr.) Cop., <i>T. subtriphylla</i> (Hk. et Arn.) Cop., <i>T. vasta</i> (Bl.) Cop., <i>Woodsia polystichoides</i> Eat.
Lomariopsidaceae	8:0	<i>Bolbitis naumanni</i> (Kuhn) Ching, <i>B. quoyana</i> (Gaud.) Ching, <i>B. rivularis</i> (Brack.) Ching, <i>B. subcrenata</i> (Hk. et Grev.) Ching, <i>Egenolfia appendiculata</i> (Willd.) J.Sm., <i>Elaphoglossum apodum</i> (Kaulf.) J.Sm., <i>E. crinitum</i> (L.) Christ, <i>E. latifolium</i> (Sw.) J.Sm.
Blechnales Blechnaceae	13:1	<i>Blechnum brasiliense</i> Desv., <i>B. ciliatum</i> Pr., <i>B. fluviatile</i> (R.Br.) Lowe, <i>B. gibbum</i> (Lab.) Mett., <i>B. indicum</i> Burm., <i>B. lanceolatum</i> (R.Br.) Sturm., <i>B. membranaceum</i> (Col.) Mett., <i>B. occidentale</i> L., <i>B. patersoni</i> (R.Br.) Mett., <i>Doodia caudata</i> (Cav.) R.Br., <i>D. media</i> R.Br., <i>Stenochlaena tenuifolia</i> (Desv.) Moore*, <i>Woodwardia virginica</i> (L.) Sm.
Polypodiales Cheiroleuriaceae	1:0	<i>Cheiroleuria bicuspidis</i> (Bl.) Pr.
Polypodiaceae	40:2	<i>Aglaomorpha meyeniana</i> Schott, <i>A. pilosa</i> (J.Sm.) Cop., <i>A. splendens</i> (J.Sm.) Cop., <i>Campyloneurum angustifolia</i> Fée*, <i>C. costatum</i> Pr., <i>C. phyllitidis</i> Pr., <i>Drynaria linnaei</i> Bedd., <i>D. quercifolia</i> (L.) J.Sm., <i>D. rigidula</i> (Sw.) Bedd., <i>D. sparsisora</i> (Desv.) Moore, <i>Goniophlebium subauriculatum</i> Pr., <i>Leptochilus lanceolatus</i> Fée, <i>Microsorium pteropus</i> (Bl.) Copel., <i>M. punctatum</i> (Linn.) Copel., <i>M. scolopendria</i> (Burm. f.) Copel., <i>Phlebodium aureum</i> J.Sm., <i>P. decumanum</i> J.Sm., <i>Photinopteris speciosa</i> Bl., <i>Platynerium bifurcatum</i> (Cav.) C.Chr., <i>P. grande</i> (A.Cunn.) J. Sm., <i>P. stemaria</i> (Beauv.) Desv., <i>P. vassei</i> hort., <i>Pleopeltis bicolor</i> (Takeda) Sledge, <i>P. lanceolata</i> Klf., <i>P. percussa</i> Hd., <i>Polypodium auriculatum</i> L., <i>P. macaronesicum</i> A. Bobrov, <i>P. proliferum</i> Klf., <i>P. richardii</i> Kl., <i>P. thyssanolepis</i> A. Br., <i>P. triangulum</i> L., <i>P. vulgare</i> L., <i>Pyrossia angustata</i> (Swartz.) Ching, <i>P. beddomeneana</i> (Giesenh.) Ching, <i>P. lingua</i> (Thunb.) Farwell, <i>P. longifolia</i> (N.L. Burm.) Morton, <i>P. nummularifolia</i> (Swartz.) Ching, <i>P. winkleri</i> (Rosenst.) Ching, <i>Selliguea plantaginea</i> Brack., <i>Thayeria hookeri</i> Cop.
Grammitidaceae	1:0	<i>Ctenopteris heterophylla</i> (Labill.) Tindale
Marsileales Marsileaceae	1:0	<i>Marsilea</i> L. sp.
Salviniales Salvinaceae	1:0	<i>Salvinia auriculata</i> Aublet.

* Arranged according to Pichi-Sermolli [12].

sampling, therefore, may result in a high proportion of negative readings as an acyanogenic form of a particular plant may have been tested. However screening in this way does give some indication of the frequency of cyanogenesis, and also suggests groups of plants in which cyanogenesis is more likely to occur.

EXPERIMENTAL

Plant material. Fresh material was sampled from mature plants growing in the greenhouses or in the gardens at the Royal Botanic Gardens, Kew. Only material which had been properly verified and identified was used. Fresh leaf samples were examined for the production of HCN by treating the

Table 2. Cyanogenesis in Gymnosperms

Order and family*	No. examined/ No. positive	Species tested (* means positive)
Coniferales Pinaceae	46:0	<i>Araucaria araucana</i> (Mol.) K.Koch, <i>Cedrus atlantica</i> Manetti, <i>C. deodara</i> (Royle ex Lamb.) Loud., <i>Chamaecyparis lawsonia</i> (A.Murr.) Parl. var. "lutea compacta", <i>C. obtusa</i> (Sieb. & Zucc.) Sieb. & Zucc. var. "nana", <i>C. pisifera</i> (Sieb. & Zucc.) Sieb. & Zucc. var. "filifera nana", <i>C. thyoides</i> (L.) Britton, Sterns & Pogg. var. "glauca", <i>Cryptomeria japonica</i> (L.f.) D. Don var. "spirilis" var. "vitellina", <i>Cunninghamia lanceolata</i> (Lamb.) Hook, <i>Juniperus communis</i> L. var. "nana", <i>J. conferta</i> Parl., <i>J. horizontalis</i> Moench, <i>J. procumbens</i> Sieb., <i>J. sabina</i> L. var. "tamariscifolia", <i>J. scopulorum</i> Sarg., <i>J. squamata</i> D. Don, <i>Larix decidua</i> Mill., <i>L. gmelini</i> (Rupr.) Litvinov., <i>L. leptolepis</i> (Sieb. & Zucc.) Murr., <i>Libocedrus decurrens</i> Torr., <i>Picea abies</i> (L.) Karst., <i>P. bicolor</i> (Maxim) Mayr., <i>P. glauca</i> (Moench) Voss., <i>Pinus aristata</i> Engelm., <i>P. ayacahuite</i> Ehrenb., <i>P. banksiana</i> Lamb., <i>P. bungeana</i> Zucc., <i>P. cembra</i> L., <i>P. cembroides</i> Zucc., <i>P. coulteri</i> D. Don, <i>P. jeffreyi</i> A.Murr., <i>P. mugo</i> Turra, <i>P. nigra</i> Arnold, <i>P. parviflora</i> Sieb. & Zucc., <i>P. peuce</i> Griseb., <i>P. pinea</i> L., <i>P. ponderosa</i> Dougl., <i>P. radiata</i> D. Don, <i>P. strobus</i> L. var. "prostata", <i>P. sylvestris</i> L., <i>Pseudolarix amabilis</i> (Nelson) Rehder, <i>Sciadopitys verticillata</i> (Thunb.) Sieb. & Zucc., <i>Sequoia sempervirens</i> (Lamb.) Endl. var. "adpressa", <i>Taxodium ascendens</i> Brongn. var. "nutans", <i>T. distichum</i> (L.) Rich., <i>Tsuga canadensis</i> (L.) Carr. var. "albo-spica" var. "pendula"
Taxaceae	2:0	<i>Taxus baccata</i> L. var. "expansa", <i>Podocarpus andinus</i> Poepp. ex. Endl.

* Arranged according to Kew "Handlist of Conifers".

material (ca 1.0 g of terminal pinnae or needles) with 2-3 drops of toluene in a sealed tube with a filter paper strip, which had been pre-treated with either sodium picrate [8,14] or copper ethylacetoacetate in CHCl_3 [9], suspended from the stopper and incubating the tube at 35° for 3 hr. Any change in the colour of the paper was observed.

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BENZYL ISOTHIOCYANATE IN *CYCLICOMORPHA SOLMSII* (CARICACEAE)

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Plant. Seeds of *Cyclicomorpha solmsii* were collected in January, 1976, by Mr. Jurgen Griesbach of the German

Agricultural Mission at about the 1500 m level on Mt. Kenya, Kenya from trees about 27 m tall. Several plants were grown from the seeds by the Horticulture department at the Hawaii Agricultural Experiment Station. The leaves of these young plants fit the description of that of *C. solmsii* [1]. However, flowering plants have not