2005

Calcium oxalate crystal macropatterns in leaves of species from groups Glycine and Shuteria (Glycininae; Phaseoleae; Papilionoideae; Fabaceae)

Teresa Cervantes-Martinez
Iowa State University

Harry T. Horner
Iowa State University, hth@iastate.edu

Reid G. Palmer
U.S. Department of Agriculture

Theodore Hymowitz
University of Illinois at Urbana-Champaign

A. H. D. Brown
Commonwealth Scientific and Industrial Research Organisation (CSIRO)

Calcium oxalate crystal macropatterns in leaves of species from groups Glycine and Shuteria (Glycininae; Phaseoleae; Papilionoideae; Fabaceae)

Abstract
Calcium oxalate crystal macropatterns in leaves were characterized for 69 species (and two Glycine tomentella cytotypes) from 14 of 16 genera in two legume groups, Glycine and Shuteria, to determine whether they share a common macropattern. A leaf clearing method was used to visualize the crystals. All 69 species (and two Glycine tomentella cytotypes) displayed prismatic crystals associated with leaf veins and vein endings. In contrast, mesophyll crystals occurred in 76.8% of 69 species and two G. tomentella cytotypes, and varied from a few to many. Conversely, only 40.9% of 22 Glycine species (in group Glycine) lacked mesophyll crystals, while 8.7% of 23 species of six genera associated with Glycine (in group Glycine) lacked mesophyll crystals. Thus 24.4% of 45 species of seven combined genera in group Glycine lacked mesophyll crystals. With seven genera in group Shuteria, 20.8% of 24 species lacked mesophyll crystals. The consistently present vein crystals varied in size and shape, so their length–width (Stubby versus Long) crystal ratios were determined for primary, secondary, and tertiary veins, and vein endings. Two trends were evident: Long-crystal ratios increased from primary veins to vein endings in species in both groups, and the perennial and annual Glycine species showed this condition to a greater extent than all the non-Glycine species. In some cases, taxonomically closely associated species were quite similar in their macropattern and presence or absence of mesophyll crystals. These results should be of value to future studies dealing with taxonomy and phylogeny of species in these two leguminous groups.

Keywords
calcium oxalate, Fabaceae, Glycine, group Glycine, group Shuteria, leaf crystals

Disciplines
Botany | Cell and Developmental Biology | Genetics | Plant Sciences

Comments
This article is published as Cervantes-Martinez, Teresa, Harry T. Horner, Reid G. Palmer, Theodore Hymowitz, and A. H. D. Brown. "Calcium oxalate crystal macropatterns in leaves of species from groups Glycine and Shuteria (Glycininae; Phaseoleae; Papilionoideae; Fabaceae)." *Botany* 83, no. 11 (2005): 1410-1421. doi: 10.1139/b05-119.

Calcium oxalate crystal macropatterns in leaves of species from groups Glycine and Shuteria (Glycininae; Phaseoleae; Papilionoideae; Fabaceae)

Teresa Cervantes-Martinez, Harry T. Horner, Reid G. Palmer, Theodore Hymowitz, and A.H.D. Brown

Abstract: Calcium oxalate crystal macropatterns in leaves were characterized for 69 species (and two *Glycine tomentella* cytotypes) from 14 of 16 genera in two legume groups, Glycine and Shuteria, to determine whether they share a common macropattern. A leaf clearing method was used to visualize the crystals. All 69 species (and two *Glycine tomentella* cytotypes) displayed prismatic crystals associated with leaf veins and vein endings. In contrast, mesophyll crystals occurred in 76.8% of 69 species and two *G. tomentella* cytotypes, and varied from a few to many. Conversely, only 40.9% of 22 *Glycine* species (in group Glycine) lacked mesophyll crystals, while 8.7% of 23 species of six genera associated with *Glycine* (in group Glycine) lacked mesophyll crystals. Thus 24.4% of 45 species of seven combined genera in group Glycine lacked mesophyll crystals. With seven genera in group Shuteria, 20.8% of 24 species lacked mesophyll crystals. The consistently present vein crystals varied in size and shape, so their length–width (Stubby versus Long) crystal ratios were determined for primary, secondary, and tertiary veins, and vein endings. Two trends were evident: Long-crystal ratios increased from primary veins to vein endings in species in both groups, and the perennial and annual *Glycine* species showed this condition to a greater extent than all the non-*Glycine* species. In some cases, taxonomically closely associated species were quite similar in their macropattern and presence or absence of mesophyll crystals. These results should be of value to future studies dealing with taxonomy and phylogeny of species in these two leguminous groups.

Key words: calcium oxalate, Fabaceae, *Glycine*, group Glycine, group Shuteria, leaf crystals.

Résumé : Afin de déterminer si ces espèces partagent un macropatron commun, les auteurs ont caractérisé les macropatrons des cristaux d'oxalate de calcium dans les feuilles de 69 espèces (et deux cytotypes du *Glycine tomentella*), appartenant à 14-16 genres des deux groupes Glycine et Shuteria, Ils ont utilisé une méthode d'éclaircissement pour visualiser les cristaux. L'ensemble des 69 espèces présente des cristaux prismatiques associés aux nervures et à leurs terminaisons. Au contraire, on n'observe les cristaux du mésophylle que chez 76,8 % des 69 espèces et les deux cytotypes du *G. tomentella*, et leur nombre varie de quelques-uns à plusieurs. Réciproquement, seulement 40,9 % des 22 espèces de *Glycine* (groupe Glycine) ne possèdent pas de cristaux dans leur mésophylle, alors que 8,7 % des 23 espèces de six genres associées au genre *Glycine* (groupe Glycine) ne possèdent pas de cristaux dans leur mésophylle. Ainsi 24,4 % des 45 espèces, appartenant à sept genres combinés du groupe Glycine, n'ont pas de cristaux dans leur mésophylle. Parmi les sept genres du groupe Shuteria, 20,8 % des 24 espèces sont dépourvues de cristaux du mésophylle. Les cristaux présents de façon constante dans les nervures varient en dimension et en forme, et les auteurs ont donc déterminé les rapports longueur–largeur (court contre allongé) des cristaux, pour les nervures de premier, deuxième et troisième ordres, ainsi que dans leurs terminaisons, chez les espèces des deux groupes. On observe deux tendances : les rapports des cristaux allongés augmentent des nervures primaires aux terminaisons, chez les espèces des deux groupes, et les espèces pérennes et annuelles du genre *Glycine* montrent ce comportement plus intensément que toutes les espèces des autres genres. Dans certains cas, des espèces étroitement associées taxonomiquement ont des macropatrons et une présence ou absence de cristaux du mésophylle, assez semblables. Ces résultats devraient s'avérer utiles pour des études ultérieures portant sur la taxonomie et la phylogénie des espèces appartenant à ces deux groupes de légumineuses.

Mots clés : oxalate de calcium, Fabaceae, *Glycine*, groupe des Glycinnes, groupe des Shuteria, cristaux foliaires.

[Traduit par la Rédaction]

Received 5 April 2005. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 30 January 2005.

T. Cervantes-Martinez. Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA.
H.T. Horner.[1] Bessey Microscopy Facility, Iowa State University, Ames, IA 50011, USA.
R.G. Palmer. USDA-ARS-CICGR and Department of Agronomy, Iowa State University, Ames, IA 50011, USA.
T. Hymowitz. Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA.
A.H.D. Brown. CSIRO, Plant Industry, Canberra, Australia.

[1]Corresponding author (e-mail: hth@iastate.edu).

 doi: 10.1139/b05-119

Introduction

The location (macropattern) and shape of calcium oxalate (CO) crystals in leaves of gymnosperms (Lin and Hu 1998) and of flowering plants (Hufford 1997; Prychid and Rudall 1999; Lersten and Horner 2000, 2004; Monje and Baran 2002) have been explored recently as useful taxonomic characters. Together, these latter studies suggest that the macropattern, shape, and hydration form of CO crystals may reflect important evolutionary changes in related taxa from the level of the family to the levels of the genus, and possibly the species.

Such characteristics are potentially important, since about 75% of the flowering plants, and many gymnosperms, display CO crystals (Horner and Wagner 1995). Zindler-Frank (1987) provided a comprehensive review of crystal distribution for the legume family, and Solereder (1908) and Metcalfe (1983) for the dicotyledons, in general. However, much less is known about the association of crystal patterns in many of the smaller taxonomic groups or species within families (e.g., Lersten and Horner 2000, 2004), and particularly within the Leguminosae (Fabaceae). Even less is known about the functional significance of these crystals (Franceschi and Horner 1980; Horner and Wagner 1995), and whether they play any nutritional role in taxa that are used as food sources, especially for soybean (*Glycine max* (L.) Merr.) (Massey et al. 2001, 2002; Horner et al. 2005).

Much needs to be learned about the biochemistry that produces CO crystals in plants. It is still unclear which pathways are involved in the synthesis of oxalate (Franceschi and Loewus 1995; Horner et al. 2000; Keates et al. 2000; Kostman et al. 2001; Loewus 1999), and in the cellular production and function of the crystals (Horner and Wagner 1995) in both vegetative (Franceschi and Horner 1980) and reproductive tissues (Ilarslan et al. 1997, 2001).

Our research continues to explore various aspects of the presence, location, and formation of CO crystals in the annual, cultivated *G. max*, and in this study it was expanded to include all but two genera in the subtribe Glycininae, which contains two groups, Glycine (eight genera) and Shuteria (eight genera) (Lackey 1981; Legume Web 2005). Recent molecular phylogenetic studies (Doyle and Doyle 1993; Kollipara et al. 1997; Lee and Hymowitz 2001; Brown et al. 2002) have focused on 20 wild perennial *Glycine* species related to the annual cultivated and wild annual soybean species. *Glycine* is associated with 17 genera in both groups. A synopsis of the taxonomic history of the Glycininae was presented by Lee and Hymowitz (2001).

The present study includes all but two unavailable genera from both groups (Lackey 1981) to obtain as broad a survey of leaf crystal macropatterns at the levels of genus and species. Our data show definite macropatterns among species, a trend in vein crystal-shape ratios, and similar crystal associations among some closely related species in each of the groups that separate them from less-related species.

Materials and methods

Data from 200 herbarium specimens representing 14 genera and 69 species (and two *Glycine tomentella* (Hayatta) cytotypes, PI 373980 and PI 441001) from all available species in the groups Glycine and Shuteria within the subtribe Glycinineae (Phaseoleae; Papilionoideae; Fabaceae) (sensu Lackey 1977, 1981; Lee and Hymowitz 2001), and field-grown *G. max* cultivar Kenwood 94 were used in this study.

Preparation of leaves:

Typically three leaf punches (cork borer, 11-mm diameter) were taken from a single leaf (one per leaflet), either in the center of a middle-age (fresh) leaflet or to one side of the main vein of a mature leaflet. With some herbarium specimens with smaller leaves, only smaller diameter punches were taken, and sometimes whole, small leaves.

Both fresh and herbarium specimens were collected and subjected to oxidizing and clearing agents (Lersten and Horner 2000, 2004), using multi-compartment trays (Horner and Arnott 1961). This chemical procedure removes all cytoplasmic contents except for the CO crystals, cell walls, and epidermal trichome walls, making the specimens essentially transparent for visualizing the crystals. The dehydrated (via ethanols) and cleared (via xylol) leaf disks or small leaflets were mounted in Permount (Fisher Scientific International, Inc., www.fisherscientific.com) on slides and cover slipped. Each leaf disk or leaflet was viewed between crossed polarizers using a Leitz orthoplan microscope (Leica-microsystems AG, www.leica-microsystems.com/Microscopes) fitted with a 35 mm automatic camera. The crystal macropattern for each species was recorded on Eastman Kodak Ektachrome T64 film (Eastman Kodak Co., www.kodak.com). The color transparencies were digitized with a Umax 3000 PowerLook 3000 scanner (Umax Technologies, Inc., www.umax.com) in the transmissive mode at 600 dpi. Digitized images were processed and sized in Adobe PhotoShop 7.0 (Adobe Systems, Inc., www.adobe.com), and organized into plates using Adobe PageMaker 6.5 (Adobe Systems, Inc., www.adobe.com).

Punches from herbarium specimens were typically from mature leaves. Punches from field-grown soybean were taken after several sets of leaves were expanded, and from older leaves later during the growing season. Punches and whole leaves or leaflets were compared quantitatively for variation in the location and size of crystals associated with the veins, vein endings, and mesophyll. Five crystals each, associated with primary, secondary, tertiary veins, and vein endings, were measured for their length and width using a Zeiss AxioVision image analysis system. Crystals with a length–width ratio of ≤1.5 were considered stubby (S) and crystals with a length–width ratio of >1.5 were considered long (L).

We acknowledge that plants growing in the wild or under cultivation in different geographical locations and elevations, and in different years, are subject to a variety of natural climatic variations. In a study such as the present one, it was impossible to provide controls. Data from other published comparative studies suggest that crystal shape, composition, and location are consistent at the species level, and most likely under genetic control (Kausch and Horner 1982; Lersten and Horner 2000). Therefore these characters are presumed not to be greatly altered by normal variations in climatic conditions.

Accession data

Collector and collection number or USDA Plant Introduc-

Table 1. Twenty perennial, two *Glycine tomentella* cytotypes, and two annual *Glycine* species in group Glycine showing crystal location associated with only veins, or veins and mesophyll, and indicating whether crystals that are associated with primary, secondary, and tertiary veins and vein endings have an average length–width ratio of ≤1.5 (S, short) or >1.5 (L, long).

Glycine group *Glycine* species	Figure No. / collection No.	Assigned cytogenetic genome for *Glycine* species*	Crystals in leaf mesophyll	Crystal ratios associated with 1°/2°/3°/vein endings
G. canescens	2/6d	A	None	L/L/L/L
G. clandestina	3/7b	A	None	L/L/L/L
G. argyrea	1*i*/5d	A	None	S/L/L/L
G. latrobeana	—/15a	A	Many	L/S/L/L
G. albicans	4/2	I	None	S/S/L/L
G. lactovirens	—/13c	I	None	L/L/L/L
G. aphyonota	—/3	I	None	S/L/L/L
G. tomentella	—/23i	D	Many	S/L/S/L
G. tomentella (78)	6/23d	DE	Few	L/S/L/L
G. arenaria	—/4b	H	Few	S/L/L/L
G. hirticaulis	7/12	H	Few	L/L/L/L
G. pindanica	1*e*/18a	H	Few	S/L/L/L
G. pullenii	1*d*,12/19	H	Few	L/S/S/L
G. microphylla	—/17a	B	Few	L/L/L/L
G. latifolia	8/14b	B	Few	S/S/L/L
G. tabacina (80)	1*h*/22b	BB	Few	L/L/L/L
G. stenophita	9/21	B	Many	S/S/L/L
G. cyrtoloba	—/9b	C	Many	S/S/L/L
G. curvata	1*a*,1*b*/8c	C	Many	S/S/L/L
G. falcata	5/11d	F	None	L/L/L/L
G. soja	10/20	G	None	S/L/S/L
G. max	11/16	G	None	L/L/L/L
G. dolichocarpa	13/10b	—	Few	L/L/L/L

Note: Chromosome numbers are in parentheses if they are other than 40.
*Assigned cytogenetic genome for *Glycine* species is by Kollipara et al. (1997).

tion number, country/state, herbarium abbreviation and accession number and (or) CSIRO G number for 201 specimens of 14 genera and 69 species (and two *Glycine tomentella* cytotypes) from members of the Glycine and Shuteria groups within the subtribe Glycininae (Phaseoleae; Papilionoideae; Leguminosae) are listed alphabetically within each group; each genus has been given a number. Mutiple collections of a given species have been given a number and letter.

Specimens were unavailable for two (*Nogra*, group Glycine; and *Diphyllarum*, group Shuteria) of the 16 genera from all of the herbaria contacted. All genera are listed for each group, and the numbers in parentheses after each genus indicated (number of identified species in the genus per number of species observed in this study) (Lackey 1977, 1981; Lee and Hymowitz 2001).

Group Glycine:

The annual, cultivated *G. max* and its wild progenitor *Glycine soja* and their related 22 wild perennial *Glycine* species (18/17), including three additional species (*G. aphyonota* and *G. pullenii* (Pfeil & Craven 2002); and *G. dolichocarpa* (Tateishi & Ohashi 1992)), were included along with six of the seven (no collections for *Nogra*) other genera (*Eminia* (5/1), *Pseudeminia* (4/1), *Pseudovigna* (1/1), *Pueraria* (20/11), *Sinodolichos* (2/1) and *Teramnus* (8/8)) (Tables 1 and 2). No synonyms were provided, so all species by name were included as identified on the herbarium sheets.

Eminia **1**. *antennulifera* (Salubeni & Balaka, 5346; Walawi; MO4078918); *Glycine* **2**. *albicans* (Craven et al. CFS4762; Australia; IL889/G2049); **3**. *aphyonota* (Grant, 1234; Australia; G2589); **4a**. *arenaria* (Craven et al. CANB375968; Australia; IL1294/G2050); 4b. *arenaria* (Grace et al. 346, PI 505293; Australia; IL805/G1949); 4c. *arenaria* (Brown et al. PI 505296; Australia; IL808/G1931); 4d. *arenaria* (Pullen, R, PI 505204; Australia, IL689/G1305); **5a**. *argyrea* (PI 595792; Australia; IL1296/G1621); 5b. *argyrea* (Grant & Pullen, 520, PI 509452; Australia; IL818); 5c. *argyrea* (PI 509451; Australia; IL817); 5d. *argyrea* (PI 505151; Australia; IL768-1); 5e. *argyrea* (PI 505151; Australia; IL768-2); 5f. *argyrea* (Hacker, JB, PI 599401; Australia; IL1305/G2448); 5g. *argyrea* (PI 595798; Australia; IL1304); 5h. *argyrea* (PI 595797; Australia; IL1303); 5i. *argyrea* (PI 595796; Australia; IL1302); 5j. *argyrea* (Grant & Pullen, PI 599400; Australia; IL1301/G2003); 5k. *argyrea* (Grant & Pullen, CANB355552; Australia; IL1300/G2002); 5l. *argyrea* (PI 595795; Australia; IL1299); 5m. *argyrea* (PI 595794; Australia; IL1298); 5n. *argyrea* (PI 595793; Australia; IL1297); **6a**. *canescens*

Table 2. Six genera and 23 species of group Glycine showing location of leaf crystals associated with only veins, only mesophyll, or both, and indicating whether crystals associated with them have an average length–width ratio of ≤1.5 (S, short) or >1.5 (L, long).

Glycine group genera and species	Figure No. / collection No.	Crystals in leaf mesophyll	Crystal ratios associated with 1°/2°/3°/vein endings
Eminia			
E. antennulifera	19/1	Few	S/S/S/L
Pseudeminia			
P. comosa	17/24	Many	L/L/L/L
Pseudovigna			
P. argentea	14/25	Few	L/S/L/S
Pueraria			
P. alopecuroides	—/26	Many	S/L/L/L
P. lobata	20/27a	Few	L/S/L/S
P. montana	—/28c	Many	L/L/L/L
P. peduncularis	1*f*,1*g*,16/29c	None	L/L/L/L
P. phaseoloides	—/30a	Many	L/S/L/L
P pulcherrima	—/31	Many	L/S/L/L
P. stricta	—/32	Many	S/L/L/L
P. subspicata	—/33	Many	L/L/L/L
P. thunbergiana	—/34j	Variable	L/L/L/L
P. tuberosa	—/35a	Many	L/S/L/L
P. wallichii	1*c*/36	Few	S/S/L/L
Sinodolichos			
S. lagopus	—/37	None	L/L/L/L
Teramnus			
T. axilliflorus	18/38	Few	S/S/S/L
T. buettneri	—/39	Few	S/L/L/L
T. flexilis	—/40	Variable	S/S/L/L
T. labialis	—/41a	Variable	S/L/L/L
T. micans	—/42	Many	S/L/L/L
T. repens	—/43	Few	S/S/S/L
T. uncinatus	15/44c	Few	S/S/S/L
T. volubilis	—/45	Variable	S/S/S/L

(Whibly, PI 440930; Australia; IL432/G1851); 6b. *canescens* (PI 440932; Australia; IL434); 6c. *canescens* (PI 440933; Australia; IL438); 6d. *canescens* (PI 440931; Australia; IL431-3); 6e. *canescens* (PI 440927; Australia; IL405); 6f. *canescens* (PI 440927; Australia; IL405-2); 6g. *canescens* (PI 440929; Australia; IL404); 6h. *canescens* (PI 440928; Australia; IL401)(6h is of unknown origin); 6i. *canescens* (PI 399478; Australia; IL379); 6j. *canescens* (PI 440933; Australia; IL438); 6k. *canescens* (PI 440934; Australia; IL455); 6l. *canescens* (PI 440934; Australia; IL1455-3); 6m. *canescens* (PI 440935; Australia; IL461); 6n. *canescens* (PI 440936; Australia; IL462); 6o. *canescens* (PI 440937; Australia; IL463); **7a**. *clandestina* (PI 246590; Australia; IL302); 7b. *clandestina* (PI 233138; Australia; IL301); 7c. *clandestina* (Grace et al. 1512; Australia; IL1419/G3004); 7d**.** *clandestina* (Grace et al. 904, PI 599402; Australia; G2357/IL991); **8a**. *curvata* (PI 499931;Australia; IL910-1); 8b. *curvata* (PI 505166; Australia; IL791-1); 8c. *curvata* (PI 505165; Australia; IL790-2); **9a**. *cyrtoloba* (PI 440963; Australia; IL481); 9b. *cyrtoloba* (PI 440962; Australia; IL480-2); 9c. *cyrtoloba* (PI 373993; Australia; IL317-2); **10a**. infrasp. *dolichocarpa* (Hsieh, JS, TOM0040; Taiwan; IL1372); 10b. *dolichocarpa* (Hsieh, JS, TOM038; Taiwan; IL1370); **11a**. *falcata* (PI 505180; Australia; IL728-1); 11b. *falcata* (PI 505179; Australia; IL674-3); 11c. *falcata* (PI 509474; Australia; IL839-1); 11d. *falcata* (PI 246591; Australia; IL321); 11e. *falcata* (PI 233139; Australia; IL320); **12**. *hirticaulis* (Craven et al.; Australia; IL1246); **13a**. *lactovirens* (Grant, 1229; Australia; IL/G2597); 13b. *lactovirens* (Grant, 1330; Australia; IL/G2598); 13c. *lactovirens* (IL/G1939); **14a**. *latifolia* (Capella, PI 253238; Australia; IL0375/G1343); 14b. *latifolia* (PI 378709; Australia; IL373); 14c. *latifolia* (PI 321394; Australia; IL0359/G1695); 14d. *latifolia* (PI 321393; Australia; IL358); **15a**. *latrobeana* (PI 509481; Australia; IL0846/G1900); 15b. *latrobeana* (Grace et al. GBG943; Australia; IL1068/G2356); **16**. *max* cultivar Kenwood 94 (Palmer, RG, Iowa, USA; ISU); **17a**. *microphylla* (PI 339665; Australia; IL0314); 17b. *microphylla* (PI 339664; Australia; IL0312/G1143); **18a**. *pindanica* (Grace et al. 01348, PI 595818; Australia; IL1285/G2951); 18b. *pindanica* (Martin, JB, 055; Australia; IL1283/G2504); 18c. *pindanica* (Grace et al. 01349; Australia; IL1263/G2952); 18d. *pindanica* (Stewart, J; Australia; IL1252/G2939); 18e. *pindanica* (Stewart, J; Australia; IL1251/G2938); **19**. *pullenii* (Latz, PK, 10069; Australia; IL/G2786); **20**. *soja* (19/May/1997; Taiwan; IL); **21**. *stenophita* (Grace et al. 806/1-3, PI 547009; Australia; IL1120); **22a**. *tabacina* (PI 320546; Taiwan; IL0329-2/G2602); 22b. *tabacina* (PI 248253; Australia; IL0323/G2762); 22c. *tabacina* (PI 193232; Australia; IL0322/

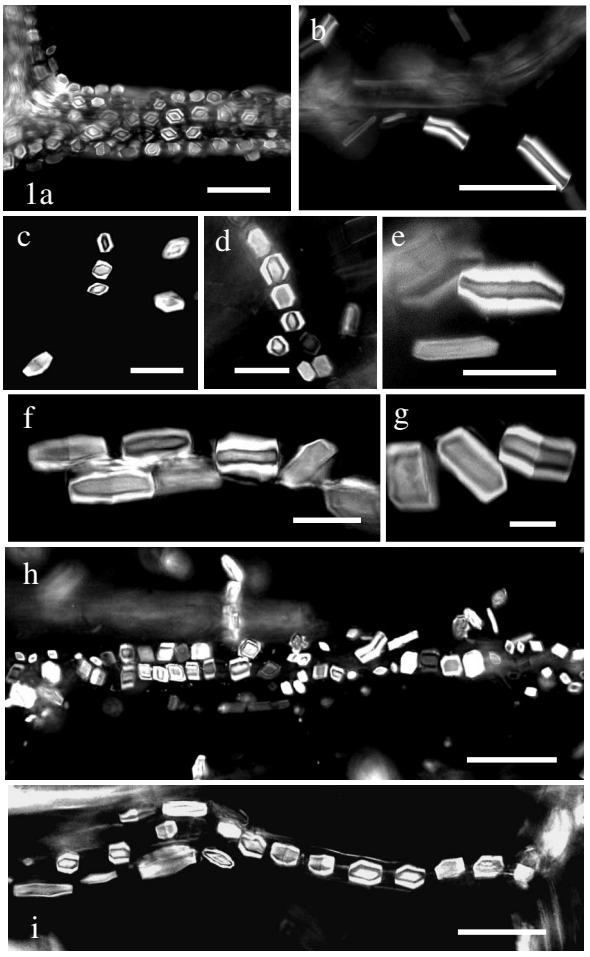
Table 3. Seven genera and 24 species of group Shuteria showing location of crystals associated with only veins, only mesophyll, or both, and indicating whether crystals associated with them have an average length–width ratio of ≤1.5 (S, short) or ≥1.5 (L, long).

Shuteria group genera and species	Figure No. / collection No.	Crystals in leaf mesophyll	Crystal ratios associated with 1°/2°/3°/vein endings
Amphicarpaea			
A. africana	23/46	Few	L/S/L/L
A. bracteata	32/47c	Few	L/L/S/L
A. edgeworthii	—/48b	Many	L/S/S/S
Cologania			
C. angustifolia var. *broussonetii*	26/49	Few	S/S/L/L
C. hirta	28/50b	None	S/L/L/L
C. intermedia	—/52b	Few	S/L/L/L
C. humifusa	—/51	Few	L/L/L/L
C. jaliscana	—/53	Many	S/S/L/L
C. lemmoni	—/54	Few	L/L/L/L
C. longifolia	—/55a	None	S/S/L/L
C. procumbens	31/56	None	S/S/S/L
C. pulchella	—/57b	Few	S/S/L/L
C. racemosa	—/58	Few	S/S/L/L
Dumasia			
D. bicolor	29/59	None	L/L/L/L
D. miaoliensis	—/60	Rare, small	S/S/L/S
D. truncata	21/61c	In groups	S/L/L/L
D. villosa	30/62h	None	S/S/L/L
Mastersia			
M. bakerii	—/63	Few	S/S/S/S
M. borneensis	22/64	Few	S/L/L/L
Neonotonia			
N. verdcourtii	33/65	Few	S/S/L/L
N. wightii	27/66b	Variable	L/L/L/L
Shuteria			
S. hirsuta	25/67b	Variable	L/L/L/L
S. involucrata	—/68	Few	S/S/S/S
Teyleria			
T. koordersii	24/69a	Few	S/L/L/L

G2763); 22d. *tabacina* (PI 320545; Taiwan; IL0326/G1335); 22e. *tabacina* (PI 272099; Australia; IL0324/G1860); 22f. *tabacina* (Grace et al. 953, PI 505200; Australia; IL785/G1925); **23a**. *tomentella* (Chen & Kao, PI 233051; Taiwan; IL0352/G1345); 23b. *tomentella* (Lu, PI 339655; Taiwan; IL0361/G1349); 23c. *tomentella* (PI 320547; Taiwan; IL0356/G1347); 23d. *tomentella* (PI 373980; Australia; IL0339/G1427); 23e. *tomentella* (Hacker, JB, 435, PI 604476; Australia; IL1333/G2476); 23f. *tomentella* (Barlow, B, BAB3922; Australia; IL1332/G2041); 23 g. *tomentella* (Barlow, B, BAB3874; Australia; IL1331/G2040); 23h. *tomentella* (Barlow, B, BAB3873; Australia; IL1330/G2039); 23i. *tomentella* (Broue, B11, PI 441001; Australia; IL489/G1133); *Pseudeminia* **24**. *comosa* (CORBY1450; Zimbabwe; CU394); *Pseudovigna* **25**. *argentea* (PI 365594; Tanzania; CU370); *Pueraria* **26**. *alopecuroides* (Maxwell, 91-99; Thailand; MO4308312); **27a**. *lobata* (det. van der Maesen, 1981; China; NY12521); 27b. *lobata* (van der Maesen, 1981; Hong Kong; NY); 27c. *lobata* (Japan; ISU324801); 27d. *lobata* (Naito and Ishikawa; Japan; ISU405440); **28a**. *montana* (Te'ang Wei Tak, 16325; China; USNH58266); 28b. *montana* (Tsui, 603; China; USNH15714); 28c. *montana* (kot T'ang Fa, 603; China; NY); 28d. *montana* (Ishigak, 4097; Japan; USNH0010942); 28e. *montana* (det. Hatusima; Japan; USNH0010943); 28f. *montana* (Furuse, 1377; Japan; USNH0010945); 28 g. *montana* (Hu & But, 23199; Hong Kong; MO5331159); 28h. *montana* (PI 009227; Japan; CU004); **29a**. *peduncularis* (det. van der Maesen, 1981; China; NY9177); 29b. *peduncularis* (69' det. van der Maesen, 1981; unknown; NY?); 29c. *peduncularis* (68'Henry; China; NY?); **30a**. *phaseoloides* (CIAT-744; Ecuador; IL/CU047); 30b. *phaseoliodes* (PI 308576; Venezuela; IL/CU378); **31**. *pulcherrima* (SUL025; Indonesia; IL/CU433); **32**. *stricta* (CIAT21069; Thailand; IL/CU404); **33**. *subspicata* (Deka, BCS-2200; India; USNH0010940); **34a**. *thunbergiana* (det. Hatusima, 1379; Taiwan; USNH0010944); 34b. *thundbergiana* (Overholt, 1950; China; USNH283291); 34c. *thundbergiana* (Piper; Virginia, USA; USNH30765); 34d. *thundbergiana* (Lyacy; Virginia, USA; USNH30764); 34e. *thundbergiana* (Harrison; Washington, DC, USA; USNH30758); 34f. *thundbergiana* (Wight, 867; Illinois, USA; USNH30755); 34g. *thundbergiana* (Tung & Shan, 2957; China; NY); 34h. *thundbergiana* (Bush, 451; Hawaii, USA; USNH0010918); 34i. *thundbergiana* (Bush, 1266; Hawaii; USNH0010917); 34j. *thundbergiana* (C.V.P.; Florida, USA; USNH296237);

34k. *thundbergiana* (Piper, 1920; Mississippi, USA; USNH296217); 34l. *thundbergiana* (Thompson, 84; Missouri, USA; USNH30767); 34m. *thundbergiana* (Safford, 1919; Washington, DC. USA; USNH30760); 34n. *thundbergiana* (Shmitt, 3671; Virginia, USA; USNH30759); 34o. *thundbergiana* (Dudley, 1963; Case Estates Arboretum, USA; USNH23112); 34p. *thundbergiana* (Jisher, Washington, DC, USA; USNH30763); 34q. *thundbergiana* (Waller, 3225; China; USNH30757); 34r. *thundbergiana* var. *hirsuta* (Overholt, 1950; China; USNH0010939); 34s. *thundbergiana* (none, 1975; unknown; USNH0010914); **35a**. *tuberosa* (Koelz, 8330; India; NY2432); 35b. *tuberosa* (Koelz, 4214; India; NY); 35c. *tuberosa* (Koelz, 8330; India; USNH0010937); **36**. *wallichi* (CIAT21287; Thailand; IL/CU405); *Sinodolichos* **37**. *lagopus* (Maxwell, 90-1120; Thailand; MO4298209); *Teramnus* **38**. *axilliflorus* (Royen, 101; Nigeria; ISU158183); **39**. *buettneri* (Geerling & Bokdam, 280; Bondoukou; MO2419408); **40**. *flexilis* (CSIRO105831; Indonesia; IL/CU451); **41a**. *labialis* (PI 200233; Kenya; IL/CU232); 41b. *labialis* (USDA/PE62315; USDA/Peoria; IL/211); 41c. *labialis* (PI 538317; US Virgin Islands; IL/CU383); **42**. *micans* (TH-0085, PI 213514?; India; IL/CU164); **43**. *repens* (FAO#Mtw-L-63, PI 406171; Kenya; IL/CU220); **44a**. *uncinatus* (PI 213514; unknown; IL/CU163); 44b. *uncinatus* (PI 321388; Australia; IL/CU223); 44c. *uncinatus* (PI 322664; Brazil; IL/CU225); **45**. *volubilis* (CSIRO51385; Venezuela; IL/CU453).

Fig. 1. Portions of cleared leaflets observed between crossed polarizers from selected species showing veins with short (length–width ratio ≤1.5 (S)), long (length–width ratio >1.5 (L)), or a combination of short and long twinned, kinked, and straight crystals (see Ilarslan et al. 1997). (*a*) *Glycine curvata*, primary vein, S. (*b*) *G. curvata*, tertiary vein, L. (*c*) *Pueraria wallichii*, primary vein, S. (*d*) *Glycine pullenii*, tertiary vein, S. (*e*) *G. pindanica*, tertiary vein, L. (*f*) *Pueraria peduncularis*, primary vein, L. (*g*) *P. peduncularis*, secondary vein, S and L. (*h*) *Glycine tabacina*, secondary vein, S and L. (*i*) *G. argyrea*, primary and secondary veins, S and L. Scale bars = 50 μm on Figs. 1*a*, 1*b*, 1*h*, and 1*i*. Scale bars = 20 μm on Figs. 1*c*–1*f*. Scale bar = 10 μm on Fig. 1*g*.



Group Shuteria

We studied seven of the eight (no collections for *Diphyllarum*) genera in this group: *Amphicarpaea* (3/2), *Cologania* (10/10), *Dumasia* (8/4), *Mastersia* (2/2), *Neonotonia* (2/2), *Shuteria* (5/2), and *Teyleria* (1/1) (Table 3). No synonyms were provided, so all species by name were included as identified on the herbarium sheets.

Amphicarpaea **46**. *africana* (Eleanor & Phillips, 2502; Malawi; CORNELL376890); **47a**. *bracteata* (IL-19; Iowa; IL/CU169); 47b. *bracteata (*none; Iowa; IL/CU200); 47c. *bracteata* (none; Illinois; IL/CU183); **48a**. *edgeworthii* (none; Korea; IL/CU210); 48b. *edgeworthii* (PI 339738; Korea; IL/CU241); *Cologania* **49**. *angustifolia* var. *broussonetii* (Fearing, 2026; Mexico; ISU242218); **50a**. *hirta* (Sousa et al., 6094; Mexico; ISU339400); 50b. *hirta* (Pringle, 4793; Mexico; ISU41239); **51**. *humifusa* (Pringle, 8270; New Mexico; ISU41240); **52a**. *intermedia* (Isely, 10938; New Mexico; ISU280232); 52b. *intermedia* (Pringle, 8241; Mexico; ISU41241); **53**. *jaliscana* (Pringle, 4403; Mexico; ISU41242); **54**. *lemmoni* (Blumer; Arizona; ISU169422); **55a**. *longifolia* (Isely, 10840; Arizona; ISU280016); 55b. *longifolia* (Hitchcock et al., 4429; New Mexico; ISU146643); 55c. *longifolia* (Isely, 10914: Arizona; ISU280252); 55d. *longifolia* (Blomer, 1679; Arizona, USA; ISU169588); **56**. *procumbens* (Pohl & Clark, 14018; Honduras; ISU356969); **57a**. *pulchella* (Pringle, 6432; Mexico; ISU41243); 57b. *pulchella* (Pringle, 5516; Mexico; ISU41244); 57c. *pulchella* (Ventura, 2348; Mexico; ISU345796); **58**. *racemosa* (Leavenworth, 602; Mexico; USNH184186); *Dumasia* **59**. *bicolor* (Lammers, 8519; Taiwan; ISU411117); **60**. *miaoliensis* (Huang, Huang & Liu, 16776; Taiwan; MO04913118); **61a**. *truncata* (none; Japan; IL/CU428); 61b. *truncata* (Liu; China; MO046873345); 61c. *truncata* (Tateishi, 470; Japan; USNH0010912); 61d. *truncata* (Hashimoto, Japan; LHBH); **62a**. *villosa* (7706115; Thailand; CANB); 62b. *villosa* (113515; Paupa and New Guinea; CANB); 62c. *villosa* (none; Taiwan; CBG9218548); 62d. *villosa* (Wolff, IA, NU45614; Pakistan; IL/CU293); 62e. *villosa* (Peterlot; Indo-China; NY3269); 62f. *villosa* (none; China; NY232); 62g. *villosa* (Ohashi, 1990; China; NY); 62h. *villosa* (Jablias, 3150; Pakistan; USNH0010914); 62i. *villosa* (Ohashi, 1990; China; USNH0010913); 62j. *villosa* (Lewalle, 964; Burundi/Muramvya/Burgarama; USNH0010911); 62k. *villosa* (Boufford et al., 24834; China; MO4020238); 62l. *villosa* subsp. *bicolor* (Ohashi et al., 20740; Taiwan; USNH0010915); *Mas-*

Figs. 2–17. Portions of cleared leaves observed between crossed polarizers of perennial species of the genus *Glycine* and non-*Glycine* taxa in group Glycine showing location of crystals associated with the leaflet veins and mesophyll. All figures are identified as to collection number in Materials and methods and Tables 1 and 2. Figs. 2–13. *Glycine* taxa. Fig. 2. *Glycine canescens*. Fig. 3. *G. clandestina*. Fig. 4. *G. albicans*. Fig. 5. *G. falcata*. Fig. 6. *G. tomentella*, cytotype-PI 441001. Fig. 7. *G. hirticaulis*. Fig. 8. *G. latifolia*. Fig. 9. *G. stenophita*. Fig. 10. *G. soja*. Fig. 11. *G. max*. Fig. 12. *G. pullenii*. Fig. 13. *G. dolichocarpa*. Figs. 14–17. Associated taxa in the group Glycine. Fig. 14. *Pseudovigna argentea*. Fig. 15. *Teramnus uncinatus*. Fig. 16. *Pseudovigna peduncularis*. Fig. 17. *Pseudeminia comosa*. All scale bars = 400 μm.

tersia **63**. *bakeri* (Vogel, 3816; Indonesia; CANB00471902); **64**. *borneensis* (none; North Borneo; CANB37817); *Neonotonia* **65**. *verdcourtii* (none; Urbana, IL greenhouse; IL/CU465); **66a**. *wightii* (J.M., 2297; India; IL/CU150); 66b. *wightii* (PI 225747; Zambia; IL/CU365); 66c. *wightii* (PI 364859; South Africa; IL/CU449); *Shuteria* **67a**. *hirsuta* (none; unknown; NY9312B); 67b. *hirsuta* (none; China; NY9312D); 67c. *hirsuta* (none; China; NY9312); **68**. *involucrata* (none; North Sumatra, Indonesia; IL/CU346); *Teyleria* **69a**. *koordersii* (Her48453; China; IL/CU042); 69b. *koordersii* (CIAT 18064; Hainan Island, China; IL/CU294).

Results

All of the observed cleared leaflet disks and leaflets of 69 species and two *G. tomentella* cytotypes in both groups, Glycine and Shuteria, displayed vein-associated prismatic crystals (most likely of the Rosanoffian type, Rosanoff 1865; and common for most members of the Papilionoideae, Leguminosae, see Zindler-Frank 1987) of CO monohydrate. Typically these crystals were found one per leaf cell. The crystals were associated with the bundle sheath cells immediately surrounding the veins (main, primary, secondary, and tertiary, and at the vein endings in the areoles).

Mesophyll crystal macropatterns

Fifty-five of the 69 species and two *G. tomentella* cytotypes displayed prismatic crystals in the mesophyll. Conversely, 40.9% of the 22 *Glycine* species did not have mesophyll crystals, and only 8.7% of the 23 associated non-*Glycine* species lacked mesophyll crystals. For all species in group Glycine, 24.4% lacked mesophyll crystals. Of the 24 species in group Shuteria, 20.8% lacked mesophyll crystals (see Tables 1–3).

Vein crystal ratios:

In a given species all of the crystals were S (Stubby crystal ratio), L (Long crystal ratio), or a mixture of S and L (Fig. 1*a*–1*i*; Tables 1–3). These data collectively showed that the percentage of L-ratio crystals increased from the primary to the secondary to the tertiary vein to the vein ending in all three groups (45, 54, 82, 92%, respectively). The 20 perennial (and two *G. tomentella* cytotypes) and two annual *Glycine* species, as one group, showed this condition to a greater extent (54, 67, 88, 100%, respectively) than the 23 species associated with the non-*Glycine* species in group Glycine (48, 48, 78, 91%, respectively), and the 24 species in group Shuteria (33, 46, 75, 83%, respectively) (see Tables 1–3).

Similarly, the perennial and annual *Glycine* species collectively had more L-ratio crystals than S-ratio crystals in all three degrees of veins and vein endings, as compared with the other two groups. In addition, this former group showed more species without mesophyll crystals than species in the other two groups.

Group Glycine

Glycine *species:*

Of the 20 perennial (and two *G. tomentella* cytotypes) *Glycine* species observed (Table 1; Figs. 2–9, 12, 13), leaf crystals in *G. canescens* (Fig. 2), *G. clandestina* (Fig. 3), and *G. argyrea* (ns, not shown) (all A-genome species), *G. albicans* (Fig. 4), *G. lactovirens* (ns), and *G. aphyonota* (ns) (all I-genome species), and *G. falcata* (Fig. 5) (F-genome species) were typically only associated with the veins and vein endings. *Glycine canescens* (Fig. 2) vein crystals were distinct from the other species vein crystals in that they appeared lateral to the veins.

Glycine tomentella (PI 373980) (ns), *G. tomentella* (PI 441001) (Fig. 6), *G. arenaria* (ns), *G. hirticaulis* (Fig. 7), *G. pindanica* (ns), *G. pullenii* (Fig. 12), *G. microphylla* (ns), *G. latifolia* (Fig. 8), *G. tabacina* (ns), and *G. dolichocarpa* (Fig. 13) displayed few crystals in the mesophyll.

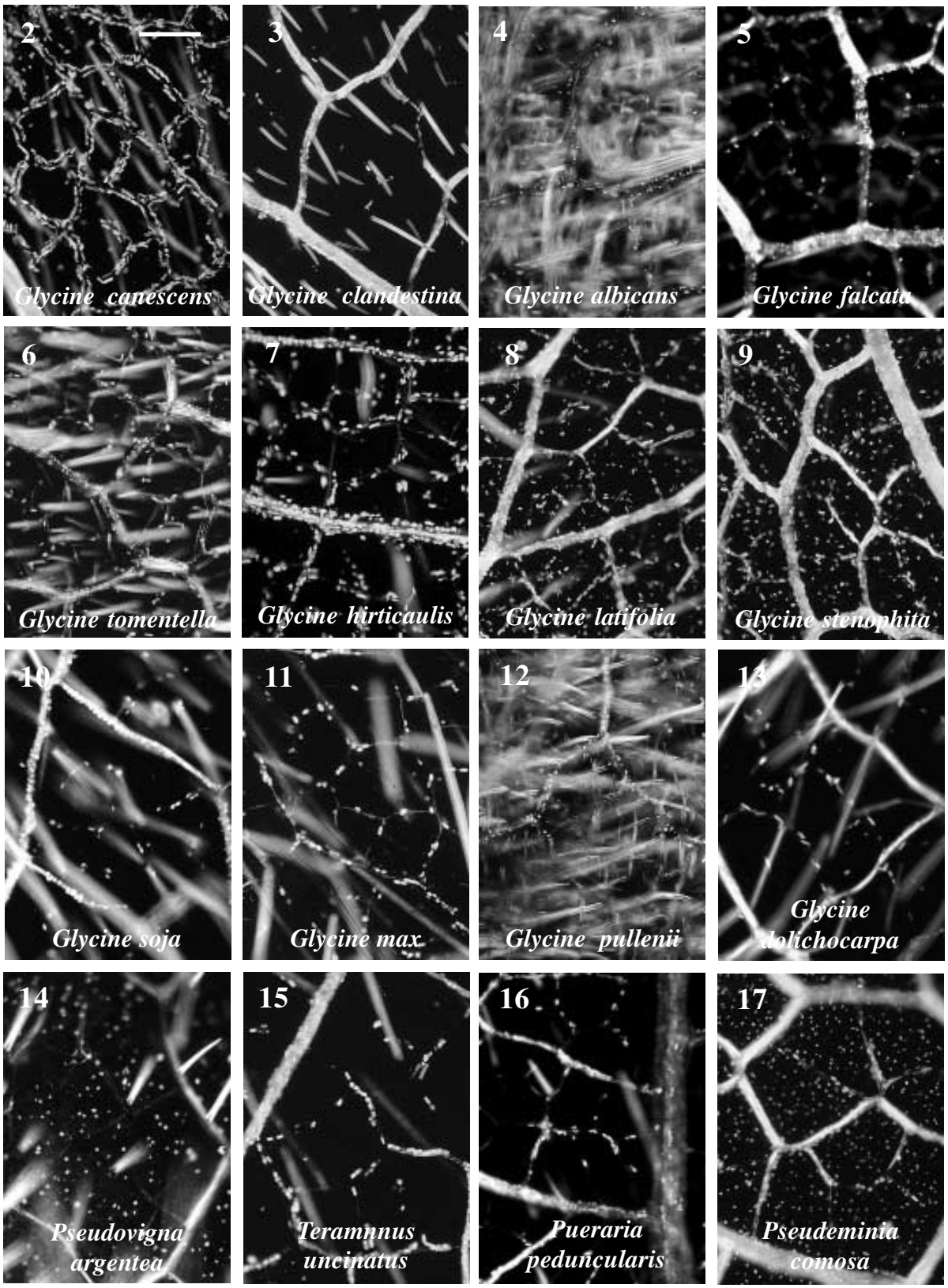
Glycine latrobeana (ns), *G. stenophita* (Fig. 9), *G. cyrtoloba* (ns), and *G. curvata* (ns) all showed many crystals in the mesophyll (Table 1). The latter two C-genome species (*G. cyrtoloba* and *G. curvata*) had very thick veins in contrast to the other *Glycine* species.

Crystals were typically associated with all of the leaf veins in the wild annual *Glycine soja* (Fig. 10) and the cultivated annual *G. max* (Fig. 11). These two G-genome species only rarely displayed crystals in the leaf mesophyll, and were scored as "None" (see Table 1).

*Non-*Glycine *species in group Glycine:*

Of the seven genera (other than *Glycine*) studied in the group Glycine, all of the observed species had crystals associated with the veins and vein endings. *Pseudovigna argentea* (Fig. 14) and *Teramnus repens* (ns) had relatively few vein crystals whereas *T. micans* (ns) and *T. uncinatus* (Fig. 15) had a large number of vein crystals. The relative number of vein crystals in *Sinodolichos lagopus* (ns) was variable, and the vein crystals in *Teramnus buettneri* (ns) were lateral, similar to those observed in *Glycine canescens* (Fig. 2). Almost all species from five of the genera displayed a variable number of crystals in the mesophyll, except for *Pueraria peduncularis* (Fig. 16) and *Sinodolichos lagopus* (ns) that had none.

The variation in numbers of crystals was as follows (Table 2): many, *Pseudeminia comosa* (Fig. 17), *Pueraria alopercuoides* (ns), *P. montana* (ns), *P. phaseoloides* (ns),



P. pulcherrima (ns), *P. subspicata* (ns), *P. thunbergiana* (ns), *P. tuberosa* (ns), *Teramnus flexilis* (ns), *T. labialis* (ns), and *T. micans* (ns); few, *Eminia antennulifera* (Fig. 19), *Pseudovigna argentea* (Fig. 14), *Pueraria lobata* (Fig. 20), *P. wallichii* (Fig. 1*c*), *Teramnus axilliflorus* (Fig. 18), *T. buettneri* (ns), *T. repens* (ns), and *T. uncinatus* (Fig. 15); few to many, *Pueraria thunbergiana* (ns), *Teramnus flexilis* (ns), and *T. labialis* (ns); none, *Pueraria peduncularis* (Fig. 16) and *Sinodolichos lagopus* (ns).

The species in this group, in general, displayed more S-ratio crystals in different veins, particularly those in the genus *Teramnus*.

Figs. 18–33. Portions of cleared leaves observed between crossed polarizers of perennial, annual, and infra (inf.) species of the non-*Glycine* genera in group Glycine and group Shuteria showing location of crystals associated with the leaf veins and mesophyll. All figures are identified as to collection number in the Materials and methods and Tables 2 and 3. Figs. 18–20. Associated taxa in the group Glycine. Fig. 18. *Teramnus axilliflorus*. Fig. 19. *Eminia antennulifera*. Fig. 20. *Pueraria lobata*. Figs. 21–33. Genera and species in group Shuteria. Fig. 21. *Dumasia truncata*. Fig. 22. *Mastersia borneensis*. Fig. 23. *Amphicarpaea africana*. Fig. 24. *Teyleria koordersii*. Fig. 25. *Shuteria hirsuta*. Fig. 26. *Cologania angustifolia* var. *broussonetii*. Fig. 27. *Neonotonia wightii*. Fig. 28. *Cologania hirta*. Fig. 29. *Dumasia bicolor*. Fig. 30. *D. villosa*. Fig. 31. *Cologania procumbens*. Fig. 32. *Amphicarpaea bracteata*. Fig. 33. *Neonotonia verdcourtii*. All scale bars = 400 μm.

Group Shuteria

Species from this group (Table 3) were more variable than group Glycine species with respect to the presence or absence of crystals associated with the veins and mesophyll, and with regard to S- and L-crystal ratios. All species displayed crystals associated with the veins (Table 3). Of the 24 species, five notably lacked mesophyll crystals; *Cologania hirta* (Fig, 28), *C. longifolia* (ns), *C. procumbens* (Fig. 31), *Dumasia bicolor* (Fig. 29), and *D. villosa* (Fig. 30). The remaining 19 species (see Table 3) displayed mesophyll crystals to varying degrees, from rare and small (*Dumasia miaoliensis*, ns), to few to many, to many in groups (*Dumasia truncata*, Fig. 21).

The S- and L-crystal ratios associated with the veins varied more in this group when compared with the species in the Glycine group. Two species, *Mastersia bakerii* (ns) and *Shuteria involucrata* (ns) only had S-crystal ratios.

Discussion

Use of clearing methods to observe the internal anatomy of plant organs, such as the vasculature (xylem and phloem), unusual-shaped cells, trichomes, and inorganic inclusions (i.e., CO crystals), has been helpful in obtaining different kinds of anatomical information that can be used with other characters to aid in defining differences between and among related plant genera and species. A few varied examples are sporangial arrangements in strobili of *Selaginella* (Horner and Arnott 1963), association of xylem and phloem in vein endings in leaf areoles of unrelated dicot taxa (Horner et al. 1994), the different forms of CO druses (spherical aggregates of crystals) in the cacti (Monje and Baran 2002), the size and location of CO druses in leaves in the Tribe Gossypeae and genus *Gossypium* (Shockey et al. 2001), and macropatterns of CO prismatic crystals and druses in leaves of the genus *Prunus* (Lersten and Horner 2000, 2004).

The study of CO crystals in leaves of *Prunus* by Lersten and Horner (2000), along with the molecular data published by Lee and Wen (2001) on the *Prunus* group, indicate that studying the macropattern of CO crystal distribution and shape (in this study crystal L–W ratios) in cleared leaves can provide additional useful information to better support phylogenetic relationships at the levels of related groups of genera and species.

When the crystal data in this study regarding the annual and perennial species of *Glycine* (Table 1) are applied to the phylogenetic tree (Kollipara et al. 1997) and one published by Brown et al. (2002; perennial species), several interesting relationships are evident. The A-genome perennial species *G. canescens*, *G. clandestina* (Pfeil et al. 2001), *G. argyrea* and *G. rubignosa* do not display any mesophyll crystals. However, *G. latrobeana*, which is sister to all other A-genome species, has mesophyll crystals. All five species are endemic to Australia (Hymowitz 2004).

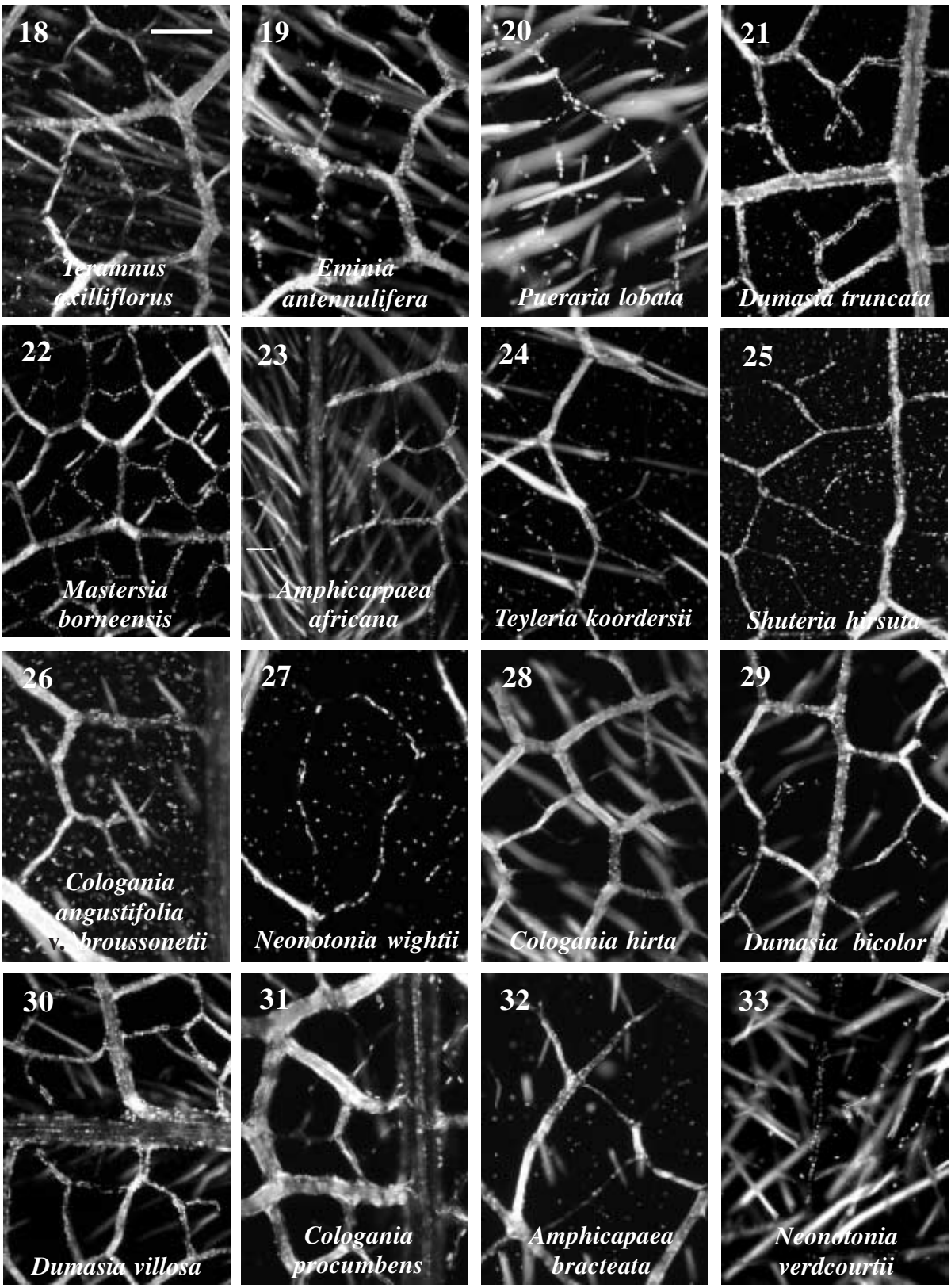
Glycine albicans, *G. lactovirens*, and *G. aphyonota* (Hymowitz 2004), all I-genome perennial species, do not display mesophyll crystals either. The only other perennial species lacking mesophyll crystals is *G. falcata*, which is the only F-genome species listed. The two annual *Glycine* species (G-genome), *max* and *soja*, also do not have mesophyll crystals. Therefore, the genus *Glycine* has the largest percentage of species lacking mesophyll crystals and L-crystal ratios associated with their veins, when compared to the related non-*Glycine* species (Table 2) and the species associated with Shuteria. We believe these results suggest reduced or advanced conditions, particularly for the two annual species.

Glycine tomentella is a widely distributed species geographically. The $2n = 40$ chromosome accessions are D-genome and are restricted to Australia. The $2n = 80$ accessions are allopolyploid of A- and D-genomes (and unknown combinations) from Australia, Papua New Guinea, Indonesia, Philippines, and Taiwan. The $2n = 38$ cytotypes are E-genomes and are endemic to Australia. The $2n = 78$ cytotypes are allopolyploids of A- and E-genomes (and unknown combinations) from Australia and Papua New Guinea. The two *G. tomentella* cytotypes display few mesophyll crystals while the $2n = 40$ accessions display many mesophyll crystals. With only one accession each of the two cytotypes examined, no conclusion can be made regarding this variation. Similarly, variation exists among these three *G. tomentella* types studied for crystal ratios associated with the veins and vein endings, but again sample size is too small to make definitive statements.

There are no published molecular phylogenetic trees at this time that only deal with the relationships of the species in group Shuteria (Table 3). Species of this latter group seem to be more variable in the presence or absence of mesophyll crystals, and the S- and L-crystal ratios associated with the veins, when compared to group Glycine species and especially to the perennial and annual *Glycine* species.

In spite of the lack of combined molecular data for all of the species in this study, the macropatterns described show consistent vein-associated prismatic crystals in all species, and the presence or absence of mesophyll crystals in some closely associated species. The emerging data on leaf crystal macropatterns, in general, suggest that the correlations and differences are useful and may be supportive of closely related species where both systematic and molecular information are available.

As a subgroup, the annual and perennial *Glycine* species have more L-ratio crystals, and fewer mesophyll crystals,



than the other genera and species from group Glycine and group Shuteria. The annual species *G. max* and *G. soja* represent the extreme in these characters. These differences suggest that between both groups, the annual and perennial species of *Glycine* are different from the other taxa in the Glycininae. We expect these differences to become more apparent, as additional molecular studies are carried out on taxa associated with both Glycininae groups.

Acknowledgements

This is a joint contribution of the USDA-ARS-CICGR Unit and the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011, Project 3769. The microscopic work was carried out in the Bessey Microscopy Facility. The authors thank Dr. Nels R. Lersten and Ms. Deborah Lewis for their helpful comments during the preparation of this manuscript, Ms. Jo Palmer of the Australian National Herbarium-CSIRO and Ms. Deborah Lewis, Curator of the Ada Hayden Herbarium, Iowa State University for providing specimens from the ISU Herbarium (ISC), and for obtaining specimens for this study from the Curators of the following herbaria (BH; CEL; CU; CANB; MO; NA). The mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or Iowa State University and does not imply its approval to the exclusion of other products that may be suitable.

References

Brown, A.H.D., Doyle, J.L., Grace, J.P., and Doyle, J.J. 2002. Molecular phylogenetic relationships within and among diploid races of *Glycine tomentella* (Leguminosae). Aust. Syst. Bot. **15**: 37–47. doi: 10.1071/SB01003.

Doyle, J.J., and Doyle, J.L. 1993. Chloroplast DNA phylogeny of the Papilionoid legume tribe Phaseoleae. Syst. Bot. **18**: 309–327.

Franceschi, V.R., and Horner, H.T., Jr. 1980. Calcium oxalate crystals in plants. Bot. Rev. **46**: 361–427.

Franceschi, V.R., and Loewus, F.A. 1995. Oxalate biosynthesis and function in plants and fungi. *In* Calcium oxalate in biological systems. *Edited by* S.R. Khan. CRC Press, Boca Raton, Florida. pp. 113–130.

Horner, H.T., Jr., and Arnott, H.J. 1961. The use of a multiple-compartment tray for processing many specimens at one time. Stain Technol. **36**: 204–205. PMID: 13715897.

Horner, H.T., Jr., and Arnott, H.J. 1963. Sporangial arrangement in North American species of *Selaginella.* Bot. Gaz. **124**: 371–383.

Horner, H.T., and Wagner, B.L. 1995. Calcium oxalate crystal formation in higher plants. *In* Calcium oxalate in biological systems. *Edited by* S.R. Khan. CRC Press, Boca Raton, Florida. pp. 53–72.

Horner, H.T., Lersten, N.R., and Wirth, C.L. 1994. Quantitative survey of sieve tube distribution in foliar terminal veins of ten dicot species. Am. J. Bot. **81**: 1267–1274.

Horner, H.T., Kausch, A.P., and Wagner, B.L. 2000. Ascorbic acid: a precursor of oxalate in crystal idioblasts of *Yucca torreyi* in liquid root culture. Int. J. Plant Sci. **161**: 861–868. doi: 10.1086/31756.

Horner, H.T., Cervantes-Martinez, T., Healy, R., Reddy, M.B., Deardorff, B.L., Bailey, T.B., Al-Wahsh, I., Massey, L.K., and Palmer, R.G. 2005. Oxalate and phytate concentrations in seeds of soybean cultivars (*Glycine max* (L.) Merr.). J. Agric. Food Chem. **53**: 7870–7877. doi: 10.1021/jf051193j.

Hufford, L. 1997. A phylogenetic analysis of Hydrangeaceae based on morphological data. Int. J. Plant Sci. **158**: 652–672. doi: 10.1086/297478.

Hymowitz, T. 2004. Speciation and cytogenetics. *In* Soybeans: improvement, production, and uses. *Co-Edited by* H.R. Boerma and J.E. Specht. No. 16 in the series AGRONOMY, ASA/CSSA/SSSA Societies of America, Inc., Madison, Wisconsin. pp. 97–136.

Ilarslan, H., Palmer, R.G., Imsande, J.M., and Horner, H.T. 1997. Quantitative determination of calcium oxalate in developing seeds of soybean. Am. J. Bot. **84**: 1042–1046.

Ilarslan, H., Palmer, R.G., and Horner, H.T. 2001. Calcium oxalate crystal idioblasts in developing seeds of soybean. Ann. Bot. (Lond.), **88**: 243–257.

Kausch, A.P., and Horner, H.T. 1982. A comparison of calcium oxalate crystals isolated from callus cultures and their explant sources. Scan. Electron Microsc. **I**: 199–211.

Keates, S.E., Tarlyn, N.M., Loewus, F.A., and Franceschi, V.R. 2000. L-ascorbic acid and L-galactose are sources for oxalic acid and calcium oxalate in *Pistia stratiotes.* Phytochemistry, **53**: 433–440. doi: 10.1016/S0031-9422(99)00448-3. PMID: 10731019.

Kollipara, K.P., Singh, R.J., and Hymowitz, T. 1997. Phylogenetic and genomic relationships in the genus *Glycine* Willd. based on sequences from the ITS region of the nuclear rDNA. Genome, **40**: 57–68. PMID: 9061914.

Kostman, T.A., Tarlyn, N.M., Loewus, F.A., and Franceschi, V.R. 2001. Biosynthesis of L-ascorbic acid and conversion of carbons 1 and 2 of L-ascorbic acid to oxalic acid occurs within individual calcium oxalate crystal idioblasts. Plant Physiol. **125**: 634–640. doi: 10.1104/pp.125.2.634. PMID: 11161021.

Lackey, R.A. 1977. A synopsis of Phaseoleae (Leguminosae, Papilionoideae). Ph.D. Dissertation. Iowa State University, Ames. pp. 293.

Lackey, R.A. 1981. Tribe 19. Phaseoleae DC. (1825). *In* Advances in legume systematics. *Edited by* R.M. Polhill and P.H. Raven. Royal Botanic Gardens, Kew, UK. pp. 301–327.

Lee, J., and Hymowitz, T. 2001. A molecular phylogenetic study of the subtribe Glycininae (Leguminosae) derived from the chloroplast DNA *rps*16 intron sequences. Am. J. Bot. **88**: 2064–2073.

Lee, S., and Wen, J. 2001. A phylogenetic analysis of *Prunus* and the Amygdaloideae (Rosaceae) using ITS sequences of nuclear ribosomal DNA. Am. J. Bot. **88**: 150–160. PMID: 11159135.

Legume Web. 2005. ILDIS World Database of Legumes, version 10, November 2005. International Legume Database & Information Service (ILDIS). Available from http://www.ildis.org/.

Lersten, N.R., and Horner, H.T. 2000. Calcium oxalate crystal types and trends in their distribution patterns in leaves of *Prunus* (Rosaceae: Prunoideae). Plant Syst. Evol. **224**: 83–96. doi: 10.1007/BF00985267.

Lersten, N.R., and Horner, H.T. 2004. Calcium oxalate crystal macropattern development during *Prunus virginiana* (Rosaceae) leaf growth. Can. J. Bot. **82**: 1800–1808. doi: 10.1139/b04-145.

Lin, J., and Hu, Y. 1998. Taxonomic significance of extracellular crystals on the phloem fibers of Taxaceae. Flora, **193**: 173–178.

Loewus, F.A. 1999. Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry, **52**: 193–210. doi: 10.1016/S0031-9422(99)00145-4.

Massey, L.K., Horner, H.T., and Palmer, R.G. 2001. Oxalate content of soybean seeds (*Glycine max*: Leguminosae) and other edible legumes. J. Agric. Food Chem. **49**: 4262–4266. doi: 10.1021/jf010484y. PMID: 11559120.

Massey, L.K., Grentz, L.M., Horner, H.T., and Palmer, R.G. 2002. Soybean and soyfood consumption increases oxalate excretion. Topics Clin. Nutr., **17**: 49–59.

Metcalfe, C.R. 1983. Secreted mineral substances: crystals. *In* Anatomy of dicotyledons, 2nd ed., Vol. II. *Edited by* C.R. Metcalfe and L. Chalk. Clarendon Press, Oxford, UK. pp. 82–92.

Monje, P.V., and Baran, E.J. 2002. Characterization of calcium oxalates generated as biominerals in cacti. Plant Physiol. **128**: 707–713. doi: 10.1104/pp.128.2.707. PMID: 11842173.

Pfeil, B.E., and Craven, L.A. 2002. New taxa in *Glycine* (Fabaceae: Phaseolae) from north-western Australia. Aust. Syst. Bot. **15**: 565–573. doi: 10.1071/SB01004.

Pfeil, B.E., Tindale, M.D., and Craven, L.A. 2001. A review of the *Glycine clandestina* species complex (Fabaceae, Phaseolae) reveals two new species. Aust. Syst. Bot. **14**: 891–900. doi: 10.1071/SB00041.

Prychid, C.J., and Rudall, P.J. 1999. Calcium oxalate crystals in Monocotyledons: a review of their structure and systematics. Ann. Bot. (Lond.), **84**: 725–739.

Rosanoff, S. 1865. Ueber die Kristalldrusen in Marke von *Kerria japonica* D. und *Ricinis communis*. Bot. Zeit. **23**: 329–330.

Shockey, K., Horner, H.T., and Wendel, J.F. 2001. Calcium oxalate crystals in the tribe Gossypeae and the genus *Gossypium* (cotton). Botanical Society of America Meeting, Albuquerque, NM (Abstract, August 14).

Solereder, H. 1908. Systematic anatomy of the dicotyledons. L.A. Boodle and F.E. Fritsch, English translation, Vols. 1 and 2. Clarendon Press, Oxford, UK.

Tateishi, Y., and Ohashi, H. 1992. Taxonomic studies on *Glycine* in Taiwan. J. Japan. Bot. **67**: 127–147.

Zindler-Frank, E. 1987. Calcium oxalate crystals in legumes. *In* Advances in legume systematics. Part 3. *Edited by* C.H. Stirton. Royal Botanic Gardens, Kew, UK. pp. 279–316.