

FLAVONOID DISTRIBUTION IN GLEDITSIA

by Frank S. Santamour, Jr.

Abstract

The major flavonoids of the leaves of *Gleditsia* species are flavone C-glycosides, with sufficient interspecific differences in distribution to allow for the identification of certain species. *G. triacanthos* was readily distinguished from all other species by high concentrations of leucocyanidin and two catechins. The involvement of *G. triacanthos* in the parentage of *G. x texana* and the verification of an artificial hybrid of *G. triacanthos* were determined by chromatographic analyses and identification of the compounds peculiar to *G. triacanthos*.

Species of the genus *Gleditsia* are not widely cultivated in American arboreta or botanic gardens. On the other hand, thousands of thornless specimens of our native honeylocust (*G. triacanthos* L.) are planted every year in urban and suburban landscapes. These thornless trees may be grown from seed or produced by vegetative propagation from thornless trees or thornless portions of thorny trees. All such plants must be considered as *G. triacanthos* f. *inermis* Schneid¹. Most of the thornless cultivars presently in the nursery trade have also been selected for fruitlessness, producing few if any functional female flowers.

The increase in popularity of the new cultivars of honeylocust over the past decade has been paralleled by an increase in the range of its most damaging insect pest, the mimosa webworm (*Homadaula anisocentra* Meyr.). Therefore, at the U.S. National Arboretum, a research project on *Gleditsia* was started in 1973 with the long-range goal of selecting or developing improved cultivars with a high degree of resistance to mimosa webworm. As a part of this project, biochemical studies were undertaken to determine the value of biochemical data in (1) identification or verification of species and hybrids, (2) determination of the nature of insect resistance.

The most recent monograph on *Gleditsia* (Gordon, 1966) recognizes 13 species and one natural hybrid, and the nomenclature of this work is followed here. Some of these species cannot be expected to be hardy in temperate zones of the United States, and many species are rare in cultivation. However, we have tried to collect and

sample living material of all cultivated species, including a new species recently described from China.

Previous reports on *Gleditsia* leaf flavonoids have been rather limited. Bate-Smith (1962) did not detect the flavonols quercetin or kaempferol in acid-hydrolyzed leaf extracts of *G. caspica* Desf. However, he did report the presence of caffeic acid and a weak concentration of cyanidin (from leucocyanidin). Bate-Smith and Lerner (1954) found that *G. triacanthos* was strongly positive for cyanidin but that *G. sinensis* Lam. gave a negative reaction.

Materials and Methods

Sexually mature trees sampled in this survey were *G. triacanthos*, including the thornless cultivars 'Imperial' and 'Moraine' at the National Arboretum; *G. japonica* Miq. and *G. x texana* Sarg. from the Arnold Arboretum, Jamaica Plain, Massachusetts; *G. aquatica* Marsh. at the Morris Arboretum, Philadelphia, Pennsylvania; and a specimen identified as *G. melanacantha* Tang & Wang found growing at the USDA Plant Introduction Station in Glenn Dale, Maryland. In addition, a large, nonflowering specimen of *G. japonica* at the National Arboretum was tested.

The remainder of the species, as well as all those mentioned above, were represented by one- or two-year-old seedlings. Seed was obtained from arboreta and botanic gardens throughout the world, either from their regular seed lists or through personal communication. Table 1 gives the primary sources. A total of 47 seedlots were tested, and in most cases we obtained seed of a given species from two or more sources. Sources other than those in the table included gardens in France, Germany, Portugal, and Tunisia.

Seed samples of all accessions were weighed, measured, color-coded, and stored dry in sealed containers in a refrigerator. Before sowing, the seed were soaked in concentrated sulfuric acid

¹ Botanical authority according to Isely (1975).

for one hour. Data on germination and seedling growth characteristics were recorded.

Seedling leaves for biochemical analyses were collected after the plants were three months old in the first growing season or during the second growing season. Leaf samples for flavonoid analyses, up to 30 grams fresh weight from mature trees and 5 to 10 grams for seedlings, were extracted in boiling 70% ethanol for one hour. Similar leaf samples were hydrolyzed in boiling 18% hydrochloric acid for 40 minutes to determine leucoanthocyanin content.

The ethanolic extracts were concentrated to about 1 ml extract for each gram of fresh leaf tissue. The extracts were allowed to cool and were cleared by centrifugation and stored in a refrigerator. For preliminary analyses, the extracts were spotted on Whatman No. 3MM filter paper (20 x 20 cm) and subjected to two-dimensional ascending chromatography in BAW (n-butanol:glacial acetic acid:water, 4:1:5, upper phase) and distilled water. These chromatograms, after being fumed with ammonia and viewed under ultraviolet light, provided tentative information on the number and behavior of compounds likely to be found in the extract. The various substances were purified by repeated banding and elution of one-dimensional runs in BAW, water, and 15% acetic acid, in whatever sequence was most appropriate for complete separation from other pigments.

Purified substances were then tested against known compounds by comparison and co-chromatography in all three solvents and occasionally in TBA (tertiary butanol-glacial acetic acid-water, 3:1:1). Pure samples of vitexin, isovitexin, and iso-orientin were supplied by Dr. S. Asen, U.S. Department of Agriculture. Commercially available material of d-catechin, epicatechin, chlorogenic acid, caffeic acid, and several other compounds were also used in this manner.

Detection and characterization of spots was sometimes aided by such chromogenic sprays as diazotized p-nitroaniline and tetrazotized benzidine.

The R_f values of purified C-glycoflavones were not altered by acid hydrolysis, and no sugars

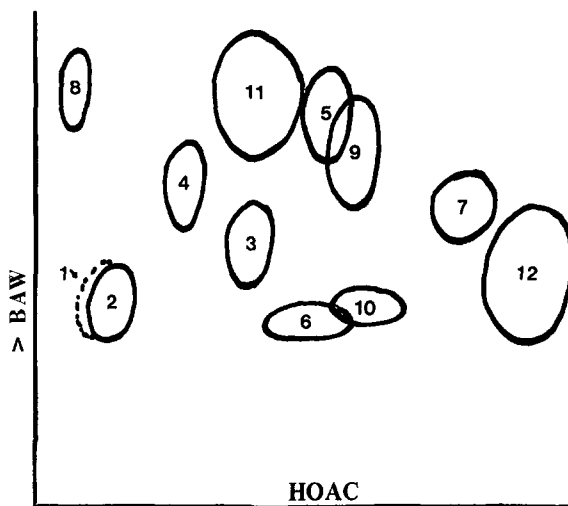


Fig. 1. Composite two-dimensional chromatogram of *Gleditsia* leaf flavonoids and related substances. Numbered substances are discussed in the text and their distribution is given in Table 2.

were detected following such treatment. C-glycoflavones have been reported as unusually resistant to acid hydrolysis.

In addition to R_f values and co-chromatography, great reliance was placed on the ultraviolet absorption spectra in 95% ethanol (compared with data of Harborne, 1967) or methanol (compared with data of Mabry *et al*, 1970). Spectral shifts with a variety of additives (sodium acetate, boric acid, aluminum chloride) also aided in identification.

The presence of leucoanthocyanins in acid-hydrolyzed leaf samples could sometimes be judged by the red coloration of the extract, especially when leucoanthocyanin content was high. The raw extract was first extracted with isoamyl alcohol, and this layer was washed twice with 1% HCL. The addition of a five-fold volume of benzene formed a one-phase system. After the addition of more 1% HCL the red pigment appeared in the lower aqueous layer. The purified

Table 1. Primary seed sources of *Gleditsia* species used in biochemical analyses.

Species	Arboretum	Country
<i>amorphoides</i> (Gris.) Taub.	Buenos Aires	Argentina
<i>aquatica</i> Marsh.	Morris Arboretum	Philadelphia, Pa. USA
<i>casatica</i> Desf.	Taschkent	USSR
<i>delavayi</i> Franch.	Taschkent	USSR
<i>fera</i> (Lour.) Merr.	Agric. & Fisheries Dept.	Hong Kong
<i>japonica</i> Miq.	Aritaki Arboretum	Japan
<i>japonica</i> var. <i>koraiensis</i> Nakai	Forest Expt. Sta.	Korea
<i>macracantha</i> Desf.	Univ. Athens	Greece
<i>melanacantha</i> Tang	U.S. Plant Introd. Sta.	Glenn Dale, Md. USA
<i>microphylla</i> Gord. (<i>heretophylla</i> Bunge)	Taschkent	USSR
<i>sinensis</i> Lam.	U.S. Plant Introd. Sta.	Chico, Calif. USA
<i>x texana</i> Sarg.	Taschkent	USSR
<i>triacanthos</i> L.	various	US and Foreign

pigment was recovered from this layer with isoamyl alcohol, and the anthocyanidins were identified by paper chromatography in Forestal solvent (acetic acid:HCl:water, 30:3:10) and by their absorption spectra.

Results and Discussion

Figure 1 shows composite chromatogram of compounds found in the various *Gleditsia* species. Spots other than flavonoids are included for the sake of completeness and for the use of future investigations. Table 2 shows the *Gleditsia* species we have studied, with notes on the presence or absence of the various substances.

The characteristics and identification of the various substances are discussed below. Some substances (#3, 4, 5, 9, 11 and 12) were compared with authentic materials, and such identifications were highly probable. Little additional data are given on them, because reference works contain full details. For other substances, some pertinent information is given.

Cyanidin (CY) is not pictured on the chromatogram because of the different solvent system used in its identification. It is assumed that this compound resulted largely from the hydrolysis of condensed tannins in the leaves.

The following substances were identified:

Substance #1. A substance that fluoresces yellow and has similar R_f values to orientin. Not studied further.

Substance #2. Probably orientin (luteolin 8-C-glucoside). Formerly called lutexin.

Substance #3. Iso-orientin (luteolin 6-C-glucoside). Formerly called lutanaretin.

Substance #4. Vitexin (apigenin 8-C-glucoside).

Substance #5. Isovitexin (apigenin 6-C-glucoside). Formerly called saponaretin.

Substance #6. Probably a vitexin derivative, and possibly a C-diglycoside. R_f values of 0.32 (BAW) and 0.48 (HOAC) are inconclusive, because Harborne (1967) and Mabry *et al* (1970) present very different values.

Substance #7. Probably rhamnosylvitexin (apigenin 8-C rhamnoglucoside). R_f values of 0.52 (BAW) and 0.74 (HOAC). UV spectral shifts similar to those reported by Mabry *et al*. (1970).

Substance #8. Possibly luteolin as a free flavone. Not studied further.

Substance #9. d-Catechin. R_f values 0.60 (BAW), 0.54 (HOAC), 0.33 (H₂O). Identity confirmed by co-chromatography with pure compounds. UV absorption maximum at 278 nm.

Substance #10. Another catechin-type compound. Does not appear to be any of the common tea components. R_f values 0.35 (BAW), 0.58 (HOAC), 0.48 (H₂O).

Substance #11. Caffeic acid, probably *trans* isomer. R_f values 0.70 (BAW), 0.37 (HOAC), Blue fluorescent in UV.

Substance #12. Chlorogenic acid. Gives green-yellow fluorescence under UV after fuming with ammonia vapor. R_f values 0.41 (BAW), 0.86 (HOAC). Co-chromatographed with commercial chlorogenic acid.

The major flavonoids of the genus *Gleditsia*, as in some other legumes, are glycoflavones, in which the sugar is attached by a carbon-carbon bond to the flavone nucleus. Species differences were not marked and no significant species-specific glycoflavone compounds were found, although thorough analyses and comparisons were used to aid in species identification on material of doubtful origin.

However, from a practical viewpoint, other flavonoids, such as catechins and leucoanthocyanidin, were used in distinguishing *G.*

Table 2. Flavonoids and related compounds found in *Gleditsia* leaves.

Species	Substance ¹												
	CY	1	2	3	4	5	6	7	8	9	10	11	12
<i>amorphoides</i>	0	+	0	0	0	+	+	+	0	0	0	0	+
<i>aquatica</i>	0	+	+	0	+	+	+	0	+	0	0	0	+
<i>Caspica</i>	0	+	+	0	+	+	+	±	+	0	0	0	+
<i>delavayi</i>	0	+	+	0	0	+	+	0	±	0	0	0	+
<i>fera</i>	0	+	+	0	+	+	+	0	+	0	0	0	+
<i>japonica</i> ²	±	+	+	0	+	+	+	0	+	0	0	+	+
<i>koraiensis</i> ²	±	+	+	0	+	+	+	0	+	0	0	+	+
<i>macracantha</i>	0	+	+	0	+	+	0	+	0	0	0	0	+
<i>melanacantha</i>	+	+	+	0	+	+	0	0	+	0	0	+	±
<i>microphylla</i>	0	+	+	0	+	+	+	±	+	0	0	0	+
<i>sinensis</i>	0	+	+	0	+	+	+	+	+	0	0	0	+
<i>x texana</i>	+	+	+	+	+	+	±	0	±	0	+	+	+
<i>triacanthos</i>	+	+	+	+	0	+	0	0	±	+	+	0	+

¹CY is cyanidin and numbers in boxhead refer to substances illustrated in Fig. 1 and described in the text. Entries indicate presence (+), trace (±), or absence (0).

²*G. japonica* var. *koraiensis*

triacanthos from all other species studied. Flavonoid tannins are polymeric condensation products of catechins (flavan-3-ols) and leucoanthocyanins (flavan-3:4-diols) and they are usually accompanied in the plant by their monomeric precursors. Thus, although the monomeric catechins could be easily determined in the alcoholic leaf extracts, the leucocyanidin could best be identified after tannin cleavage by acid hydrolysis.

G. x texana has long been considered a natural hybrid between *G. aquatica* and *G. triacanthos*. The chemical evidence from mature trees and open-pollinated seedlings of *G. x texana* indicated that *G. triacanthos* was definitely involved in the parentage of that species.

When leaf tissue from *G. triacanthos* was hydrolyzed, the solution turned red after about 10 minutes of boiling. The degree of "redness" was less for mature trees of *G. x texana* and still less for seedlings derived from *G. texana*. Although no precise quantitative data were taken, the crude extracts and the purified material clearly showed that the amount of leucocyanidin diminished with the reduction in the contribution of *G. triacanthos* germplasm.

No reddish color was apparent in the acid-hydrolyzed extract of *G. japonica*, and leucocyanidin could only be determined after purification.

Leaves of the Chinese *G. melanacantha* were similar to *G. triacanthos* in giving a marked red coloration to the solvent during acid hydrolysis. After purification, leucocyanidin was identified chromatographically. In addition, what appeared to be two or three other leucoanthocyanidins were detected, although all had absorption peaks similar to cyanidin. No catechin compounds were found in *G. melanacantha*, and it was possible that the leucoanthocyanins in this species were derived from monomeric flavan-3:4 diols.

A few young hybrid seedlings of *G. melanacantha* x *G. triacanthos* derived from controlled pollination were also studied. True hybridity of these plants was verified by the presence of the catechin compounds in the alcoholic extract.

It may be noteworthy that we also found these catechin compounds in the leaves of *Albizia julibrissin* Durazz. and *A. kalkora* Prain, two other hosts of the mimosa webworm. Apparently the high tannin content in the leaves of both honeylocust and "mimosa" is no deterrent to feeding by this insect pest.

Conclusions

In any breeding program to develop superior and insect-resistant cultivars of honeylocust, our native species *G. triacanthos* should be the key species. The chemical characteristics peculiar to *G. triacanthos* should allow rapid

chromatographic verification of interspecific hybrids involving *G. triacanthos* as the male parent. The fact that most selected thornless cultivars of *G. triacanthos* are also predominantly male-flowering virtually eliminates the possibility of reciprocal crosses and suggests that this species will be more often used as a male. In view of the marked morphological similarity among many *Gleditsia* species, chemical criteria for judging hybridity will be critical factors in progeny evaluation.

Literature Cited

- Bate-Smith, E.C. 1962. *The phenolic constituents of plants and their taxonomic significance*. Jour. Linn. Soc. (Bot.) 58:95-173.
- Bate-Smith, E.C. and N.H. Lerner. 1954. *Leuco-anthocyanins 2. Systematic distribution of leuco-anthocyanins in leaves*. Biochem. J. 58: 126-132.
- Gordon, Donald. 1966. *A revision of the genus Gleditsia Leguminosae*. Ph.D. Thesis, Indiana Univ., 115pp.
- Harborne, J.B. 1967. *Comparative Biochemistry of the Flavonoids*. Academic Press. 383pp.
- Isely, Duane. 1975. *Leguminosae of the United States: II. Subfamily Caesalpinioideae*. Mem. N.Y. Bot. Gard. 25(2): 1-228.
- Mabry, T.J., K.R. Markham, and M.B. Thomas. 1970. *The Systematic Identification of Flavonoids*. Springer-Verlag, 354pp.

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CREW EVALUATION BY THE CONTRACTOR¹

by Howard L. Eckel

Evaluation methods of a crew's performance by a contractor must, by necessity of profits and growth, take a somewhat different approach than the evaluation methods used by the customer.

The contractor and the customer have a mutual goal—production. However, the necessity of profits and growth causes the contractor to use a different evaluation procedure.

The contractor has four subject areas that he must consider and include in his crew evaluation procedure. The contractor must consider: 1) personnel, 2) equipment, 3) cost, and 4) production.

The contractor usually uses two basic techniques in evaluating these four subject areas. The two basic techniques are: 1) records and/or statistics, and 2) actual on-site inspection.

In the record and/or statistical evaluation area, the contractor should keep records and statistics of the individual crew's performance on eight topics: accident control, damage claims, complaints, absenteeism, equipment costs, personnel development, expense accounts, and labor turnover.

These eight topics are extremely important in the contractor's evaluation of his crews. These

areas have a definite influence on profits, growth and production.

If we examine these eight topic areas briefly, it is obvious that they must be included in crew evaluation by the contractor:

Accidents: The frequency and the cost of accidents and the type of accidents are recorded as incurred by individual crews. This, then, allows the contractor to determine if there is a particular accident trend or repetitive situation either in the personnel or automotive area. Once this is outlined, corrective action by re-training can be initiated. As an example, we once had a crew that had a continual history of poison ivy infection. The supervisor, once alerted to the numerical repetitiveness of this accident type, displayed a picture of poison ivy to the crew members and found out that no one knew what poison ivy looked like. The solution, of course, was an instructional program, training them to spot poison ivy.

Property damage claims and complaints: Again, statistical records should be kept, by crew, of the type and cost incurred. These individual crew statistics can then be compared to corporate averages or service line averages which will

1. Presented at the annual convention of The International Society of Arboriculture in St. Louis, Missouri in August 1976