Am. Midl. Nat. 137:266–273

Growth and Biomineralization of Celtis occidentalis (Ulmaceae) Pericarps

MARLINA R. COWAN, MARK L. GABEL[1]
Biology Department, Black Hills State University, Spearfish, South Dakota 57799

A. HOPE JAHREN
Department of Soil Science, University of California, Berkeley 94720

AND

LARRY L. TIESZEN
Department of Biology, Augustana College, Sioux Falls, South Dakota 57197

ABSTRACT.—A study of the growth and biomineralization of extant pericarps of *Celtis occidentalis* was conducted to clarify the chemical nature of the abundant fossil endocarps of *Celtis*, and to track changes in elemental concentrations with time in a drupe with a highly mineralized endocarp. Fruits were collected at 7–10 days intervals through 1 growing season (155 days). A comparison of size and weight changes showed cyclic growth patterns comprising three distinct periods. Pericarp differentiation into three discrete layers was observable after 46 days of fruit growth. Simultaneous formation of columnar cells of the mesocarp and generation of a silica framework accompanied calcium carbonate deposition within the endocarp. Energy dispersive x-ray spectrometry showed an increasing accumulation of calcium within the endocarp from 206 to 904 counts per sec (cps), while silicon increased from 4 to 133 cps. X-ray diffraction analysis showed aragonite and opal within mature endocarps. Increasingly positive stable carbon isotope ratios ($\delta^{13}C$) from −26.6‰ to −21.0‰ were obtained from the endocarp while those for the exo/mesocarp became slightly more negative as the growing season progressed. This is the first report of the growth and mineralization of drupaceous fruits which accumulate high concentrations of silica and calcium carbonate. Furthermore, the dramatic changes in mineralization, microstructure and stable carbon isotope ratios during maturation must be considered in understanding modern plants and interpreting the fossil record.

INTRODUCTION

Celtis L., often known as hackberry, includes approximately 70 species worldwide and about five in North America (Preston, 1989). In the United States, the genus extends from the East Coast to the Rocky Mountains and in scattered areas of the far West. Although *Celtis* fruits (Fig. 1) have been the subject of several past studies (Yanovsky *et al.*, 1932; Fulbright *et al.*, 1986), observations of their growth and mineralization are lacking. Among drupaceous fruits cultivated commercially, including peaches, apricots, plums and cherries, researchers have discovered characteristic patterns of development. Lilleland (1930, 1932) reported cyclic growth patterns in apricots (*Prunus armeniaca* L.) and peaches (*P. persica* Batsch) which he described as "rapid growth," "depressed growth" and "final swell." Lott (1933) indicated a similar pattern in peach (*P. persica* Batsch) drupes. Tukey (1934) reported a three-stage sequential development in *P. cerasus* L. (sour cherry). Growth of at least one variety of plum (*P. domestica* L.) has only slight cyclic growth (Lilleland, 1934).

Simkiss and Wilbur (1989) defined biomineralization as the conversion by organisms of

[1] Author to whom correspondence should be addressed

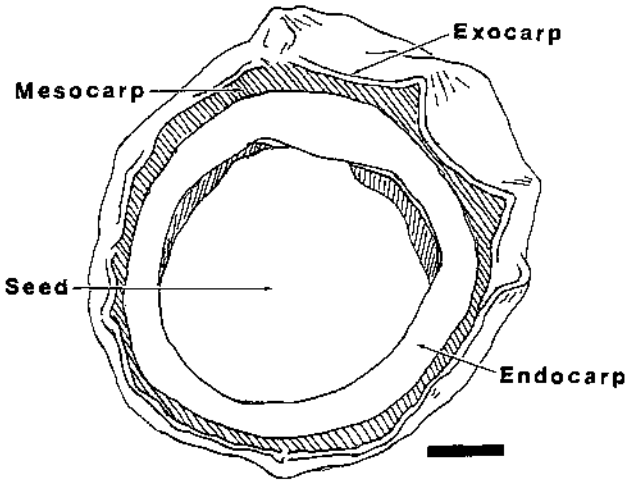

FIG. 1.—Cross section of an air-dried fruit of *Celtis occidentalis*. Bar = 1 mm

ions in solution into solid minerals. In 1982, Arnott outlined three systems of biomineralization in plants, with products including calcium oxalate, carbonates and silicon.

Cystoliths and calcified or silicified cells have been widely reported. Solereder (1908) noted the presence of silica and carbonates in the wood and leaves of many genera within the Urticales (his Urticaceae). Satake (1931) and Pireyre (1961) found cystoliths in leaves of *Celtis*. Werner (1931) reported the presence of "Nebencystolithen" (secondary cystoliths) in leaves of *Celtis occidentalis* L. Metcalfe and Chalk (1950) described hairs, as well as epidermal cell walls, of *Celtis* leaves as calcified or silicified, and noted the presence of cystoliths in the epidermis and silicification in the cortex of the young stems of *Celtis* and *Ulmus*. In an anatomical study of the Ulmaceae, Schweitzer (1971), found cystoliths composed of both calcium carbonate and silica and that trichomes were often silicified. Setoguchi *et al.* (1986) discovered calcium, silicon and magnesium in cystoliths in the leaves of *Celtis sinensis*. Okazaki *et al.* (1991) described similar results on the same species. Yanovsky *et al.* (1932) reported the presence of silica and carbonates in fruits of extant *Celtis*, but there are no reports of the chronology or levels of deposition in the fruit walls.

Celtis is extensively represented in the fossil record of North America (Chaney, 1925; Berry, 1928; Segal, 1966; Thomasson, 1979) and Europe (Nagalhard, 1922). In a detailed study, Kordos-Szakály and Kordos (1985) reported six morphotypes of fossil *Celtis* endocarps from Europe.

Analysis of carbon isotope ratios ($^{13}C/^{12}C$) is an important tool in the study of plants and terrestrial ecosystems (O'Leary, 1981; Tieszen, 1991; Tieszen and Boutton, 1989). It is known that several factors including ambient carbon dioxide concentrations, water stress, nutrient contents and temperature, as well as genetically determined photosynthetic pathway types, influence these ratios (Tieszen, 1991). Carbon isotope ratios of fossils have also been studied (Nambudiri *et al.*, 1978). *Celtis* endocarps, both fossil and extant have been the subject of recent investigations (Haffner *et al.*, 1990; Backlund *et al.*, 1991, 1992).

The purposes of this paper are (1) to compare the growth of *Celtis* to other drupaceous fruits; (2) to investigate the biomineralization of modern *Celtis* fruits and (3) to determine if stable carbon isotope ratios are consistant over the growth of the fruit. We initiated this

study as a preliminary investigation of the minerals and especially organic carbon in fossil *Celtis* fruits to facilitate the understanding of paleoenvironments.

MATERIALS AND METHODS

One hundred *Celtis occidentalis* flowers or fruits were collected from cultivated trees in Spearfish, S.D., every 7 to 10 days, over a period of 155 days, beginning with the opening of flower buds until the fruit reached maturity. A similar number were collected in a comparable manner from Austin, Minn. Twenty percent of the specimens were killed and fixed in FAA, and others were air-dried. Ten air-dried fruits per sampling were weighed and length and width were measured using a dissecting microscope with an ocular micrometer. Due to the importance and abundance of *Celtis* endocarps in the fossil record, that layer was isolated from other pericarp layers and weighed.

Two air-dried fruits from each sampling date were sectioned, mounted onto carbon stubs and carbon-coated. Three areas in the middle of each endocarp wall were examined using energy dispersive x-ray (EDX) analysis (Goldstein *et al.*, 1981) with area analysis at 10kX magnification and at 15kV. Element compositional changes were detected and proportional comparisons were attained by average intensity values acquired as counts per second (cps) over a 200-sec sampling period. Other air-dried endocarps from three collection dates were subjected to x-ray diffraction (XRD). To observe structural changes, FAA preserved samples, whole and cross-sectioned, were prepared for scanning electron microscopy by an ethanol dehydration series and by critical point drying, mounting onto aluminum stubs, and sputter-coating (Goldstein *et al.*, 1981) with 60%Au/40%Pd.

To determine stable carbon isotope ratios, exocarps and mesocarps were separated as a unit from endocarps of 10 fruits from each sampling date. Endocarps were opened and seeds removed. The pooled exocarp and mesocarp layers (exo/mesocarp), and endocarps were pulverized separately. Carbonate was removed with treatment by 1 M HCl under vacuum for 2 h. Samples were rinsed twice in distilled water, centrifuged, dried at 60 C for 24 h, weighted and loaded into a Carlo Erba elemental analyzer. Carbon and nitrogen levels were monitored to determine the relative levels of organic compounds within layers of the fruit walls. After combustion, the gas was separated and introduced to a VG Isogas isotope ratio mass spectrometer (IRMS). Delta ^{13}C values were calculated using the formula:

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{standard}}{^{13}C/^{12}C_{standard}} \times 1000.$$

RESULTS

Celtis occidentalis trees usually flower in the spring, with young flowers bearing two stigmas, six basifixed anthers, and abundant basal trichomes. Ovaries were greatly enlarged by 32 days after full bloom, with sepals, trichomes and stigmas remaining. After an additional 19 days of growth, trichomes and sepals were shed, with the expanding ovary and stigmas persisting. Advanced embryo development coincided with the outward signs of fruit ripening. Cells of the pericarp were thin-walled and undifferentiated initially, but three pericarp layers were observable by 46 days (Table 1). Formation of the stony endocarp occurred with the appearance of a skeletal framework, within which an amorphous deposition subsequently formed.

For the 1st 58 days of development, there was a constant increase in average length and width of the *Celtis occidentalis* fruit ("rapid growth"). This was followed by a period of little or no size increase for 60 days ("depressed growth"), and finally an additional period of less rapid ("final swell") growth (Table 1 and Fig. 2a). Average increase in length was 5.0

TABLE 1.—Time line of fruit growth in *Celtis occidentalis*

Growth cycle	Days	Event
	0	full bloom
Beginning of rapid growth	10	ovary wall cells undifferentiated
	20	
	30	
	40	
	50	three layers of pericarp trichomes and sepals shed
End of rapid growth ("depressed growth")	60	
	70	
	80	rapid increase in silicon
	90	endocarp matrix observable
	100	endocarp at approximately full weight
	110	
Initiation of second stage of growth ("final swell")	120	endocarp at lowest % C and N
	130	
	140	endocarp at most positive $\delta^{13}C$
	150	endocarp at highest silicon levels
	160	fruit mature

mm, and in width was 5.3 mm for the period from 10 days to 155 days. In addition to increased size, the drupes became increasingly spherical, as the ratio of average fruit length to width decreased from 1.8 to 1.0 after the 1st 66 days of development. Distinct stages of weight gain were not observed in the fruit of *C. occidentalis* (Fig. 2b). The mean weight of endocarps was 0.03 g at 46 days of development, which was 89% of the average whole fruit weight for that date. At the conclusion of the maturation period, average endocarp weight was 0.12 g, representing 54% of average mature whole fruit weight (Fig. 2b).

Potassium, magnesium, sulfur, silica and calcium were detected in the endocarps simultaneously by energy dispersive x-ray analysis. Total average counts initially acquired for silica were low, at 4 cps, and increased rather steadily to 133 cps by 155 days, whereas detected counts for calcium were consistently higher than all other detectable elements throughout the season, with an overall increasing trend from 206 cps to 904 cps by 155 days (Fig. 3a). X-ray mapping showed no large concentrations of silica or calcium, but rather dispersed small depositions of each. Counts for all other detectable elements decreased throughout

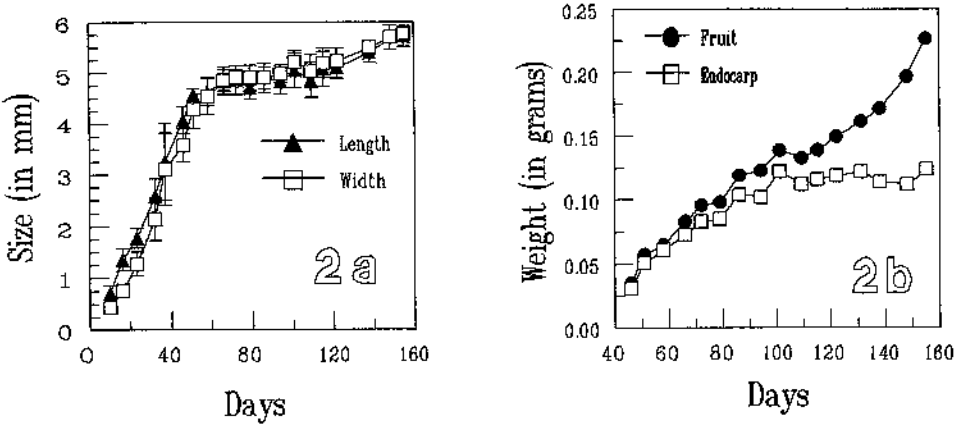

FIG. 2.—Growth of *Celtis occidentalis* fruits over 1 growing season (155 d). Each point is an average of 10 fruits. (a) Length and width of fruits. (b) Average weight of whole fruits and endocarps

endocarp development. X-ray diffraction analysis demonstrated the presence of aragonite and opal within *Celtis occidentalis* endocarps.

Mass spectrometry revealed different trends in $\delta^{13}C$ values of endocarps and exo/mesocarps (Fig. 3b). The values for endocarps became more positive with endocarp development, increasing from −26.6‰ at 46 days to −21.0‰ at maturity (155 days). There was a slight tendency toward more negative $\delta^{13}C$ values in the exo/mesocarp (Fig. 3b), with the most positive value −26.9‰ at 51 days and the most negative −30.3‰ at 148 days.

Carbon and nitrogen content decreased throughout growth of the endocarp. At 46 days, carbon content was highest at 44.0%; it was lowest at 12.7% on 122 days (Fig. 3c). Nitrogen content was initially 3.2%, and decreased to ca. 0.5% at 155 days. The carbon content of the exo/mesocarp increased slightly from an initial 45.6% to over 50% at maturity, while the nitrogen levels steadily declined from 3.3% to 1.9% (Figs. 3c and 3d).

Two-tailed t-tests showed no significant differences between fruits from Minnesota and South Dakota in calcium and silicon levels ($P = 0.72$ and 0.23, respectively) or in organic carbon isotope ratios ($P = 0.67$).

DISCUSSION

The presence of three distinct growth stages in the fruit of *Celtis occidentalis* is consistent with the results of other studies of drupaceous fruits (Lilleland, 1930, 1932; Lott, 1933; Tukey, 1934). Unlike most previously reported drupes, hackberry exhibited the greatest size increase in the first stage of development rather than the last stage.

In studies by Lott (1933) of peaches and Lilleland (1934) of plums, development of the endocarp with respect to total fruit wall structure showed the same general pattern as *Celtis*. In *Celtis*, however, the endocarp comprises a larger portion of the total fruit throughout development. Peach endocarp peaked at 53% of the total weight of the fruit, after beginning at 28%, and ended with 26% of total fruit weight. In data calculated from Lilleland's (1934) study of cherry, endocarps never exceeded 25% of the total fruit weight. *Celtis* endocarps became a smaller proportion of the fruit weight from ca. 90% early in the study, to just over 50% at the end of the growing season.

Endocarp and other fruit wall layers in *Celtis occidentalis* formed similarly to those ob-

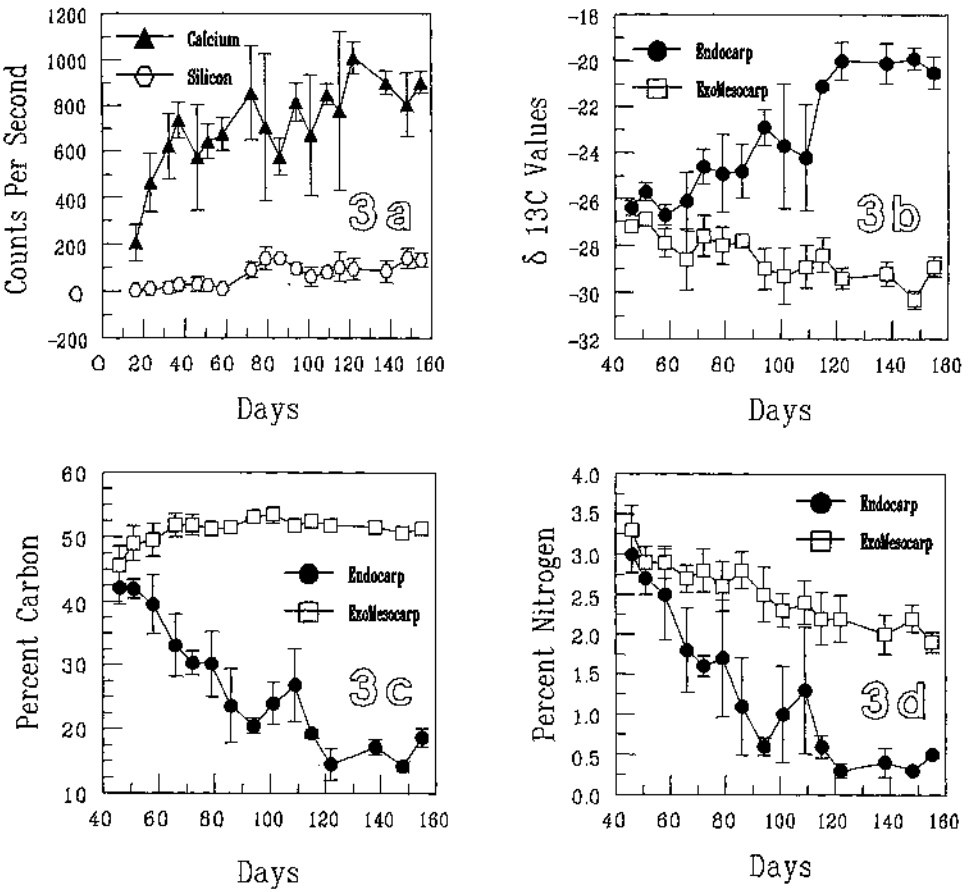

FIG. 3.—Changes in amounts of calcium, silicon, $\delta^{13}C$, carbon and nitrogen in *Celtis occidentalis* during 1 growing season (155 days). (a) Energy dispersive x-ray analyses of calcium and silicon in counts per second. Each point is an average of three areas on each of two endocarps. (b) Stable carbon isotope ratios ($\delta^{13}C$) for endocarps and exo/mesocarps. Each point is an average of 10 samples. (c) Percent carbon present in endocarps and exo/mesocarps. Each point is an average of 10 samples. (d) Changes in percent nitrogen present in endocarps and exo/mesocarps. Each point is an average of 10 samples

served in peaches (Lott, 1933). Endocarp development in *Celtis*, as measured by weight gain, occurred primarily over 40–100 days, a period without a large overall fruit size increase, but a time of significant weight gain. The increase in fruit weight (Fig. 2b) after 101 days was largely due to the increasing weight of the exo/mesocarp.

Lott (1933) reported a decrease of nitrogen in the "flesh" (mesocarp) of the peach from 0.2% to 0.1%, and from the "stone" (endocarp) from 0.2% to 0.1%. In *Celtis*, exo/mesocarp levels decreased slightly (from 3.3% to 1.9%), but not as dramatically as the endocarp levels (3.2% to 0.5%).

The weight increase of the endocarp was synchronous with the escalation of the number of cps acquired for calcium and, to a lesser degree, silica, in this layer of the fruit wall (Fig.

3a). As development progressed, the reduction in EDX count values for potassium, magnesium, and sulfur, and decrease in percent nitrogen and carbon in the endocarps, may indicate the translocation of nutrients from the endocarp to other parts of the fruit. Nutrients may be concurrently replaced by calcium and silica through biomineralization. The cps for calcium increased very rapidly between 16 days and 79 days, while cps for silicon increased most after 80 days. Correlation of SEM observations, EDX values, and XRD results indicate that as the endocarp develops, a silica (opal) framework is constructed with concurrent deposition of aragonite.

The strong increasing (positive) trend in $\delta^{13}C$ in maturing endocarps may be the result of secondary fractionation (O'Leary, 1981) and discrimination against ^{13}C in the pathways involved in transport of carbon containing molecules such as sugars. The increase in percent carbon of the exo/mesocarp and the accompanying increasingly negative $\delta^{13}C$ values indicate the transfer of carbon from the endocarp to the exo/mesocarp.

While the use of stable carbon isotopes is a very powerful tool and well-suited for ecological and paleoecological studies, the variation in $\delta^{13}C$ values during the growth of endocarps must be considered in interpretation of isotope ratios in other studies.

It is possible to compare *Celtis* growth with that of other drupaceous fruits, but no other data have been published for drupes with high concentrations of silica and calcium. It seems probable that this highly mineralized condition may be a major contributing factor to the excellent preservation of *Celtis* in the fossil record.

Acknowledgments.—The authors thank Mr. C. Jahren for assistance with collections. We also thank the Black Hills State University Faculty Research Committee, The National Geographic Society (grant 4618-91), and the National Science Foundation (grants BIO-9200345 and EAR-9223153).

LITERATURE CITED

ARNOTT, H. J. 1982. Three systems of biomineralization in plants with comments on the associated organic matrix, p. 199–218 *In:* G. H. Nancollas (ed.). Biological mineralization and demineralization. Springer-Verlag, New York.

BACKLUND, D. C., M. L. GABEL AND L. L. TIESZEN. 1991. An environmental gradient in the Tertiary Great Plains as indicated by stable carbon isotopes from organic compounds in plant fossils. *Proc. S. Dak. Acad. Sci.*, **70**: 99–108.

———, ——— AND ———. 1992. Stable carbon isotope ratios in extant *Celtis* (Ulmaceae) in the Great Plains with a comparison to fossil *Celtis*. *Proc. S. Dak. Acad. Sci.*, **71**:77–83.

BERRY, E. W. 1928. Stones of *Celtis* in the Tertiary of the western United States. *Am. Mus. Novit.*, **298**: 1–5.

CHANEY, R. W. 1925. Notes on two fossil hackberries from the Tertiary of the western United States. *Contrib. Palaeontol.*, **51**:51–57.

FULBRIGHT, T. E., K. S. FLENNIKEN AND G. L. WAGGERMAN. 1986. Enhancing germination of spiny hackberry seeds. *J. Range Manage.*, **39**:552–554.

GOLDSTEIN, J. I., D. E. NEWBURY, P. ECHLIN, D. C. JOY, C. FIORI AND E. LIFSHIN. 1981. Scanning electron microscopy and x-ray microanalysis. Plenum, New York. 673 p.

HAFFNER, J., M. L. GABEL AND L. L. TIESZEN. 1990. Stable carbon isotope ratios of Miocene sediments and fossil *Celtis* (Ulmaceae) and *Berriochloa* (Gramineae) reproductive structures from the northern Great Plains. *Proc. S. Dak. Acad. Sci.*, **69**:145–152.

KORDOS-SZAKÁLY, M. AND L. KORDOS. 1985. Morphotypes of Hungarian fossil *Celtis* (Urticales) stones. *Ann. Hist.—Nat. Mus. Natl. Hung.*, **77**:35–63.

LILLELAND, O. 1930. Growth study of the apricot fruit. *Proc. Am. Soc. Hortic. Sci.*, **27**:237–245.

———. 1932. Growth study of the peach fruit. *Proc. Am. Soc. Hortic. Sci.*, **29**:8–12.

———. 1934. Growth study of the plum fruit—the growth and changes in chemical composition of the climax plum. *Proc. Am. Soc. Hortic. Sci.*, **30**:203–208.

LOTT, R. V. 1933. The growth rate and chemical composition of the Hiley peach from stone formation to fleshy maturity. *Proc. Am. Soc. Hortic. Sci.*, **29**:1–7.

METCALFE, C. R. AND L. CHALK. 1950. Anatomy of the dicotyledons, Vol 2. Clarendon Press, Oxford. 1500 p.

NAGALHARD, K. 1922. Fossilium Catalogus II. Plantae Pars 10, Ulmaceae. W. Junk, Berlin. 84 p.

NAMBUDIRI, E. M. V., W. D. TIDWELL, B. N. SMITH AND N. P. HEBBERT. 1978. A C_4 plant from the Pliocene. *Nature*, **276**:816–817.

OKAZAKI, M. H., H. SETOGUCHI AND E. HISANAGA. 1991. Inorganic composition of cystoliths isolated from leaves of higher plants, p. 173–177 *In:* S. Suga and H. Nakahara (eds.). Mechanisms and phylogeny of mineralization in biological systems. Springer-Verlag, Tokyo.

O'LEARY, M. H. 1981. Carbon isotope fractionation in plants. *Phytochemistry*, **20**:553–567.

PIREYRE, N. 1961. Contribution à l'étude morphologique, histologique et physiologique des cystolithes. *Rev. Cytol. Biol. Vég.*, **23**:93–320.

PRESTON, R. J. 1989. North American trees (exclusive of Mexico and tropical Florida), 4th ed. Iowa State University Press, Ames. 407 p.

SATAKE, Y. 1931. Systematic and anatomical studies on some Japanese plants. I. Systematic importance of spodograms in the Urticales. *J. Fac. Sci. Univ. Tokyo Sect. 3 Bot.*, **3**:485–511.

SCHWEITZER, E. M. 1971. Comparative anatomy of Ulmaceae. *J. Arnold Arbor.*, **52**:523–585.

SEGAL, R. H. 1966. A review of some Tertiary endocarps of *Celtis* (Ulmaceae). *Southwest. Nat.*, **11**:211–216.

SETOGUCHI, H., M. OKAZAKI AND S. SUGA. 1986. Calcification in higher plants with special reference to cystoliths, p. 409–418. *In:* R. E. Crick. Origin, evolution and modern aspects of biomineralization in plants and animals. Plenum Press, New York.

SIMKISS, K. AND K. M. WILBUR (EDS.). 1989. Biomineralization: cell biology and mineral deposition. Academic Press, New York. 337 p.

SOLEREDER, H. 1908. Systematic anatomy of the dicotyledons, Vol. II. (Translated from German) Clarendon Press, Oxford. 1172 p.

THOMASSON, J. R. 1979. Late Cenozoic grasses and other angiosperms form Kansas, Nebraska, and Colorado. *Univ. Kans. Publ. Bull.*, **218**:1–68.

TIESZEN, L. L. 1991. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *J. Archaeol. Sci.*, 18:227–248.

——— AND T. W. BOUTTON. 1989. Stable carbon isotopes in terrestrial ecosystems research, p. 176–195. *In:* P. W. Rundel, J. R. Ehleringer and K. A. Nagy (eds.). Stable isotopes in ecological research. Ecological Study Series. Springer, New York.

TUKEY, H. B. 1934. Growth of the embryo, seed, and pericarp of the sour cherry (*Prunus cerasus*) in relation to season of fruit ripening. *Proc. Am. Soc. Hortic. Sci.*, **31**(suppl. vol.):125–144.

WERNER, O. 1931. Haar-und Cystolithenscheiben in Blattgeweben bei Urticales, bei *Bryonica dioica* and *Zexmenia longipetiolata*. *Oesterr. Bot. Z.*, **80**:81–97.

YANOVSKY, E., E. K. NELSON AND R. M. KINGSBURY. 1932. Berries rich in calcium. *Science*, **75**:565–566.

SUBMITTED 22 MAY 1996 ACCEPTED 20 SEPTEMBER 1996