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Biomineralization in seeds: developmental trends in isotopic signatures of hackberry

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Abstract

Experiments on hackberry (*Celtis*) fruits collected over the course of a growing season at two localities were undertaken to assess the isotopic composition of the intricate mineral ($CaCO_3$ and $SiO_2 \cdot nH_2O$) and organic structure of hackberry endocarps. Hackberry fruits contain biomineralized aragonite-rich endocarps and have been documented as fossils in a variety of sites in North America and Eastern Europe ranging in age from historical to millions of years old, giving them potential as paleoclimate indicators. In the modern hackberries studied, three distinct stages of development were observed: stage 1 (0–55 days) shows a rapid increase in the whole mass of the hackberry drupe as basic components differentiate; stage 2 (day 55–85) involves a short (30-day) period of slow growth overall, but is concurrent with a sharp rise in the mass of calcium carbonate mineral; stage 3 (day 85 to full maturity) involves a sharp increase in total mass due to mesocarp development. Fruit tissues were found to have lower $\delta^{13}C$ values than stem tissues at both sites of collection; there was very little difference between the $\delta^{18}O$ values in the endocarp carbonate at the two sites. The most variable $\delta^{13}C$ and $\delta^{18}O$ values of endocarp were observed in stage 1 development. After stage 2 begins, the $\delta^{18}O$ values in endocarp carbonate stabilized. The $\delta^{13}C$ value of opal-occluded organic carbon appeared to be constant throughout the growing season. © 1998 Elsevier Science B.V.

Keywords: biomineralization; paleoclimate; stable isotope; phytolith; fossil seed

1. Introduction

The hackberry (*Celtis*) is a deciduous tree in the elm family (Ulmaceae). This irregularly shaped tree sometimes occurs as a shrub and has a wide, but fragmented, distribution throughout North America and Eastern Europe, including Anatolia (Turkey). The hackberry is viable in a wide range of habitats, including deciduous riparian woodlands, various shrub communities, in rocky ravines, and as scattered individuals in grasslands (Ginski, 1977; Plummer, 1977; Carmichael et al., 1978; Brown, 1982; Albee et al., 1988; DeBolt, 1992), and is able to grow at elevations ranging from 200 to 2000 m above sea level (Elias, 1980).

Celtis produces a fleshy, globose drupe containing a single seed. Fruits ripen in late fall and are dispersed by birds, rodents, other small mammals, and gravity. Occasionally, the seed has been

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observed to remain on the tree until the following spring (Lamb, 1915). The drupe contains a fleshy mesocarp and a biomineralized aragonite-rich endocarp, all of which encase the endosperm. The durability of the 'biomineralized' aragonite endocarp contributes to its preservation in the fossil record: fossil *Celtis* endocarps are found throughout the Quaternary sediments of the Great Plains, and are common botanical remnants in sediments of the Miocene and Oligocene epochs. Because the *Celtis* fruit is edible and was a favorite condiment of some American Indian groups, fossilized *Celtis* endocarps are commonly found in Great Plains Holocene archeological sites (Thomasson, 1991). Fossil hackberry endocarps have been excavated from a variety of sites ranging in age from historical to millions of years old, but at present there is very little information on their structure, isotopic composition and relation to local climate.

In this paper, we report on our investigation of the morphological, mineralogical, and isotopic development in hackberry fruits collected from two locations in the Northern Great Plains during the growing season of 1993. The motivation of this research was our interest in the suitability of the stable isotope composition of hackberry endocarps as paleoclimatic indicators (Jahren, 1996). Elsewhere, we have researched the durability of the endocarp through time in terms of its mineralogy and ^{14}C composition (Wang et al., 1997); this work suggests that the general composition of endocarps is well-preserved, at least through the Quaternary.

2. Materials and methods

2.1. Field collection

Celtis occidentalis fruits were collected from two planted trees in Austin, Minnesota and one in Spearfish, South Dakota during the growing season of 1993. Samples were gathered over a period of 18 weeks beginning with the first appearance of fruit, and continuing until the fruits had reached maturity. Each collection consisted of picking 10–20 fruits from each tree. Following collection, specimens were air-dried for several weeks and then weighed. One fruit per tree was analyzed isotopically for each collection, and at least three replications were made of each endocarp, with less than 0.2‰ variability between replications.

2.2. Chemical analyses

The mass of total carbonate in fruit was determined by reacting a known mass of ground sample with 100% phosphoric acid at 25 C, cryogenically purifying the CO_2 produced, and determining its quantity manometrically (McCrea, 1950).

Components of drupes (meso+exocarps, endosperm) were either separated physically, through cutting and scraping, or chemically, with the following chemical treatments: whole endocarp was treated chemically with 35% H_2O_2 for 24 h at room temperature in order to remove oxidizable organic materials, then acidified with 6 M HCl for 12 h in order to remove all carbonate mineral. Persisting endocarp remains were exposed to 60% HF for 2 weeks in order to completely dissolve minerals, leaving a network of occluded organics. Cellulose was isolated from plant tissue using a method adapted from Harborne (1984) in which plant samples were boiled in H_2O, then bleached with sodium chlorite and acetic acid to remove lignin, and rinsed. Hemicellulose was removed with 17% NaOH; and further rinsed. The remaining pure cellulose was lyophilized and stored.

Whole hackberry drupes were impregnated under vacuum in Epo-tek® 301-2 optically transparent epoxy, then thin-sectioned to a thickness of 30 μm according to standard petrographic techniques. Slides were then examined under a Nikon Optiphot-2 petrographic microscope with camera attachment.

Endocarp fragments examined via scanning electron microscope (SEM) were mounted on aluminum stubs and coated with Au. All SEM work was done on a IDIS microscope (stage 2) at 10 kV. Magnification levels ranged from 27 to 580×, with most features showing best resolution at about 400×.

Selected samples were subjected to X-ray diffraction (XRD) analysis in order to ascertain mineralogy, and to Fourier Transform Infra-Red

(FTIR) analysis in order to characterize organic components. Samples were introduced to the FTIR instrument as a 1–2% concentration in KBr.

Organic carbon samples were prepared for stable isotope analysis via combustion in sealed vycor tubes containing Cu, CuO, and Ag (Minagawa et al., 1984). The released CO_2 was purified cryogenically, measured manometrically, and collected for $^{13}C/^{12}C$ measurement on a stable isotope ratio mass spectrometer.

Carbonate samples were prepared for stable isotope analysis through a pretreatment in bleach designed to minimize the effects of organic carbon upon carbonate reaction (Jahren, 1996) and then by reaction with 100% phosphoric acid (McCrea, 1950) in separate reaction vessels (as described above) or at 90°C in an automated 'common acid bath' carbonate device. Measurement of stable isotope ratios of the liberated CO_2 in both methods was performed on a VG Prism stable isotope mass spectrometer at the Lawrence Berkeley National Laboratory.

All isotope values are reported in the delta notation:

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000\ [‰]$$

where the standard is VSMOW for $R = {}^{18}O/{}^{16}O$, and the standard is VPDB for $R = {}^{13}C/{}^{12}C$.

3. Results and analyses

3.1. Light microscopy and SEM results

The mesocarp, the mineral endocarp, and the endosperm are illustrated in cross-section under light microscopy (Fig. 1). Selective chemical treatments of the mineral endocarp (physically separated from mesocarp and endosperm) reveal an intricate structure. Whole endocarp (treated chemically with 35% H_2O_2 for 24 h at room temperature in order to remove oxidizable organic materials) is illustrated in Fig. 2. This SEM image clearly reveals ~30 μm diameter, 'honeycomb' mineral units of the endocarps that are more visible at higher magnification (Fig. 3). Upon acidification with 6 M HCl (for 12 h in order to remove all carbonate mineral), a persisting endocarp remains (Fig. 4), consisting of a skeletal framework, or 'scaffolding', upon which the carbonate mineral was overlaid. Combustion analysis indicated that this 'scaffolding' is ~15% organic carbon by weight, indicating that it consisted predominantly of minerals insoluble in HCl. The remaining skeletal framework was exposed to 60% HF for 2 weeks in order to completely dissolve minerals, leaving a network of occluded organics (Fig. 5).

3.2. Characterization of mineral components

X-ray diffraction of bulk endocarp indicates that endocarps consist primarily of aragonite ($CaCO_3$) and that the acid-resistant mineral 'skeleton' consists of opal ($SiO_2 \cdot nH_2O$). Cystoliths in the Acanthacea plant *Beloperone californica* have also been found to be composed of both calcium carbonate and silica (Hiltz and Pobeguin, 1949), and this deposition of both calcium carbonate and silica, apparently in a single cell, is thought to be most unusual (Simkiss and Wilbur, 1989). We find interlaced amorphous silica and aragonite precipitated in the biomineralized endocarp of the hackberry in most specimens, but occasionally the opal is not present, for unknown reasons.

3.3. Characterization of organic components

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize organic compounds present in different components of the hackberry fruit. Results are presented in Table 1. Results indicate the presence of three compounds in all components: $RCH{=}CH_2$ (alkene), fatty acid or ester, and alkane. In addition to these compounds, the mesocarp and opal-skeleton occlusion contain additional alkane and alcohol compounds, while the mesocarp only contains ether compound. The fact that several compounds are found in all components suggests that the organic chemistry is rather homogeneous throughout the fruit, but that cellulose and lignin are not found in the endocarp.

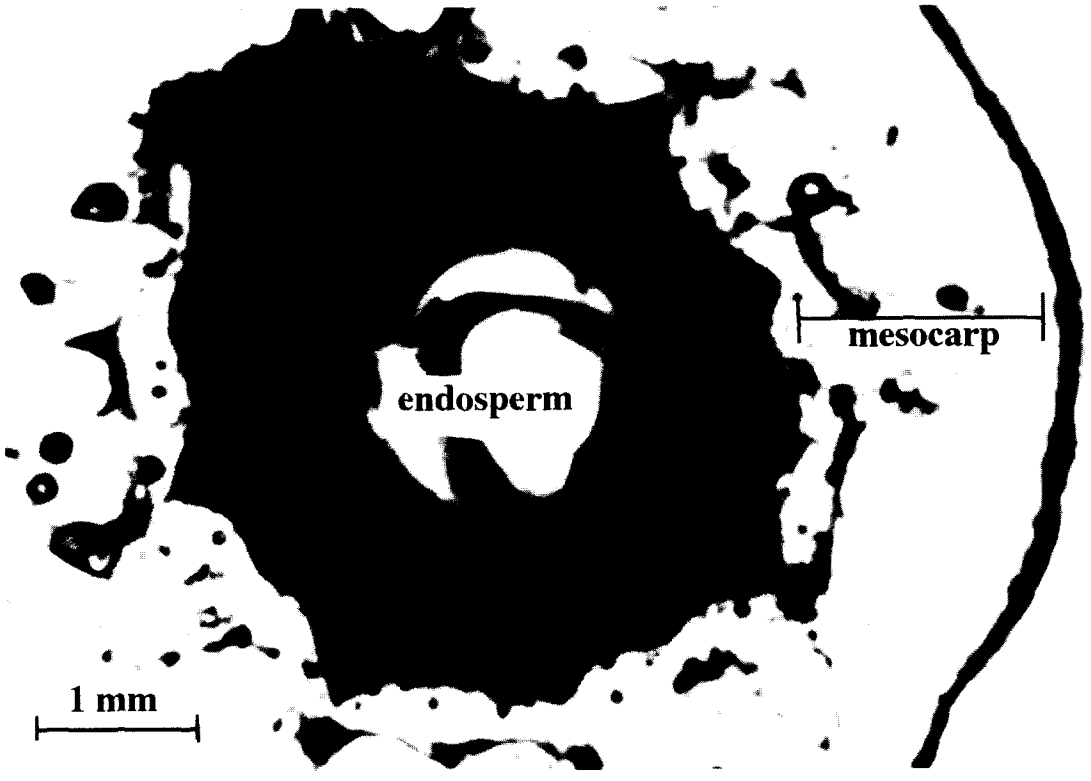

Fig. 1. Photograph of hackberry in cross-section.

Fig. 2. SEM image of hackberry endocarp that has been treated with 35% H_2O_2 in order to remove oxidizable organics. ×166.

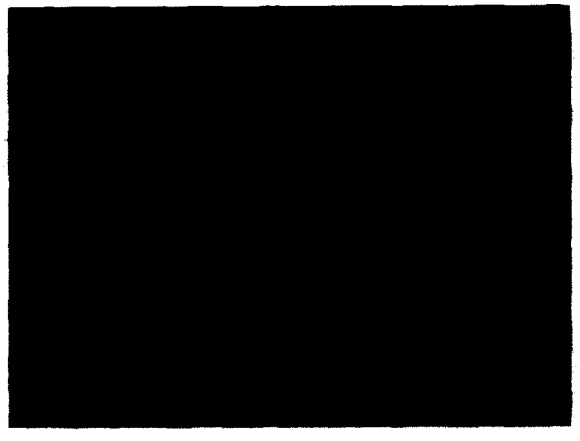

Fig. 3. High-magnification SEM image of hackberry endocarp that has been treated with 35% H_2O_2 in order to remove oxidizable organics; here the honeycomb mineral units are distinctly visible. ×580.

3.4. *Thin-section petrography results*

Development of the mineral endocarp is clearly apparent in a time-series collection of photographs taken under the petrographic microscope (Figs. 6–9). Extensive development in mineral endocarps was observed to occur between 74 and

Fig. 4. SEM image of hackberry endocarp after treatment with both 35% H_2O_2 and 6 M HCl. Only the skeletal framework of the honeycomb mineral structure remains. ×424.

Fig. 5. SEM image of hackberry endocarp after treatment with 35% H_2O_2, 6 M HCl and 60% HF. Only the organic material occluded within the skeletal framework of the honeycomb mineral structure remains. ×3030.

133 days after appearance, both with respect to added mineral mass, and definition of mineral structure.

3.5. *Stable isotope analysis results*

Selected portions of the hackberry tissue collected were analyzed for carbon and oxygen stable isotope content: (stems, stem cellulose, organic carbon occluded within the opal skeletal structure of the endocarp, endocarp carbonate, and the fleshy mesocarp). Components were isolated using the methods described earlier in this section, and the results of the isotopic analyses are presented in Table 2.

The stability of the isotopic composition of the endocarp throughout the growing season is depicted in Figs. 10–12. Fig. 10 plots the $\delta^{13}C$ values of endocarp carbonate against time; Fig. 11 plots the $\delta^{18}O$ values of endocarp carbonate against time; and Fig. 12 plots the $\delta^{13}C$ of organic carbon occluded within the endocarp's opal skeletal structure against time.

4. Discussion and conclusions

Williamson and Coston (1989) have described a three-stage growth pattern in the development of peach fruit as the following: stage 1, rapid cell division and increase in cell size in the pericarp; stage 2, a short period of slow pericarp growth; and stage 3, enlargement of the mesocarp cells. This general 3-stage model of fruit development has been confirmed in peaches, as well as other fruits by many other workers, including Ognjanov et al. (1995), and Luthra et al. (1980). Three distinct stages of development were also observed in the hackberry at our two sites. Fig. 13 shows mass of hackberry drupe plotted against days after its first appearance. The various 'stages' of development have been labeled according to patterns observed in the data and in the morphology of the hackberry drupes. Stage 1 (0–55 days) shows a rapid increase in the whole mass of the hackberry drupe as the basic components begin to differentiate themselves. Stage 2 (day 55–85) involves a short (30-day) period of slow growth overall, but it is concurrent with a sharp rise in the mass of calcium carbonate mineral in the drupe. The final stage (3) involves a sharp increase in total mass as the mesocarp acquires most of its mass.

Three-stage development in the hackberry drupe has implications for the timing of biomineralization in the organism. Fig. 14 plots percent calcium carbonate (by mass) against day after first appearance of the drupe. The striking feature of Fig. 14 is that during stage 2 the total mass of the drupe is found to be up to 20% carbonate in composition

Table 1
Results of FTIR analysis of the three main morphological components of hackberry fruit[a]

Hackberry component	Significant peak absorption (cm^{-1})	Class of compounds present	Interpretation
Mesocarp	1056	C–O	ether
	1645	C=C	$RCH=CH_2$ (alkene)
	1745	C=O	fatty acid or ester
	2854	C–H	alkane
	2925	C–H	alkane
	3337	O–H	alcohol
Endosperm	1655	C=C	$RCH=CH_2$ (alkene)
	1745	C=O	fatty acid or ester
	2926	C–H	alkane
Opal-skeleton occluded organics	1100	C–O	R_2CHOH (alcohol)
	1642	C=C	$RCH=CH_2$ (alkene)
	1736	C=O	fatty acid or ester
	2918	C–H	alkane
	3346	O–H	alcohol

[a]Sample was a mature fruit sample from Austin, MN (1993).

Fig. 6. Photograph of hackberry thin-section; taken under the petrographic microscope with crossed polars and quartz plate inserted. Scale: the long axis of the image = 1 mm. The endocarp is shown at 41 days after appearance, and lacks any visible pattern in the mineral component.

Fig. 7. Photograph of hackberry thin-section; taken under the petrographic microscope with crossed polars and quartz plate inserted. Scale: the long axis of the image = 1 mm. The endocarp is shown at 74 days after appearance, small, honeycomb-shaped mineral units become apparent.

(during stages 1 and 3 the total mass contains a much smaller % carbonate). This pattern is apparent in petrographic thin sections (Figs. 6–9) where most of the mineral mass of the endocarp appears to have been added during a short period in the middle of drupe development.

The pattern of organic material addition/mineral material addition/organic material addition is intriguing from a plant physiological perspective. Many studies clearly illustrate the ion-specific nature of element translocation in plants (Sale and Campbell, 1980; Dornbos and McDonald, 1986; Iskander, 1987; Laszlo, 1990), but with respect to the hackberry, there is a clear timing of selective element transport and deposition. During fruit development, C-substrates travel from their synthesis sites in the vegetative parts of the plant through the phloem to the site of the developing

Fig. 8. Photograph of hackberry thin-section; taken under the petrographic microscope with crossed polars and quartz plate inserted. Scale: the long axis of the image = 1 mm. The endocarp is shown at 133 days after appearance; the mineral endocarp has undergone extensive development both in added mineral mass, and definition of mineral structure.

Fig. 9. Photograph of hackberry thin-section; taken under the petrographic microscope with crossed polars and quartz plate inserted. Scale: the long axis of the image = 1 mm. The endocarp is shown at 150 days after appearance; the mineral portion of the endocarp has apparently stabilized, and the periphery has become more defined in structure.

fruit (Thorne, 1985; Patrick, 1990). Studies have shown that phloem-mobile elements, such as K^+, Mg^{2+}, C and N can also be *re*-translocated from stem and leaf tissue to the developing fruit (Pitman, 1975; Mauk and Noodén, 1992), and that the 'signal' for this retranslocation mobilization is hormonal (Neuman and Noodén, 1983; Noodén, 1987).

Carbon in hackberries is ultimately derived from the atmosphere as the plant fixes CO_2 into organic

Table 2
Stable isotope composition of several parts of the hackberry fruit and related plant tissues

Location	Stem		Fruit				
	1993 season stem cellulose $\delta^{13}C$ (‰)	1993 season stem $\delta^{13}C$ (‰)	Opal-occluded organic carbon $\delta^{13}C$ (‰)	Endosperm $\delta^{13}C$ (‰)	Mesocarp $\delta^{13}C$ (‰)	Endocarp $CaCO_3$ $\delta^{13}C$ (‰)	Endocarp $CaCO_3$ $\delta^{18}O$ (SMOW) (‰)
Austin, MN[a]	-26.08 ± 1.40 ($n=3$)	-27.44 ± 1.58 ($n=5$)	-27.23 ± 0.39 ($n=12$)	-28.15 ± 1.69 ($n=6$)	-29.35 ± 1.35 ($n=5$)	-15.02 ± 2.36 ($n=8$)	$+29.21\pm0.58$ ($n=8$)
Spearfish, SD[a]	not analyzed	-26.72 ± 0.47 ($n=2$)	-26.68 ± 1.40 ($n=14$)	-27.03 ± 0.63 ($n=6$)	-27.92 ± 1.44 ($n=8$)	-14.03 ± 1.63 ($n=11$)	$+29.00\pm0.98$ ($n=12$)
$\Delta\delta$ (Spearfish – Austin)[b]	–	$+0.72$	$+0.55$	$+1.12$	$+1.43$	$+0.99$	-0.21

[a] Values presented are *mean value of n samples* $\pm$ *standard deviation seen in n samples*, where *n* is given above.
[b] $\Delta\delta$ values represent mean $(\delta X_{\text{Spearfish}})$ – mean $(\delta X_{\text{Austin}})$ for the same sample type, where $X = {}^{13}C$ or ^{18}O, as appropriate.

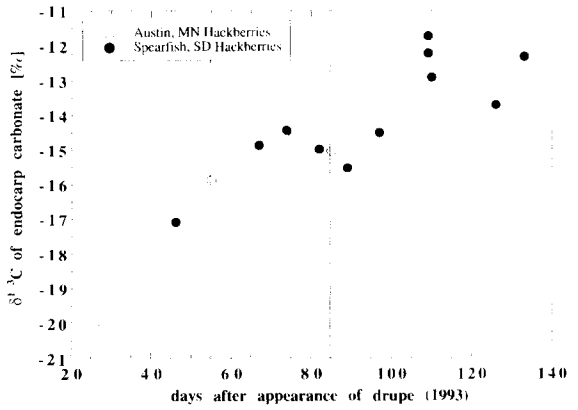

Fig. 10. Plot showing change in $\delta^{13}C$ value of endocarp carbonate with time.

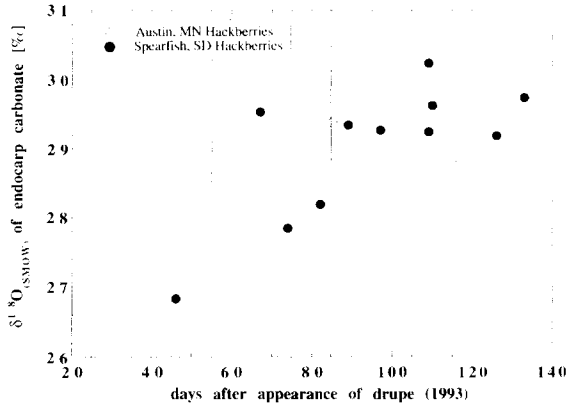

Fig. 11. Plot showing change in $\delta^{18}O$ value of endocarp carbonate with time.

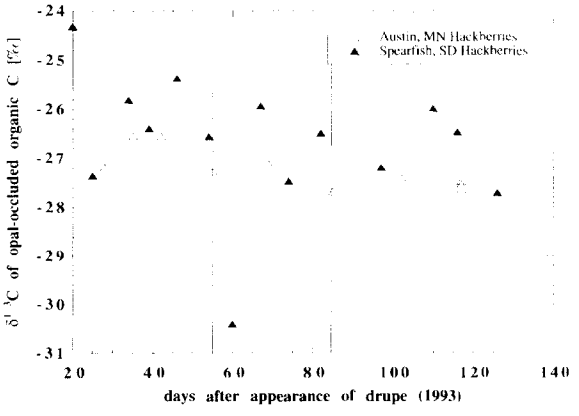

Fig. 12. Plot showing change in $\delta^{13}C$ value of organic carbon occluded within the opal-skeletal structure of the endocarp with time.

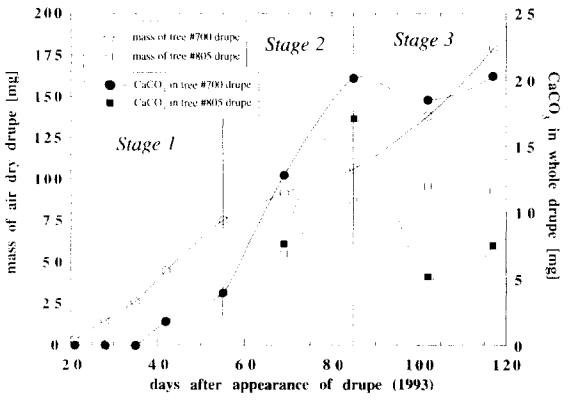

Fig. 13. Plot showing change in mass of hackberry drupe with time. For this figure, hackberries were collected from two trees, #700 and #805 in Austin, Minnesota during the growing season of 1993. Mass (air-dry) of the entire drupe is shown in open symbols and is scaled against the left *y*-axis. Mass of calcium carbonate mineral in the drupe is shown in closed symbols and is scaled against the right *y*-axis. The dotted line is a simple interpolation from mass to mass in the tree #700 whole drupe measurements.

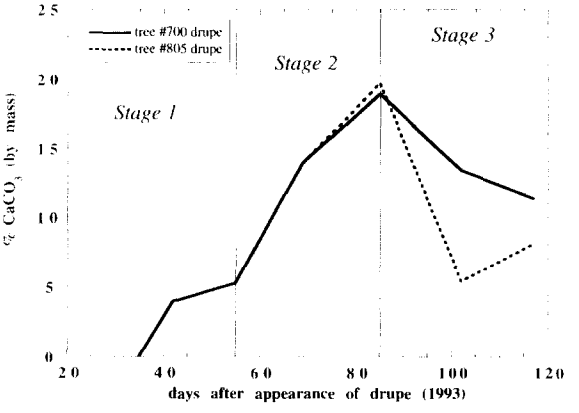

Fig. 14. Plot showing change in percent calcium carbonate (by mass) in whole hackberry drupe with time. All hackberries in this plot were collected in Austin, MN during the growing season of 1993.

carbon compounds. These organic carbon compounds are then either transported to a site of tissue development as an incomplete sugar, or are respired back to CO_2. Because CO_2 is a relatively soluble gas, respired CO_2 can be 'stored' by solubilizing it into plant tissue waters. After it has been translocated and concentrated in subcellular compartments, dissolved CO_2 acts as a means to

mediate cellular pH, as supply of readily available protons and minor reorganization creates carbonic acid (Simkiss and Wilbur, 1989).

The other important component of mineral material addition is the incorporation of Ca^{2+} into the plant. The ultimate source of Ca^{2+} in all plants is dissolved calcium in soil water. It is unlikely that gross uptake of Ca^{2+} by the plant is a limiting factor for fruit development, based upon the quantification of standard metabolic Ca^{2+} uptake rates inferred from Sr^{2+} tracing studies (Laszlo, 1994). Interestingly, both xylem flow rates and the concentration of Ca^{2+} in xylem fluid have been shown to fluctuate substantially during fruit development (Noodén and Mauk, 1987), suggesting that supply to developing fruit tissue may be a major sink for xylem-dissolved Ca^{2+}. Patterns of Ca^{2+} demand may even be genetically informed: *P. vulgaris*, fruit Ca^{2+} is supplied predominantly by redistribution from the pod (Mix and Marschner, 1976), suggesting that translocation from storage tissue may be a major source of fruit Ca^{2+}. Another important factor is that Ca^{2+} is phloem-immobile, and must be transported via a different route, such as the xylem or other non-vascular routes (Pate, 1975; Pate and Hocking, 1978; van Bel, 1990).

The working hypothesis for the development of the hackberry fruit that we wish to put forward as a result of these observations includes the following: (1) development of early season structures – during this phase of development preliminary fruit structures are constructed from phloem-supplied organic carbon compounds. Components, such as the fruit stem, the basic definition of the pericarp, and the organic matrix for subsequent mineral deposition are constructed. This phase is equivalent to developmental stage 1 described by Williamson and Coston (1989); (2) at about 55 days after first appearance, the hackberry drupe enters the second phase of its development – during which the bulk of the mineral endocarp forms and other late-season structures, such as the mesocarp and the endosperm, add substantial mass. The switch from stage 1 to stage 2 development is probably triggered hormonally, and the most important driver of this hormonal switch is probably a large increase of Ca^{2+} in the xylem and in cell walls due to either translocation from storage tissues in stems and leaves or increased Ca^{2+} uptake by the plant. Mineral endocarp development continues and gradually plateaus into the fully developed endocarp; (3) the mesocarp continues to develop until senescence.

The different phases of development in the hackberry are expressed in the way that ^{13}C is partitioned into the various components of hackberry tissue. Table 2 shows that fruit organic structures have more negative $\delta^{13}C$ values than stem organic structures at both field sites. This may be the result of the photosynthetic capability of 'green' fruit tissue: photosynthesizing structures have previously been found to be 2–4‰ more negative in $\delta^{13}C$ values than non-photosynthesizing tissues in the same plant (O'Leary, 1988).

Table 2 also illustrates the systematic difference between the stable isotope values at Spearfish, SD and Austin, MN ("Spearfish - Austin" row, Table 2). Specifically, stem tissue $\delta^{13}C$ values in Spearfish, SD plant tissue tend to be $\sim$0.60‰ greater than $\delta^{13}C$ comparable structures from Austin, MN. In fruit tissue, the $\delta^{13}C$ isotopic differences between the two sites increases to 1.0‰ or more in comparable structures. Growing season temperatures in Austin, MN are very stable and unchanging during the months of June, July and August, with an average daily value of 22.1°C. In contrast, during the month of June in Spearfish, SD average daily temperatures only reach 17.1°C; then in July and August, average daily temperatures increase to 23.1°C. All reported temperatures are 30-year averages of 1960–1990 data (Bair, 1992). It is probably this increase in temperature during the late season that drives the increase in $\delta^{13}C$ isotopic difference between similar structures at the two sites. It is interesting to note that there is very little difference between the $\delta^{18}O$ values in the endocarp carbonate of hackberries at the two sites. As many workers have noted, the $\delta^{18}O$ of calcium carbonate is a strong function of the $\delta^{18}O$ of the equilibrium solution and temperature (McCrea, 1950). Temperature of precipitation has been shown to affect the $\delta^{18}O$ of resultant calcium carbonate (Urey, 1947), but in this system, the differing temperatures of the two sites have not made the $\delta^{18}O$ in endocarp carbonate strongly different; however, it is likely that the temperature

Table 3
Estimates of within-site total variabilities based upon the data of Figs. 10–12

Component of hackberry	Variability through growing season (‰)	Variability in late development phase only (‰)
$\delta^{13}C$ in endocarp carbonate	8.0	3.0
$\delta^{18}O$ in endocarp carbonate	3.0	1.2
$\delta^{13}C$ in opal-occluded organic C	0.9	0.9

effect has been offset by differences in the $\delta^{18}O$ of tree source-water at the two sites, as well as differences in relative humidity which determined water use efficiency. In order to pursue these speculations, we are presently completing studies designed to evaluate possible correlations between $\delta^{18}O$ values in hackberry endocarp carbonate, the $\delta^{18}O$ value of site environmental water and site growing season temperature (Jahren et al., submitted).

Despite the fact that the $\delta^{13}C$ difference between similar organic components changes by about +0.5‰ from early to late season at the two sites, organic components are roughly equally different from endocarp carbonate $\delta^{13}C$ at both sites throughout the season, as is shown by a comparison of mesocarp organic C and endocarp $CaCO_3$ columns in Table 2.

One of the goals of this study was to assess the variability of stable isotope composition in hackberry structures during the course of the growing season, in order to evaluate fossil hackberry endocarps as possible paleoclimate indicators. For example, the $\delta^{13}C$ value of endocarp carbonate was observed to fluctuate greatly over the course of the growing season (Fig. 10). The majority of this fluctuation, however, is encompassed by the stage 1 development and represents early stages of carbonate precipitation and the possible use of translocated metabolized C. The trend in variability of $\delta^{18}O$ in endocarp carbonate is similar, but on a more restricted scale (Fig. 11). After stage 1 development ends, and the phase involving substantial carbonate precipitation begins, the value of $\delta^{18}O$ in endocarp carbonate has stabilized greatly. In contrast, the value of $\delta^{13}C$ in opal-occluded organic carbon appears to be stable throughout the growing season (Fig. 12).

From these results, the within-site variabilities are estimated as presented in Table 3, and indicate that $\delta^{13}C$ in endocarp carbonate is much more variable than $\delta^{18}O$ in endocarp carbonate, and that variability of both signatures decreases significantly with fruit maturity.

Therefore, when choosing hackberry endocarps for analysis in a paleoclimate context, it is clearly best to select against obviously immature, largely unformed 'stage 1' endocarps when possible. Furthermore, in evaluating the variability in terms of potential uncertainty in a paleoclimate reconstruction, it is important to remember that the numbers expressed in Table 3 represent both growing season variability and within tree variability, since sampling over the growing season was necessarily done on different fruits at each stage in development.

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