Article

Content of starch in walnut organs (*Carya illinoensis* Koch) in two phenological stages

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Abstract

The study was carried out in Torreon, Coahuila, with the purpose of analyzing the starch dynamics (mg g^{-1} MS) and its contribution to the total carbon of the biomass in the perennial organs of the walnut tree (*Carya illinoensis* Koch) in two phenological stages. The sampling was systematic. The results showed a significant difference in the concentration of starch between root (U= 7, p= 0.007) and trunk (U= 0, p= 0), but not between branches (U=26, p= 0.574) and annual growth (U= 31, p = 0.959). The means of concentration of starch obtained in production were: root= 56.22 mg g^{-1} MS, trunk= 61.52 mg g^{-1} MS, branch= 32.76 mg g^{-1} MS, annual growth= 31.87 mg g^{-1} MS. In production, the starch was concentrated in trunk ($\bar{x}$= 86.79 mg g^{-1} MS), followed by root ($\bar{x}$= 42.36 mg g^{-1} MS) and in smaller amount in branch ($\bar{x}$= 33.08 mg g^{-1} MS) and annual growth ($\bar{x}$= 31.85 mg g^{-1} MS). In dormancy the results were: root ($\bar{x}$= 70.08 mg g^{-1} MS), trunk ($\bar{x}$= 36.26 mg g^{-1} MS) and in smaller amount in branch ($\bar{x}$= 32.44 mg g^{-1} MS) and annual growth ($\bar{x}$= 31.89 mg g^{-1} MS). The total carbon contained in the biomass ranges from 42.62 to 43.36%. The contribution of the starch carbon represented from 1.34 to 3.64% with respect to the total biomass of the tree.

Keywords: biomass, cycle of C., reserves, walnut.

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Introduction

The pecan walnut, also known as the incarcerated walnut (*Carya illinoensis* Koch), is a deciduous fruit tree of the Juglandaceae family (Chávez *et al*., 2009) that reaches a height of 30 m and a longevity of more than 100 years (Madero, 2000). The reproductive age of these trees begins after the fifth or sixth year (Valentini *et al*., 2010). This species is native to Mexico and the United States of America dominated both countries nut production worldwide (Orona *et al*., 2007).

In northern Mexico, this species is cultivated under irrigation conditions for commercial use in a considerable area and in regions that have an arid to semiarid climate regime (Chávez *et al*., 2009). In Mexico, the approximate total surface of the pecan tree in irrigated conditions is 75 thousand hectares (García *et al*., 2009). The states with the highest nut production at a national level are Chihuahua, Coahuila, Nuevo León, Durango and Sonora with a total of 92.36% (Orona *et al*., 2006), particularly the states of Chihuahua and Coahuila together contribute 82% of the National nut production (González *et al.*, 2014).

The first walnut plantations in the Comarca Lagunera were established in 1948 (Potisek *et al*., 2010) and currently the value of walnut production occupies the first place among the fruit trees of the region, with an approximate value of 200 million pesos (García *et al*., 2009), the area harvested in the Comarca Lagunera in 2003 was 5 534 ha with a production of 7 600 tons (SIAP-SAGARPA, 2015). The varieties and proportions harvested pecans in the state of Coahuila for the year 2006 were: Western (50%), Wichita (33%) and other varieties (17%). Other varieties include creole, Barton, Mahan, Texas, Pawnee and Cheyenne nuts (Orona *et al*., 2013). Urban trees, such as walnut, in addition to contributing to carbon capture (Mexhal and Herrera, 2014) have beneficial effects on the urban population in different aspects (Donovan *et al*., 2011; Donovan and Prestemon, 2012; Pilat *et al*., 2012; Donovan *et al*., 2013).

Global warming is one of the events that characterize climate change and for agriculture represents a condition of extraordinary importance because the development of crops is closely related to the climate conditions that occur during each phenological stage (Gardea *et al*., 2010). During the development and growth of a tree it is subjected to constant stress by biotic or abiotic factors and in turn has a direct effect on its carbohydrate reserves. On the other hand, the concentration of carbohydrates, product of photosynthesis, varies according to the environmental conditions and the phenological stages of the trees (Valenzuela, 2014).

The growth of trees is driven by photosynthesis, which is carried out in the presence of carbon dioxide, water, oxygen and sunlight. The final product of this chemical reaction is glucose, a simple carbohydrate that is later associated with fructose to form a disaccharide commonly known as table sugar (sucrose) or in complex sugars such as starch (Martínez *et al*., 2013). Carbohydrates are the main products of photosynthesis, considered a source of energy for trees (Martínez *et al*., 2013).

The reserves stored in the plants can be used for growth and maintenance (Kramer and Kozlowski, 1979; Stepien *et al*. 1994; Sauter and Witt 1997). In woody trees, the roots seem to be the organ where the largest amount of reserve carbohydrates accumulates in the period before flowering, which is consumed in the development of flowers and fruits and during new episodes of vegetative growth (Kozlowski *et al*., 1991; Piispanen and Saranpää, 2001; Barbaroux and Breda 2002; Hoch *et al*., 2003). These reserves change constantly depending on the redistribution of carbohydrates in support of growth (Kozlowski 1992, Gamboa and Marín 2012).

During the dormancy stage, the roots and stems of the deciduous trees reach the maximum value of storage of reserves, which decreases from the sprouting of buds and the first stages of intensive growth of shoots and leaves according to Martínez *et al*. (2013); Valenzuela *et al*. (2011). In deciduous trees in winter the growth of the root increases, while that of the aerial biomass (trunk, branches) stops, presenting an inverse effect in summer (Valenzuela, 2006; Valenzuela *et al*., 2011; Valenzuela *et al*., 2014).

The concentration of reserves decreases during the vegetative growth stage, beginning its accumulation in the plant tissues at the end of summer and early autumn, of these reserves, a part will be used in the breathing process during the winter stage, another part for root growth and one more for sprouting in spring (Valenzuela *et al*., 2014).

Given the economic importance of walnut cultivation in the Comarca Lagunera, the objective was to analyze the carbohydrate balance in starch (mg starch g^{-1} MS) in different walnut vegetable organs (*Carya illinoensis* Koch) Western variety in two phenological stages (production and dormancy).

Materials and methods

Study area

The study was developed in the experimental field of the Universidad Autonoma Agraria Antonio Narro Laguna Unit (25° 33' 22.63'' north latitude and -103° 22' 07.77'' west longitude) in Torreon, Coahuila (Figure 1). The climate of the region is dry desert with an average annual rainfall of 230 mm (IMTA, 2005) and an altitude of 1 120 m (INEGI, 2012).

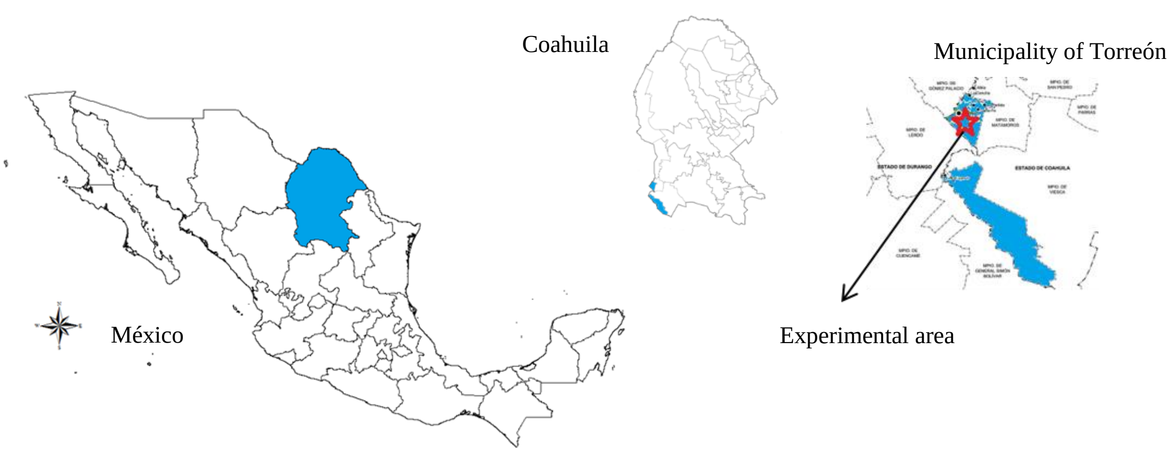

Figure 1. Location of the experimental area.

Sampling. The sampling was systematic and consisted of selecting trees and samples using a randomly established order. To do this, four adult trees were selected with an average age of 40 years interspersed in the middle part of the plot to avoid the edge effect. In each tree, two samples of root, trunk, branch and annual growth were taken (outbreak emitted by the tree in the year in which the study was conducted). The phenological stages that were evaluated were: production (month of august, when the tree is in fruit production and maximum vegetative growth) and dormancy (months of December and January, when the tree is completely defoliated without aerial vegetative growth).

The samples of trunk and branches were taken in the form of chips with a Pressler® drill, the samples of annual growths were obtained with a conventional saw and for the root a peak was used making a small trench to locate the main root and extract the sample. The samples were carefully cleaned by removing traces of soil and placed in perforated and labeled aluminum bags, then frozen in liquid nitrogen to stop all biochemical processes in the tissues. The samples were transferred to the Forest Ecology Laboratory of the Faculty of Biological Sciences of the Juárez University of the state of Durango.

Laboratory work. The samples were stored in a freezer (Revco Value Plus® ThermoScientific®) at a temperature of -70 °C for one week and then subjected to a lyophilization process (Lyophilizer Labconco Freezone Triad® Freeze Dry Systems®) for seven days a temperature of -40 °C in order to dehydrate the samples and avoid enzymatic activity. Samples were ground in a knife mill (Fritsch® Pulverisette 15®) to a fine powder and 10 mg of dry matter were weighed into microtubes (MCT-200-C Clear Axygen Scientific®) using an analytical balance (PW 250 Adam®).

Determination of the concentration of starch. To determine the concentration of starch, 1 ml of distilled water was added to the microtubes containing the dry matter and vortexed (Maxi Mix II® Thermo Scientific®) for 1 min. Subsequently they were boiled for 10 min to gelatinize the starch. The samples were centrifuged at 2500 rpm (Spectrafuge 16M Labnet®) for 2 min, 300 μl of the supernatant was removed and placed in clean microtubes. Then, 900 μl of absolute ethanol was added and centrifuged at 10,000 rpm for five minutes to precipitate the starch. The alcohol was carefully drained from the microtube leaving only the starch precipitated to the bottom and 1 ml of distilled water was added. The microtubes were placed in the vortex for three minutes and 50 μl of iodine solution was added to each one. Finally, the absorbance was measured in a UV-Visible spectrophotometer at 595 nm (Genesys 20® Thermo Scientific®), using as a control 1 ml of distilled water and 50 μl of iodine with rice starch as standard (Sigma-Aldrich®).

Estimation of the biomass of the trees. To convert the concentrations of starch in the wood of the tree-level samples, the biomass of each of the trees was estimated. Barbaroux (2002) showed that the heartwood does not contain any type of reserves and if there is one, they are not remobilized, since it is made up of dead cells. The length of the sapwood was measured directly in the wood chips sampled. The walnut sapwood is visually recognizable by its lighter color and the absence of large vessels in the early wood. In branches and roots, wood corresponding to heartwood was not displayed, so it is considered that all the wood was sapwood. To calculate the volume of each organ of the trees, the density values of the wood determined for walnut by the INTI CITEMA (2003) and using the allometric equations in *Carya* according to Rodríguez *et al*. (2006). Root biomass was determined according to the methodology of Drexhage *et al*. (1999).

[1] log(biomasa de raíz kg)=-1.56+2.44∗log(diameter)

The branch biomass was estimated by the difference between the total tree biomass [2] and the trunk biomass [3] according to Brucciamacchie (1982):

[2] total tree biomass (g)= - 484.7 ∗ $Diam_{1.30}$ ∗ 414.4 ∗ $(Diam_{1.30})^2$; [3] trunk biomass (g)= -320.9 ∗ $Diam_{1.30}$ ∗ 332.2 ∗ $(Diam_{1.30})^2$ branch biomass (g) = [1] - [2]

Determination of the C-total. The determination of total carbon in the samples was carried out by the procedures established in ASTM D02-84R07: Test Method for Ash in Wood.

Starch contribution to C-total. The starch content was converted into mg C g^{-1} of MS in order to estimate the contribution of starch to total C using the conversion factor derived from the molecular mass of glucose, which corresponds to 0.4 g of C g^{-1} of glucose (Valenzuela, 2006).

Statistical analysis

To compare the concentrations of starch between the different perennial organs of the walnut, the Kruskall-Wallis test was used, in case of a significant difference, the multiple comparison test of means of Conover was used. Likewise, to test a significant difference between phenological stages, the Mann-Whitney test was used. These tests were considered significant at a significance level of $p \leq 0.05$.

Results and discussion

Concentration of starch in perennial organs

The results obtained in the production stage showed that the starch was significantly concentrated in the trunk ($\bar{x}$= 86.79 mg starch g^{-1} MS), followed by the root ($\bar{x}$= 42.36 mg starch g^{-1} MS) and in less quantity in the branch ($\bar{x}$= 33.08 mg starch g^{-1} MS) and annual growth ($\bar{x}$= 31.85 mg starch g^{-1} MS), H= 22.92, Gl= 3, $p<0.001$ (Table 1 and 2).

Table 1. Descriptive statistics of the concentration of starch (mg g^{-1} MS) of the perennial organs of root (R), trunk (T), branch (RM) and annual growth (CA) in the phenological stage of production.

Variables	Production			
	R	T	RM	CA
N	8	8	8	8
Mean	42.36	86.79	33.08	31.86
Typic error of the mean	2.94	10.47	0.93	0.51
Deviation typical	8.31	29.61	2.63	1.44
Minimum	31.86	64.01	30.89	29.51
Maximum	53.85	153.79	38.73	34.2

Table 2. Kruskal-Wallis test for the comparison in the concentration of starch (mg g^{-1} MS) between perennial organs in walnut in the phenological stage of production.

Test of Kruskal-Wallis		22.9205
Gl		3
p		0.000042
Perennial organs	Mean (mg g^{-1} starch)	Homogeneous groups
Trunk	87.7925	A
Root	42.3688	B
Branch	33.0863	C
Annual growth	31.86	C

In the dormancy stage an inverse effect was observed in root and trunk with respect to the production stage, since the starch was significantly concentrated in the root ($\bar{x}$= 70.08 mg starch g^{-1} MS), followed by trunk ($\bar{x}$= 36.26 mg starch g^{-1} MS) and in less quantity in branch ($\bar{x}$= 32.44 mg starch g^{-1} MS) and annual growth ($\bar{x}$= 31.89 mg starch g^{-1} MS), (H= 20.5, Gl= 3, p<0.001 (Table 3 and 4).

Table 3. Descriptive statistics of the concentration of starch (mg g^{-1} MS) of the perennial organs of root (R), trunk (T), branch (RM) and annual growth (CA) in the phenological stage of dormancy.

Variables	Dormancy			
	R	T	RM	CA
N	8	8	8	8
Mean	70.08	36.26	32.44	31.89
Typic error of the mean	7.79	1.85	1.26	0.83
Typical deviation	22.05	5.25	3.58	2.35
Minimum	40.79	32.06	29.12	29.26
Maximum	100.3	48.75	39.39	36.07

The analysis of the concentration of starch in perennial walnut organs between the two phenological stages of production and dormancy showed significant difference in the root (U= 7, p= 0.007) and trunk (U= 0, p< 0.001), while in the branch (U= 26, p= 0.574) and annual growth (U= 31, p= 0.959) no significant difference was observed between both stages (Figure 2).

Table 4. Kruskal-Wallis test for comparison in the concentration of starch (mg g^{-1} MS) between perennial organs in walnut in the phenological stage of dormancy.

Test of Kruskal-Wallis		20.5028
Gl		3
p		0.000134
Perennial porgans	Mean	Homogeneous groups
Trunk	70.0825	A
Root	36.2613	B
Branch	32.4488	C
Annual growth	31.8925	C

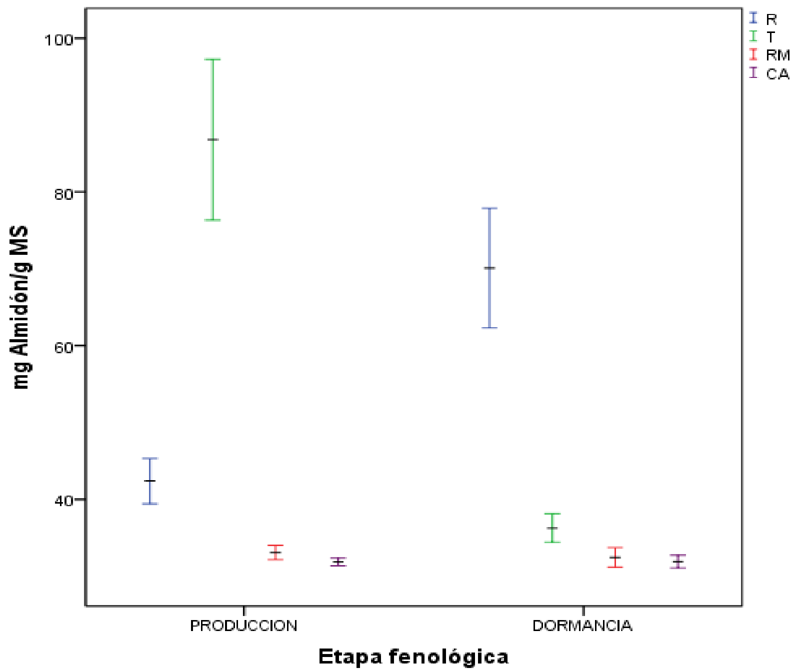

Figure 2. Comparison between the means of starch concentration (mg g^{-1} MS) of perennial walnut organs in two phenological stages (production and dormancy). Root (R), trunk (T), branch (RM), annual growth (CA). The vertical bars show the typical error of the mean.

According to Kozlowski *et al*. (1991) in woody trees, the roots seem to be the organ where the largest amount of reserve carbohydrates accumulates in the period before the maximum, which are consumed in the development of new tissues. The results in this study showed that it is in the trunk where the highest amount of starch accumulates during the phenological stage of production ($\bar{x}$= 86.79 mg starch g^{-1} MS) followed by the root ($\bar{x}$= 42.36 mg starch g^{-1} MS), which is when the tree is at its highest point of fruit production.

With regard to the phenological stage of dormancy, according to Martínez *et al*. (2013) and Valenzuela *et al*. (2011) during the dormancy stage the roots and stems of the deciduous trees reach the maximum value of storage of reserves, the root being the one that presents the most accumulation, which coincides with the results found by Rodríguez *et al*. (2002); Navarro-Cerrillo and Calvo (2003); Sanz-Pérez *et al*. (2004); Valenzuela (2006); Gamboa and Marín (2012). In addition, Kozlowski *et al*. (1992) report that it is at the root where the largest amount of reserve carbohydrates accumulates in the period prior to vegetative growth, Valenzuela *et al*. (2011) reports a significantly higher concentration of starch in the root with respect to the rest of the organs in deciduous trees in the dormant stage. The results observed in the present study coincide with the previous, since the root reached the maximum value of storage of reserves ($\bar{x}$= 70.08 mg starch/g MS) and in smaller proportion the annual growth ($\bar{x}$= 31.89 mg starch g^{-1} MS). The root was the most important starch storage organ according to what was reported by Lacointe (2001) and Ludovici *et al*. (2002) in studies carried out on trees.

In this study it was found that in the two phenological stages the accumulation of starch occurred in greater proportion in root and trunk, which shows a storage of starch in a balanced way that prevents plugging that would prevent the transport of other elements such as sugars and amino acids to accumulate in the winter stage, since the accumulation of the starch is carried out in the vicinity of the cells near the xylem vessels (Tromp, 1983; Fisher and Höll, 1992; Sauter and Van Cleve, 1994) and allows a transport fast at the time of sprouting.

This may also indicate that this growth takes place during the production stage, while root growth is carried out in the resting stage as reported by Valenzuela (2006); Valenzuela *et al*. (2011); Valenzuela *et al*. (2014). This pattern of growth and reactivation of tissues at different phenological stages is very common behavior of porous wood species (Zimmermann *et al*.,1971) as is the case of walnut (González *et al*., 2014).

During dormancy, trees remain defoliated and growth depends exclusively on stored reserves, in latitudes such as those in the Comarca Lagunera, the dormancy stage is characterized by a decrease in photoperiod and environmental temperature, as a consequence, trees enter a period of dormancy, which is preceded during periods of high temperatures in order that the tree can resume the regrowth without problems in spring (Rowland and Arora, 1997; El Zein *et al*., 2011). During this period, C is stored in the form of soluble starch and sugars (Kozlowski and Pallardy 2002).

The results in this study show a higher concentration of starch in the root in relation to the trunk in dormancy, while in the trunk an inverse effect was observed, this could be explained by the fact that in regions with marked climatic seasons in which winter presents Temperatures near the freezing point at certain times of the day, although they are few, can cause irreversible cellular damage in species that are not adapted to these conditions (Repo *et al*., 2008) and as a consequence the adapted trees have developed mechanisms to avoid damage of tissues by low temperatures, in regard to starch, Kramer and Koslowski (1979); Piispanen and Saranpää (2001); Poirier *et al*. (2010); El Zein *et al.* (2011) observed that it is transformed into soluble sugars as a response under these conditions.

Contribution of C-starch to C-total in tree-level biomass

The dry weight in kg of the total biomass of the four trees sampled by perennial organs is presented in Table 5.

Table 5. Total biomass (dry weight in kg) of four trees calculated using the models of Drexhage *et al*. (1999) and Brucciamacchie (1982) in the experimental site.

Characteristics	Tree 1	Tree 2	Tree 3	Tree 4
Biomass of the trunk (kg)	281.56	234.39	521.31	425.61
Biomass of branches (kg)	67.17	55.72	125.61	102.25
Biomass of roots (kg)	107.14	86	224.74	176.03
Total biomass of the tree (kg)	408.85	337.19	783.19	632.01

The contribution of C-total to total biomass in dry weight of the four trees and the amount expressed in percentage of C-total, as well as of C-starch in the two phenological stages obtained for each of the trees sampled are presented in in Figure 2.

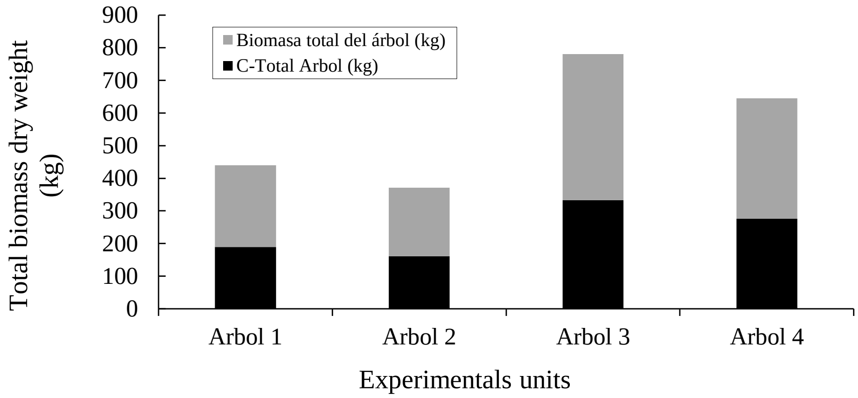

Figure 3. Contribution of total carbon to the biomass of four walnut trees in the experimental plot of the UAAAN-UL in Torreon, Coahuila.

The total carbon contained in the biomass found in this study ranges from 42.62 to 43.36% and coincides with the data obtained by Figueroa (2001); Gayoso and Guerra (2005); Pimienta *et al*., (2007); Vigil (2010); Valenzuela *et al*. (2011); Calderón-Reyes and Solis-Urbina (2012) for trees. These data are a starting point from the point of view of environmental services (Mexhal and Herrera, 2014). The contribution of carbon from starch in walnut represents 1.34 to 3.64% with respect to the total tree biomass according to Barbaroux *et al*., (2003); Valenzuela *et al*., (2011); Hoch *et al*., (2003); Damesin and Lelarge (2003).

Conclusions

The perennial organs in walnut that showed variation in the accumulation of starch were the root and the trunk, these organs presenting an inverse behavior in concentration depending on the phenological stage. The walnut presented a behavior in the distribution of starch and total carbon and the carbon coming from the starch inside the perennial organs similar to that of other deciduous species, both forest and fruit trees and that present porous wood. The root turns out to be the organ that has the highest concentration in starch during the physiological stage of dormancy or winter rest, this can be explained by the growth of this organ during this vegetative stage, while the trunk presents a higher concentration in the phenological stage of production, since it is at this stage when the growth in diameter occurs, a behavior characteristic of the deciduous species.

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