Dynamics of nonstructural carbohydrates and biomass yield in a fodder legume tree at different harvest intensities

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Summary  Tropical tree fodder is harvested by frequent prunings, and resprouting depends on nonstructural carbohydrate reserves in the remaining tree parts. We studied the effects of three pruning intensities (removal of all leaves and branches leaving 1 m of stem once a year (T-12), or every 6 months (T-6), and about 50% pruning every 2 months (P-2)) on regrowth and the dynamics of soluble sugars and starch in the legume tree Gliricidia sepium (Jacq.) Walp. growing under humid tropical conditions in Guadeloupe, Lesser Antilles. Carbohydrates were sampled in roots, stems and branches. Among pruned trees, trees in the T-6 harvest regime had the highest leaf fodder yield (0.73 kg tree–1 year–1). High litter loss reduced leaf yield of T-12 trees, but compared with the other treatments, T-12 trees produced the most branch biomass (3.43 kg tree–1). Among treatments, P-2 trees had an intermediate leaf fodder yield and the lowest branch production. Sucrose, glucose and fructose were the most common sugars in all biomass compartments. Mannose, pinitol and an unidentified cyclitol were relatively abundant in branches. Root sugar and starch concentrations were unaffected by harvest regime. There was a significant interactive effect of harvest intensity and regrowth time on stem sugar concentration. Stem starch concentration was highest in T-12 trees. After a year of fodder harvesting, whole-tree reserves of nonstructural carbohydrates were highest in T-12 trees; however, a larger proportion of reserves were located in roots and stems of T-6 and P-2 trees. These reserves, which were not lost in pruning and contributed to regrowth of G. sepium after pruning, may explain the relatively small effects of harvesting regime on soluble sugar and starch concentrations.

Keywords: agroforestry, defoliation, Gliricidia sepium, humid tropics, soluble sugars, starch.

Introduction

Alley cropping is a production system, widely used in tropical agroforestry, in which crop or forage species are cultivated in alleys between tree hedgerows. The trees are periodically pruned during the cropping season to prevent shading and provide green manure for the companion crop (Kang et al. 1981), or the tree prunings may be used for fuelwood or forage (Singh et al. 1989). Pruning interval and frequency depend on management objectives: long intervals are used for fuel-wood production and short intervals are used for forage production, because leaf production peaks soon after pruning (Duguma et al. 1988).

Gliricidia sepium (Jacq.) Walp. (Papilionaceae: Robinieae) is an important tree forage crop in many parts of the tropics. The leaves have a high nutritive value with protein comprising 20–30% of dry matter (Simons and Stewart 1994). The optimum pruning interval for forage production by G. sepium is 3–6 months, because a longer cutting interval decreases the proportion of leaves in new biomass (Chadhokar 1982). In an association of G. sepium and Panicum maximum Jacq. cv. Ntchisi, the highest tree and grass forage production was obtained when pruning was performed every 3 months (Ezenwa et al. 1995). Shortening the harvest interval from 8–12 weeks to 4 weeks decreased leaf yields and increased tree mortality to 50% (Ezenwa and Atta-Krah 1992). A cutting interval of 3 months lowered survival and biomass production of Sesbania grandiflora (L.) Pers. (Duguma et al. 1988). In Erythrina berteroana Urb., highest biomass production was achieved by cutting every 6 months. A 2-month pruning interval resulted in high tree mortality, whereas a 4-month pruning interval decreased biomass production over time (Romero et al. 1993).

There is evidence that regrowth of pruned trees depends on carbohydrate reserves. Several studies on temperate deciduous trees have shown that carbohydrate reserves are depleted as a consequence of defoliation and coppicing regimes (Blake 1983, Kays and Canham 1991, Tschaplinski and Blake 1994, 1995). Defoliation of silver birches (Betula pendula Roth) late in the growing season decreases the concentration of soluble sugars, and delays leaf emergence after dormancy (Raitio et al. 1994). In contrast, pruning Acer rubrum L., Betula populifolia Marshall, Fraxinus americana L. and Prunus serotina Ehrh. trees at the beginning or end of the growing season increased
starch concentration, whereas starch accumulation was decreased in trees pruned in the middle of the growing season (Kays and Canham 1991).

Usually, the most marked effects of pruning are seen in roots and the lower parts of the stem. In hybrid poplar (Populus maximowiczii × P. nigra L. “MN9”), complete shoot defoliation decreased stem starch concentration to half that of intact plants. Partial shoot defoliation (50%) and total removal of buds did not affect starch reserves. In both treatments, the sucrose concentration was depleted (Tschaplinski and Blake 1994). In citrus trees, removal of one third of the canopy reduced the soluble sugar and starch concentration in fine roots (Eissenstat and Duncan 1992). Under humid tropical conditions, defoliation of Cedrela odorata L. caused a decrease in starch reserves, especially in large roots and the lower parts of the stem (Rodgers et al. 1995).

The aim of the present work was to study the effects of three forage harvesting intensities on the dynamics of regrowth and nonstructural carbohydrate reserves in Gliricidia sepium growing under humid tropical conditions. We tested the hypothesis that regrowth rate following pruning depends on nonstructural carbohydrate reserves in the remaining tree biomass. Gliricidia sepium was grown with tropical forage grasses in a cut-and-carry forage production system, in which both pruned branches and cut grass were removed from the site as feed for animals. The objective of this production system is to improve land-use efficiency in the densely populated Caribbean islands by means of two-storied forage production.

Materials and methods

Field site

The study was carried out on the experimental farm of the Antillean Research Centre of the Institut National de la Recherche Agronomique (INRA) in Prise d’Eau, Guadeloupe (16°12' N, 61°39' W, 125 m a.s.l.). Soils are alluvial Oxisols with a pH of 6.0 and relatively high cation exchange capacity (13.5 meq 100 g⁻¹) and calcium concentration. The organic matter content of upper soil layers is about 35 g kg⁻¹ and nitrogen content is about 2.5 g kg⁻¹. The soil is uniform to a depth of 0.5 m (Nygren and Cruz 1998).

The experiment was established in May 1993. Gliricidia sepium trees were planted in rows with 0.7 m between trees and 3 m between rows, totalling 4760 trees per hectare. The total area of the experiment was 0.4 ha. The between-row alleys were initially planted with Pangola grass (Digitaria decumbens Stent.), but Bahia grass (Paspalum notatum Flügge) soon invaded the plot. At the time of the study, P. notatum dominated the grass layer (about 80% of grass biomass), and D. decumbens was the second most common grass species.

Between May 1993 and March 1995, all trees were partially pruned every 3–6 months, depending on tree growth, to minimize excessive shading of the grass layer. In April 1995, an 800 m² area comprising nine rows of G. sepium trees and eight grass alleys was separated from the main experiment for studies of pruning intensity (Nygren and Cruz 1998). On February 29, 1996, the trees were pruned completely and the experiment was divided into nine 7 × 12-m plots, each extending over four G. sepium hedgerows and three grass alleys. Three harvesting regimes (each replicated three times) were assigned randomly to the plots: removal of all leaves and branches leaving a 1-m high stem once a year (T-12), or every 6 months (T-6), and partial pruning every 2 months (P-2). In the P-2 regime, defoliation intensity averaged 56.1 ± 11.3% in five prunings. The experiment was completed on January 31, 1997.

Weather conditions during the experiment were typical for this humid tropical area (Figure 1). Total rainfall was 2388 mm and potential evapotranspiration estimated by the formula of Penmann (Rosenberg et al. 1985) was 1167 mm. A slight hydrologic deficit of 36 mm developed during the first 2 months of the experiment from March 1 to April 26, 1996. Mean maximum temperature for the 2-month pruning intervals of the P-2 regime varied from 27.9 to 30.0 °C, and the corresponding mean minimum temperature varied from 19.5 to 22.6 °C. Mean global radiation varied from 12.7 to 19.9 MJ m⁻² day⁻¹.

Biomass measurements

At each pruning, aboveground biomass of G. sepium was estimated by applying the pipe model theory (Nygren et al. 1993, Nygren and Cruz 1998). The diameters of all branches of eight trees per plot were measured below the lowest green leaf. Fresh leaf area, and leaf and branch dry mass (48 h at 70 °C) were determined for two branches per sample tree in the partial pruning treatment (P-2) at the first two prunings (April 26 and June 20, 1996), and thereafter for a branch in each sam-

![Figure 1. Cumulative rainfall, potential evapotranspiration (PET) and mean (± SE) of daily maximum temperature (Tmax), minimum temperature (Tmin) and global radiation (Rg) at the study site during the 2-month pruning intervals of the P-2 harvest regime (50% pruning every 2 months) of Gliricidia sepium trees. The first pruning interval for trees in the T-6 regime (total pruning every 6 months) was through Week 24 and the second pruning interval was from Weeks 24 to 48. The carbohydrate sampling of trees in the T-6 and P-2 regimes was carried out from Week 23 to 47 (August 9, 1996 to January 24, 1997). The study started on February 29, 1996.](attachment:image)
ple tree, as the canopy structure was modified by the cutting regimes. Linear regression equations were fit to the data to estimate the variables as a function of branch cross-sectional area.

Three 30 × 30-cm litter collectors were placed on each plot after 10 weeks of regrowth. Litter was collected weekly and its dry mass (48 h at 70 °C) determined. Litterfall was computed on a land area basis (kg ha⁻¹), and this value was divided by the number of trees (4760 ha⁻¹) to estimate litter loss per tree. Flowers of *G. sepium* were observed only in Week 48. The flowers on each sample branch were dried and weighed separately. The dry mass of flowers per tree was estimated as:

\[
M_t \text{(sample)} = \frac{b_t \cdot b_f}{n_t},
\]

where \(M_t\) (tree) is dry mass of flowers per tree, \(M_t\) (sample) is total mass of flowers in the sample branches of a plot, \(b_t\) is number of branches with flowers, \(b_f\) is number of branches in the sample, \(b_s\) is total number of branches per plot and \(n_t\) is number of trees per plot.

In February 1997, a randomly selected tree per plot was cut at the root collar to determine stem mass. The entire stem was weighed. Four discs, distributed evenly from 5 cm above the root collar to 5 cm below the stem top, were cut and weighed fresh and after drying (48 h at 70 °C) to determine wood humidity. Stem fresh mass was multiplied by mean dry-to-fresh mass ratio of the discs (0.50 ± 0.024 standard deviation) to estimate stem dry mass. Cumulative biomass production was estimated as the sum of standing biomass change, harvest and litterfall during the 2-month-period between the measurements of standing biomass.

### Carbohydrate sampling

In the T-12 cutting regime, monthly sampling of trees for carbohydrate analyses was initiated on March 8, 1996, 1 week after the initial pruning. Sampling of trees in the T-6 and P-2 regimes began on August 9, 1996, just before the total pruning of trees in the T-6 regime, and the third partial pruning of trees in the P-2 regime. Thereafter, sampling of trees in all regimes was carried out every 2 weeks from August 9 to October 4, 1996 and then monthly until the end of the experiment on January 31, 1997.

A pool of 16 trees per plot was selected for carbohydrate sampling. On each sampling date, four randomly selected trees were sampled. A stem core per tree, extending from bark to the center of the stem, was removed with an increment borer at a height of 15–25 cm above the ground. For root sampling, the soil around the stem was removed until a main root was found. The root sample was taken by cutting a piece from the bark to the center of the root with a sharp knife. We sampled roots of different sizes from every tree to get a representative sample of coarse root biomass. The branch samples were taken just before each P-2 pruning from Week 23 through Week 47. One branch per tree was cut and divided into unlignified green twig and woody branch. Sample discs were taken from the top, center and base of both parts. No leaves were sampled. All samples were pooled by plot and biomass compartment for analyses. If a tree was included in two consecutive samplings, it was excluded from the two following samplings to avoid excessive sampling damage.

Samples were placed in ice in the field and transported to a freezer (−20 °C) within an hour. Root samples were washed with deionized water before freezing to remove soil. Samples were stored in 95% ethanol at −20 °C until analyzed.

### Carbohydrate analyses

Samples were removed from the ethanol in which they had been stored and dried for 2 to 3 days at room temperature. The dried samples were frozen in liquid N and ground with a planetary mill (Pulverisette 6, Fritsch GmbH, Idar-Oberstein, Germany). The ground samples were suspended in storage ethanol to extract soluble sugars and then centrifuged at 14,360 g for 10 min. The supernatant was saved, and the pellet re-extracted in 5 ml of 95% ethanol and re-centrifuged. The pellets were combined and oven dried. The supernatants were combined and the ethanol evaporated in a rotary evaporator.

The dry sugar extract was diluted with 5 ml of MQ-filtered water. The solution was stored at –20 °C until analyzed by high performance liquid chromatography (HPLC).

The oven dried pellet was weighed and its mass summed with the mass of sugar extracted to determine dry mass of the samples. Starch in the pellet was alkali extracted and solubilized with 18 ml of 0.5 M NaOH for 30 min. The pH of the sample was adjusted to 4.8 with 2.0 M acetic acid (Dekker and Richards 1971). Starch was hydrolyzed to glucose by the addition of lyophilized amyloglucosidase (EC 3.2.1.3.) from *Aspergillus niger* (Sigma Chemicals A7420) (Method VTT-4336-91 of the Food Laboratory, State Technical Research Centre (VTT), Helsinki, Finland) and analyzed by HPLC.

The HPLC analyses were performed on a Waters 600 E (Waters, Milford, MA) equipped with a pulsed amperometric detector (PAD) (DECADE; ANTEC Leyden, Leiden, The Netherlands). Sample injections were made with a 10-µl sample loop. The soluble sugars were separated in a Waters Sugar Pak Ca-cation exchange column protected with a Sugar Pak guard column, with 0.01 M Ca EDTA as the eluent. The flow rate of the mobile phase was 0.5 ml min⁻¹ and the column temperature was 90 °C. A post-column pump was used to adjust the pH to 12 with NaOH to ensure proper PAD function.

Detection was achieved by triple-pulsed amperometry (Johnson and LaCourse 1990) with a gold working electrode. The pulse potential (E) and duration (τ) were: \(E_1 = 150 \text{ mV}, \quad τ_1 = 400 \text{ ms} \) for measuring carbohydrate oxidation, \(E_2 = 750 \text{ mV}, \quad τ_2 = 120 \text{ ms} \) for cleaning the electrode surface, and \(E_3 = −800 \text{ mV}, \quad τ_3 = 130 \text{ ms} \) for reducing gold oxide to gold.

The HPLC data were analyzed with Baseline HPLC software (Waters). Soluble sugars were identified and quantified by comparing peak retention times and areas with those of standard sugars. The reproducibility of the HPLC analyses...
was good.

Peak identification was confirmed by gas chromatography–mass spectrometry (GC–MS) analysis on a randomly selected subset of 24 samples (10% of all samples). The soluble sugars were analyzed as trimethylsilyl (TMS) derivatives. After a 30-min incubation at 80 °C, the TMS-derivatized sugars were subjected to gas chromatography (5890 series II, Hewlett Packard Co., Palo Alto, CA) followed by mass spectrometry (5988A, Hewlett Packard Co.) to quantify soluble sugars (Brittain et al. 1971). The TMS-derivatives of sugars were determined in a 25 m HP-5 (5% phenyl methyl siloxane) column (Hewlett Packard Co.) with an internal diameter of 0.2 mm and a film thickness of 0.33 μm. The column-temperature program started at 110 °C. The temperature increased 10 °C min⁻¹ to the final temperature of 300 °C, which was held for 36 min. Helium with 100 kPa inlet pressure served as the carrier gas. TMS-Sugars were identified by co-chromatography of authentic TMS-derivatives by GC–MS (HP 6890, Hewlett Packard Co.).

The GC–MS analysis revealed two cyclitols that were not detected in the HPLC analysis. One of them was pinitol-like but could not be identified. The other cyclitol, pinitol, had the same retention time in the HPLC analysis as glucose from which it could not be separated. Thus, these two carbohydrates were expressed as glucose in the HPLC results. Mean error in total sugar contents due to the overlap of glucose and pinitol peaks was estimated on the basis of HPLC peaks for pure glucose and pinitol standards, and the mass proportions of these sugars in the GC–MS analysis. We first calculated the maximum error ($E_m$), or the case if the whole peak quantified as glucose were pinitol, from the regression coefficients of the HPLC calibration lines for glucose ($\beta_g$) and pinitol ($\beta_p$) as:

$$E_m = \frac{\beta_g - \beta_p}{\beta_g}.$$  

The value of $E_m$ was 0.431; the glucose standard peak was higher than the pinitol standard peak. Mean error in the glucose-pinitol group ($E_g$) and total sugar ($E_t$) concentrations determined by HPLC was calculated as:

$$E_g = E_m \frac{P_p}{P_p + P_g},$$  

$$E_t = E_m P_g,$$

where $P_p$ and $P_g$ are mean mass proportions of pinitol and α-glucose + β-glucose, respectively, in the GC–MS analyses.

**Results**

**Aboveground biomass production of Gliricidia sepium**

Standing foliage biomass increased in the T-12 trees during the 6 months following pruning, through August 1996, and decreased afterwards because of high litterfall (Figure 2a). Growth of T-6 trees followed the same pattern as T-12 trees until they were pruned on August 15, 1996 (Figure 2c). The productivity of T-6 trees was lower during the second half of the year than at the beginning. Standing foliage biomass of P-2 trees remained relatively low throughout the study (Figure 2e). Cumulative foliage production was highest in T-6 trees, but both cumulative branch production and standing branch biomass were highest in T-12 trees at the end of the study (Figure 2b). The trees were preparing for flowering by the end of the experiment, as indicated by the decreases in foliage biomass in the T-6 and T-12 trees (Figures 2a and 2c). However, at the end of the experiment (January 31, 1997), flower biomass was low (70.5 g tree⁻¹) in T-12 trees and negligible in T-6 and P-2 trees.

Among treatments, total annual forage harvest was highest for T-6 trees (Table 1). Although considerable loss of foliage occurred from T-12 trees as a result of leaf fall, branch harvest was highest for these trees. The P-2 regime resulted in the lowest litter loss and branch production, and the forage harvest was 56% lower than in the T-6 regime (Table 1). Differences
in harvestable biomass and litterfall were significant between all pruning regimes for all biomass compartments (ANOVA followed by Duncan’s multiple range test (MRT) at P = 5%). At the end of the study, mean (± SE) stem biomass was significantly lower in T-6 trees (1.78 ± 0.23 kg tree−1) and P-2 trees (1.34 ± 0.13 kg tree−1) than in T-12 trees (3.02 ± 0.44 kg tree−1). In the plot adjacent to our experiment, trees were subjected to consecutive complete and partial prunings every three months, two trees were sampled for root mass in March 1997 and four trees in June 1997. Mean root biomass of these six trees was 2.06 kg tree−1 (Lecompte 1997).

**Identification of sugars**

Based on GC–MS analysis, sucrose was the predominant sugar in all biomass compartments, followed by glucose. Mannose, pinitol and an unidentified cyclitol occurred in quite high concentrations in branches (Table 2).

Among tree organs, the error in the HPLC quantification of the glucose-pinitol group was highest in green twigs and woody branches (Table 3). The error in the glucose-pinitol group in roots and the error in total sugars in all biomass compartments were small. The total sugar + cyclitol concentration was quantified as glucose, based on HPLC analyses. The very low sugar concentrations in both stem and root of trees in all pruning regimes on October 31, 1996 were probably caused by a sample handling error in the field, and are thus omitted from the following analyses. Within-treatment variation was high, especially in root tissue and in trees in the T-12 regime.

The total concentration of soluble sugars plus pinitol was higher in roots than in stems in all pruning regimes (Figure 3). During the first half of the year, the root sugar concentration in T-12 trees was over 30 mg g−1 (Figure 3a). During the latter half of the year, the root sugar concentrations fluctuated between 10 and 20 mg g−1 in all harvest regimes. The dominant sugar in roots was sucrose. In stem tissue, the sugar concentration fluctuated in all harvest regimes; however, it was somewhat lower in P-2 trees, although it increased at the end of the experiment (Figure 3c). In all treatments, there was a gradual increase in stem sugar concentration over time. By the end of the study, stem sugar concentration increased and root concentration decreased in all pruned trees.

Factorial ANOVA of the effects of harvest intensity and sampling date on the concentration of total soluble sugars in root and stem indicated that the interaction of harvesting regime and date caused the most pronounced effects on total soluble sugar concentration; in roots it explained 29% of the variation and in stem tissue about 37% (Table 4). However, the effects were significant in stem tissues, but not in roots. Although the F-test failed to detect significant differences between harvest regimes (Table 4), Duncan’s MRT indicated that mean total sugar concentration in stem tissues was significantly higher in T-6 trees than in P-2 trees. The value was intermediate in T-12 trees.

**Stem and root starch dynamics**

Among pruning treatments, highest starch concentrations were observed in T-12 trees (Figure 4). Within-treatment variation was higher in root than in stem in all harvest regimes. In stem tissue, the highest starch concentrations were above 100 mg g−1 (Figure 4a). Mean stem starch concentrations were 69.6, 56.3 and 48.9 mg g−1 in T-12, P-2 and T-6 trees, respectively. Starch concentrations fluctuated in T-6 trees, and there was a gradual decrease toward the end of November. In P-2 trees, starch concentration in stem tissue was low during the first four samplings and increased in September.

In root tissue, the starch concentration was relatively stable in T-12 trees on most days (Figure 4b). In T-6 trees, root starch concentration increased sharply from the middle of August onward and then decreased again in October. Starch concent-

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**Table 1.** Annual harvestable aboveground biomass production (kg tree−1 year−1) and litterfall (kg tree−1 year−1) of *Gliricidia sepium* trees subjected to three forage harvest intensities (T-12 = total pruning every 12 months, T-6 = total pruning every 6 months, and P-2 = 50% pruning every 2 months). Values are means ± standard errors of three plots.

<table>
<thead>
<tr>
<th>Harvest intensity</th>
<th>Foliage harvest</th>
<th>Foliage litter</th>
<th>Branch harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-12</td>
<td>0.147 ± 0.010</td>
<td>0.667 ± 0.040</td>
<td>3.428 ± 0.411</td>
</tr>
<tr>
<td>T-6</td>
<td>0.725 ± 0.062</td>
<td>0.403 ± 0.039</td>
<td>2.310 ± 0.208</td>
</tr>
<tr>
<td>P-2</td>
<td>0.410 ± 0.114</td>
<td>0.205 ± 0.010</td>
<td>0.970 ± 0.304</td>
</tr>
</tbody>
</table>

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**Table 2.** Mean mass sugars (%) observed in biomass compartments of *Gliricidia sepium* trees based on gas chromatography–mass spectrometry.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Arabinose</th>
<th>Fructose</th>
<th>α-Glucose</th>
<th>β-Glucose</th>
<th>Mannose</th>
<th>Myo-inositol</th>
<th>Pinitol</th>
<th>Sucrose</th>
<th>Unidentified Cyclitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>1.6</td>
<td>2.6</td>
<td>9.9</td>
<td>13.3</td>
<td>1.6</td>
<td>1.5</td>
<td>2.8</td>
<td>61.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Stem</td>
<td>0.4</td>
<td>1.2</td>
<td>5.3</td>
<td>7.9</td>
<td>0.7</td>
<td>0.6</td>
<td>4.2</td>
<td>76.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Green twig</td>
<td>1.1</td>
<td>3.1</td>
<td>10.1</td>
<td>13.4</td>
<td>9.3</td>
<td>1.3</td>
<td>9.1</td>
<td>31.7</td>
<td>9.1</td>
</tr>
<tr>
<td>Woody branch</td>
<td>3.5</td>
<td>2.9</td>
<td>10.6</td>
<td>13.0</td>
<td>5.1</td>
<td>1.1</td>
<td>10.0</td>
<td>41.6</td>
<td>12.1</td>
</tr>
</tbody>
</table>

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**Figure 3** shows the dynamics of the main soluble sugars, including the glucose-pinitol group quantified as glucose, based on GC–MS analysis.
Trations were lower in the P-2 trees than in the other pruned trees and fluctuated more. The trends in starch concentration in root tissue were similar in trees in all treatments. Date had the most important effect on starch concentration in both root and stem, explaining 21% of the variation in roots and 33% of the variation in stem (Table 5). All of the analyzed effects were statistically significant in stems, but not in roots. Mean stem starch concentration was significantly higher in T-12 trees than in the other pruned trees (Duncan’s MRT).

Nonstructural carbohydrates in branches

The mean error in glucose + pinitol concentration estimated by HPLC and the mass proportion of unidentified cyclitol were higher in branches than in other plant compartments (Tables 2 and 3). Therefore, we present only the total sugar estimates for woody branches and green twigs (Figures 5a and c). Soluble sugar concentration was higher in green twigs than in woody branches. In green twigs, the concentration of soluble sugars was highest in T-6 trees followed by P-2 trees.

Differences in starch concentrations between twigs and branches were small (Figures 5b and d). Starch concentration was higher in T-12 trees than in the other pruned trees, and it increased gradually toward the end of the experiment. The percentage of soluble sugars comprising total nonstructural carbohydrates (sugars and starch) was higher in the frequently pruned T-6 and P-2 trees (68 and 62%, respectively) than in T-12 trees (42%).

Total nonstructural carbohydrate reserves

Total nonstructural carbohydrate reserves were estimated by multiplying tree biomass (g tree⁻¹) by the carbohydrate concentration (g g⁻¹), and assuming that each biomass compartment had about the same carbohydrate concentration as the analyzed sample. For branches, the accumulation of total carbohydrate reserves was calculated for the period August 9, 1996 to January 24, 1997 (Table 6). In all harvest regimes, the total starch content increased throughout the experiment. The sugar content showed no clear pattern, except in T-6 trees (Table 6), where it increased at the end of the experiment with increasing tree biomass. In the T-12 and P-2 trees, the decrease

Table 3. Mean overestimation of mass concentration of the glucose-pinitol group and total sugars in different compartments of *Gliricidia sepium* trees resulting from the overlap of glucose and pinitol peaks following HPLC. The estimate is based on mean mass percentages of both α- and β-glucose and pinitol determined by gas chromatography–mass spectrometry (Table 2). See Equations 2–4 for details of calculation.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Mean error %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose-pinitol group</td>
<td>Total sugars</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>4.6</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td>10.4</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Green twig</td>
<td>12.0</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Woody branch</td>
<td>12.8</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Mean concentrations of the four most common soluble sugars in the stems (above thick line) and roots (below thick line) of *Gliricidia sepium* trees subjected to three forage harvest intensities (T-12 = total pruning every 12 months, T-6 = total pruning every 6 months, and P-2 = 50% pruning every 2 months). During the sampling period, trees in the T-12 harvest regime were pruned on February 29, trees in the T-6 regime were pruned on August 15, and trees in the P-2 regime were pruned on August 15, October 10 and December 5.

Table 4. Summary of the factorial analysis of variance of effects of forage harvest intensity (T-12 = total pruning every 12 months, T-6 = total pruning every 6 months, and P-2 = 50% pruning every 2 months) and sampling date on the total concentration of soluble sugars in the roots and stems of *Gliricidia sepium* trees. The ANOVA was computed for the period from August 9, 1996 through January 24, 1997 when data were available for all pruning regimes. The data for October 31, 1996 were excluded from the analyses.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Root</th>
<th></th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>SS</td>
<td>P &gt; F</td>
</tr>
<tr>
<td>Harvesting</td>
<td>2</td>
<td>168</td>
<td>0.605</td>
</tr>
<tr>
<td>Date</td>
<td>7</td>
<td>807</td>
<td>0.670</td>
</tr>
<tr>
<td>Interaction</td>
<td>14</td>
<td>3325</td>
<td>0.180</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>6950</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>11,333</td>
<td>0.39</td>
</tr>
</tbody>
</table>

r²
in biomass at the end of the study caused a decrease in the total reserves of soluble sugars.

When whole-tree nonstructural carbohydrate reserves were estimated at the end of the experiment (Figure 6), we found that the biggest effect of harvest regime was on the nonstructural carbohydrate content of branches. In T-12 and T-6 trees, more than 20% of tree total soluble sugars was found in branches, whereas in P-2 trees only 2% of tree total sugar reserves was localized in branches. Branch starch reserves comprised 35, 10 and 6% of the whole-tree starch reserves in T-12, T-6 and P-2 trees, respectively.

Discussion

Starch was the major reserve carbohydrate in most biomass compartments of all pruned trees and the highest concentration of starch was observed in T-12 trees. The total concentration of soluble sugars + pinitol was higher than the starch concentration only in green twigs of T-6 and P-2 trees. Starch concentration in stem tissue was lower than starch concentration in root tissue in T-12 and T-6 trees. In T-6 trees, starch concentration was about 40% lower in the stems than in the roots, indicating that the effects of pruning are first seen in the stem. Erdman et al. (1993) observed that stem starch reserves were enough to support initial coppice shoot growth in G. sepium. In P-2 trees, which were most affected by forage harvest treatment, starch concentration was low in both roots and stems, perhaps indicating that the root starch reserves were mobilized toward the canopy in P-2 trees.

Sucrose was the most abundant soluble sugar. Among tree organs, the highest sucrose concentration was observed in root tissue, where it may be needed to maintain symbiotic dinitrogen fixation (cf. Vance 1990). In T-12 trees, the root sucrose concentration was high especially at the beginning of the experiment, but at the end of the experiment, the sucrose concen-
trations in stems and roots were similar. If N₂ fixation by *G. sepium* follows the same yearly dynamics as observed at another site in Guadeloupe (Nygren et al. 2000a), N₂ fixation may have been more active during the first half of the experiment, and the high root sucrose concentration may reflect the energetic requirements of N₂ fixation. Sucrose transport to the stem and branches during the second half of the experiment may have corresponded to the preparation for flowering.

Higher sugar concentrations than starch concentration in green twigs, especially in T-6 and P-2 trees, may have been a consequence of pruning. It has been shown that pruning changes carbohydrate metabolism so that monosaccharides are mobilized instead of sugar polymers, and starch reserves are hydrolyzed to soluble sugars (Tschaplinski and Blake 1989). The high sugar concentration in twigs is probably an indication of active metabolism before resprouting.

Starch concentrations in roots and branches of P-2 trees increased toward the end of the experiment. In a previous study, Nygren et al. (2000b) found that 50% defoliated *G. sepium* seedlings accumulate amino acids under greenhouse conditions. Nodule biomass in relation to leaf biomass has also been observed to increase (Nygren et al. 2000a).

In a short-term study, forage harvest was 30% higher in the P-2 regime than in the T-6 regime (Nygren and Cruz 1998). However, in the longer term, biomass production and forage yield in heavily pruned trees are likely to decrease (Table 1; cf. Ezenwa and Atta-Krah 1992, Romero et al. 1993). In our study, the highest forage harvest was obtained from T-6 trees in which pruning did not greatly affect reserve carbohydrates because most of the reserves were in roots and stems that were not harvested.

Table 6. Total sugar and starch contents in branches (green twigs and woody branches combined) of *Gliricidia sepium* trees at three forage harvest intensities (T-12 = total pruning every 12 months, T-6 = total pruning every 6 months, and P-2 = 50% pruning every 2 months). The sampling on August 9, 1996 was conducted before the T-6 and P-2 prunings, the sampling on January 24, 1997 was conducted before all of the pruning treatments, and other samplings were conducted before the P-2 prunings. Abbreviation: n.d. = no data.

<table>
<thead>
<tr>
<th>Date</th>
<th>T-12</th>
<th></th>
<th></th>
<th>T-6</th>
<th></th>
<th></th>
<th>P-2</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Regrowth</td>
<td>Starch</td>
<td>Sugar</td>
<td>Regrowth</td>
<td>Starch</td>
<td>Sugar</td>
<td>Regrowth</td>
<td>Starch</td>
<td>Sugar</td>
</tr>
<tr>
<td></td>
<td>(week)</td>
<td>(g tree⁻¹)</td>
<td>(g tree⁻¹)</td>
<td>(week)</td>
<td>(g tree⁻¹)</td>
<td>(g tree⁻¹)</td>
<td>(week)</td>
<td>(g tree⁻¹)</td>
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</tr>
<tr>
<td>August 9, 1996</td>
<td>23.0</td>
<td>18.0</td>
<td>28.7</td>
<td>23.0</td>
<td>13.4</td>
<td>10.8</td>
<td>7.1</td>
<td>6.5</td>
<td>1.6</td>
</tr>
<tr>
<td>October 4, 1996</td>
<td>31.0</td>
<td>128.6</td>
<td>4.2</td>
<td>7.1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>7.1</td>
<td>7.9</td>
<td>0.9</td>
</tr>
<tr>
<td>December 2, 1996</td>
<td>39.4</td>
<td>156.2</td>
<td>35.3</td>
<td>15.6</td>
<td>13.3</td>
<td>3.5</td>
<td>7.6</td>
<td>15.6</td>
<td>7.6</td>
</tr>
<tr>
<td>January 24, 1997</td>
<td>47.0</td>
<td>286.5</td>
<td>38.8</td>
<td>23.1</td>
<td>38.8</td>
<td>17.0</td>
<td>7.1</td>
<td>17.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Seasonal variation in weather at the study site was small (Figure 1). *Gliricidia sepium* is native to the seasonally dry Pacific lowlands of Central America, where it drops leaves and flowers during the dry season. Leaf emergence occurs at the beginning of the rainy season, after seed dispersal (Simons and Stewart 1994). *Gliricidia sepium* apparently maintains the rhythm of its native region when growing in the humid tropics. In our experiment, only the T-12 trees started to flower. However, trees in all treatments seemed to accumulate carbohydrates in their upper parts at the time of anthesis. We have also observed nitrogen accumulation in twigs of pruned *G. sepium* at the beginning of the flowering season (Nygren et al. 2000a). If *G. sepium* trees growing in their natural range are pruned at the beginning of the dry season, they resprout and remain leafy throughout the dry season (Hernández and Benavides 1994).

Whole-tree carbohydrate reserves were highest in T-12 trees, and similar in T-6 and P-2 trees. Branches contained a minor fraction of the whole-tree reserves of nonstructural carbohydrates, which may partially explain the apparent lack of response of sugar and starch reserves to pruning. We conclude that only a small part of whole-tree reserves was removed in the pruning, and that stem and root reserves are sufficient to supply the carbohydrate required for regrowth. Trees in the P-2 treatment were most affected by pruning, even though loss
of carbohydrate reserves in branch biomass was low in this regime, and stem and root reserves were relatively stable. In response to the P-2 regime, reduced photosynthetic production may reduce productivity more in the long term than the lack of carbohydrate reserves for regrowth.

Acknowledgments

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References


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