Variation in Carbohydrate Reserves and Dry Matter Production of Bush Tea (*Athrixia phylicoides*) Grown under Different Environmental Conditions

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Abstract. Reserve carbohydrates are critical for herbage yields, productivity as well as management strategies of bush tea (*Athrixia phylicoides* DC). This study was conducted to evaluate carbohydrate accumulation in response to pruning seasons (summer, autumn, winter, and spring) involving different organs grown under different conditions and to determine mean dry matter production of bush tea. Three separate parallel trials were conducted under wild, field, and glasshouse conditions. Seasons and different growing sites were considered as treatments. Treatments for all controlled trials (field and glasshouse) consisted of seasonal pruning (winter, spring, summer, and autumn). Trials were arranged using a randomized complete block design with 25 single plants as replicates per treatment. Seasonal responses revealed that winter had the highest starch (145.0 mg g⁻¹) in the stems and reserve carbohydrates (480.6 mg g⁻¹) in the roots, whereas in the roots the sugar (400.6 mg g⁻¹) was highest in summer. The highest significant root reserve carbohydrates occurred in winter (594.6 mg g⁻¹) and the lowest in autumn (fall) (313.3 mg g⁻¹). Bush tea plants pruned during winter had the highest overall reserve carbohydrates in the stem (598.7 mg g⁻¹). Under glasshouse conditions, the highest dry matter production was observed in December (midsummer) (170 g per plant); while in field-grown plants in the same month dry matter production was 400 g per plant. Therefore, the best time to maximize production of bush tea is during the spring and summer seasons.

Several studies have demonstrated that reserve carbohydrate levels in trees decrease considerably during periods of rapid growth (Demirtas et al., 2010). Reduced levels of reserve carbohydrates during regrowth have been reported for tea (*Camellia sinensis*) (Kandiah et al., 1984; Selvendran and Selvendran, 1972) and this is attributed to pruning frequency as standard practice during harvesting. It is generally hypothesized that plants mobilize reserve carbohydrates to rebuild photosynthetic tissue after cutting (Demirtas et al., 2010), defoliation (Teague and Smit, 1992), or seasonal loss of foliage (Latt et al., 2000). The authors further reported that soluble sugar concentrations in roots and stems were being maintained consistently at or above the levels in their control, suggesting that sugar levels were maintained through the hydrolysis of starch reserves, whereas no starch replenishment occurred shortly after pruning.

Besides genetic factors, plant physiological responses can vary due to cultural practices such as mineral nutrition (Mudau et al., 2006), harvesting practices (Mudau et al., 2010), and climatic factors during a growing season (Cui et al., 2013; Mudau et al., 2005). The seasonal variation has been reported by Mudau et al. (2007a) and can be ascribed to agronomic practices such as pruning (Mudau et al., 2010). Pruning removes substantial amounts of leaves and branches, resulting in a drastic reduction of photosynthesis products (Marasha et al., 2013). The ability of a bush tea plant to recover from pruning depends on the plant’s starch reserves in the roots (Ndunguru 2004; Panda, 2011). Traditionally, the whole bush tea plant is harvested for medicinal and tea purposes (Maudu et al., 2010; Mudau et al., 2007b); hence the pruning treatments were synchronized as if pruning for tea harvest.

Pruning herbal tea plants is an intensive agronomic practice that affects productivity and quality (Marasha et al., 2013). Tea quality is inevitably related to phytochemical composition and indirectly influences the health benefits of herbal tea. Fluctuations in carbohydrate reserves are also thought to be directly influenced by pruning frequency (Latt et al., 2000), as regrowth of flush depends on the abundance of carbohydrates stored. The quality of tea is determined by pruning frequencies (Panda, 2011), and no studies on bush tea to date have investigated the seasonal variation of carbohydrate accumulation and dynamics as affected by pruning frequencies. Currently, there are small plantations of bush tea cultivation in South Africa and data are lacking, which elucidates the growth pattern and its effect on vegetative flush after pruning. The objectives of the study were a) to determine the response of sugar, starch, and total carbohydrate reserve in all four seasons; b) to investigate the influence of three growing environments (glasshouse, field condition, and wild bush tea) on the reserve carbohydrates allocation on roots, stem, and leaves; and c) to determine the influence of seasons on biomass/mean production of the bush tea.

Materials and Methods

**Study sites.** Wild bush tea. Wild bush tea materials were collected from Mudzidzidzi village in the Limpopo province of South Africa (24°50′ S, 31°17′ E; altitude 610 m above mean sea level), with subtropical climate of summer rainfall, and cold and dry winters. The annual rainfall ranged from 600 to 800 mm.

**Glasshouse trial.** A glasshouse trial was conducted at the University of South Africa Science Campus in Florida, South Africa. Temperatures in the glasshouse were kept between 20 and 24 °C, with humidity at 60%. The photosynthetically active radiation ranged from 134.38 to 157.24 units, whereas chlorophyll content ranged from 30.96 to 44.65 units. The planting materials made up of mature bush tea stock plants were collected from Mudzidzidzi village. Planting materials were selected on the basis of true to name and type, free of disease and insects, and in a healthy physiological state. During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut about 7–8 cm long and were treated with Seradix No. 2 [0.3% Indole-3-butyric acid (IBA)] (Bayer® Pretoria, South Africa) and planted on seedling trays.
on a mist bed, supplied with a misting system operating through misting nozzles. The mist bed used was 3 m long, 1.5 m wide, and 0.5 m high. Light irrigation was done three times a day every day except on rainy days.

Bush tea seedlings were allowed to grow on seedlings trays for 3 months. Rooted cuttings (seedlings) were ready and were transplanted directly into 20-L bags. The medium used during transplanting was pine bark and sand at a ratio of 2:1, respectively. In an attempt to achieve optimum growth, the growing bush tea plants in plastic bags were treated to a split application with N, P, and K at rates 300, 300, and 200 kg ha⁻¹, respectively, as reported by Mudau et al. (2007a) 2 weeks after transplanting and later transplanted to the field.

Field cultivated trial. The experiments on cultivated bush tea were carried out on Morgenzon commercial farm (22°56' 60S, 30°28' 60E; altitude 709 m; summer rain-fall and dry winter). Planting materials were selected on the basis of true-to-type and name, free of disease and insects, and in a healthy physiological state. Meteorological data on temperature (°C), rainfall (mm), relative humidity (%), and evaporation (mm) were supplied by the Agrometerological Division of the Department of Agriculture, Limpopo province (Table 1).

Plant material preparations. Plant materials were collected using methodology stated by Mudau et al. (2006). Plant material was then prepared as described in the glasshouse trial, the rooted cuttings were transplanted directly into the open fields.

Treatments, design, and experimental details

Trial 1. The seasonal effects on the response of root, stem, and leaf carbohydrate reserves of bush tea under field conditions.

The treatments consisted of bush tea cultivated in four seasons viz., autumn, winter, spring, and summer during the 2013/2014 seasons. The experiments started on 1 Mar. 2013 (beginning of autumn) and ended on 28 Feb. 2014 (end of summer). In each season, 50 single plants were planted in a randomized complete block design in the field.

Trial 2. Effects of growing environments on the partitioning of carbohydrates in the root, stem, and leaf of bush tea.

Treatments consisted of bush tea grown separately under glasshouse, field, and wild growing conditions. In the glasshouse and field conditions, 50 single bush tea plants were planted in a randomized complete block design. Thirty bush plants were used as replicates leaving buffer trees. In wild bush tea, 50 plants were randomly tagged.

Trial 3. Seasonal and cumulative dry matter production in bush tea under field conditions and in wild bush tea.

Field conditions. In each season, 50 single plants were planted in a randomized complete block design in the field during 2013 and 2014, respectively. Thirty bush plants were used as replicates leaving buffer trees. Thirty single bush plants were used as replicates leaving buffer trees.

Wild bush tea. Fifty single plants were randomly tagged.

Parameters recorded. At harvest, parameters recorded were soluble sugars (sucrose, glucose, and fructose), starch, and total reserve carbohydrates (the sum of sugar and starch). Samples were separated in plant organs as roots, stems, and leaves, and freeze-dried for analyses.

Sample preparation and carbohydrate analysis

Carbohydrate analysis was conducted using a method described by Latt et al. (2000). Samples were analyzed using the perchloric acid/anthrone method to measure starch and soluble sugars (McCready et al., 1950; Yemm and Willis, 1954). The ethanol was used to extract soluble sugars from the ground samples and the washing was done four times. The supernatants of each sample were then kept in separate flasks to determine the sugar content. The remaining pellets (solid residues) were washed twice with 52% perchloric acid to solubilize the starch in the samples. The solubilized starch solutions were treated with a mixture of an anthrone and sulfuric acid to hydrolyze the starch to glucose, producing a green color. Absorption was measured using a spectrophotometer at 620 nm wavelength; absorbances were regressed against readings from glucose standard solutions. A correlation factor of 0.9 was used in converting glucose equivalents to starch (McCready et al., 1950). Glucose content in the soluble sugar solution was determined by treatment with the anthrone/sulfuric acid mixture (Latt et al., 2000).

Data collected and statistical analysis

Data were subjected to analysis of variance using the general linear model of SAS version (9.2; SAS Institute, Cary, NC). Variables considered for roots, stems, and leaves included soluble sugar, starch, and total reserve carbohydrates (the sum of sugar and starch). Mean separations were done using Duncan multiple range test.

Results

Seasonal response of root carbohydrates allocation of bush tea. Results in Table 2 show that winter exhibited highest starch contents (230.1 mg g⁻¹) followed by autumn (124.0 mg g⁻¹) compared with other seasons. The lowest starch contents were during spring (85.0 mg g⁻¹). The range of highest and the lowest starch content was 147 mg g⁻¹. The soluble highest soluble sugars content was in summer (400.6 mg g⁻¹) with the lowest in autumn (219.6 mg g⁻¹). The range between the highest and the lowest was 180.4 mg g⁻¹ (Table 2). The highest reserve carbohydrates content was observed in winter (550.8 mg g⁻¹), followed by summer, spring, and autumn (483.6 mg g⁻¹, 433.3 mg g⁻¹, and 343.6 mg g⁻¹), respectively.

Table 1. Average seasonal variation in temperature, rainfall, relative humidity, and evaporation during growth of bush tea (2013–14) in Morgenzon commercial farm in the Limpopo province of South Africa.
followed by the field-grown plants (452.8 mg·g⁻¹) and the lowest observed in the wild plants (436.0 mg·g⁻¹) (Table 3). As similar production in the glasshouse (248.7 mg·g⁻¹), followed by the wild (263.8 mg·g⁻¹) and the lowest observed in the glasshouse (248.7 mg·g⁻¹) (Table 3). No significant differences in sugar content were observed in all growing conditions. The content of carbohydrates was higher in bush tea grown under field conditions (433.9 mg·g⁻¹), followed by bush tea from the wild (420.1 mg·g⁻¹). The lowest total carbohydrates were in bush tea grown under glasshouse conditions (409.1 mg·g⁻¹) (Table 3).

Response of partitioning of carbohydrates on the stem, under different growing environments. Starch content was highest in wild bush tea (320.0 mg·g⁻¹), followed by bush tea grown under field conditions (231.3 mg·g⁻¹) and the lowest from bush tea grown under glasshouse conditions (138.7 mg·g⁻¹) (Table 3). A similar trend as to what was observed in starch was seen with sugar content. Sugar content was highest in wild bush tea (140.3 mg·g⁻¹), followed by bush tea grown under field conditions (130.9 mg·g⁻¹) and the lowest from bush tea grown under glasshouse conditions (111.8 mg·g⁻¹) (Table 3). The content of carbohydrates was higher in wild bush tea (460.3 mg·g⁻¹), followed by bush tea grown under field conditions (362.2 mg·g⁻¹). The lowest total carbohydrates were in bush tea grown under glasshouse conditions (250.5 mg·g⁻¹) (Table 3). There was no interaction between growing sites and plant organs.

Seasonal and cumulative dry matter production in wild bush tea. Summer had the highest mean dry matter production ranging from 143.4 to 170.4 g/tree, followed by spring (46.9 to 90.4 g/tree), then autumn (30.8 to 49.1 g/tree) (Table 5). The lowest was recorded from the winter months ranging from 10 to 15 g/tree. Similarly, the lowest number of cuts was in winter (one and two cuts) and the highest obtained during summer (8 to 10 cuts). There was a strong correlation of 91% between number of cuts and mean production g/tree in cultivated bush tea, whereas the correlation of 75% was pronounced in the wild bush tea (Table 6).

Discussion

There is a clear difference in carbohydrate reserves between seasons, and different seasonal trends are observed with variable conditions (Hoffman et al., 2001). Plants generally have been known to have higher carbohydrate reserves in winter (Loescher et al., 1990;
Table 4. Seasonal and cumulative dry matter production in wild bush tea.

| Seasons | Month   | Number of cuts | Mean production (g/tree) |
|---------|---------|----------------|--------------------------|
| Autumn  | March   | 5              | 9.0 f                    |
|         | April   | 6              | 14.0 f                   |
|         | May     | 6              | 45.0 e                   |
|         | June    | 1              | 9.0 f                    |
| Winter  | July    | 2              | 10.0 f                   |
|         | August  | 3              | 80.0 cd                  |
|         | September | 6        | 200.4 c                  |
| Spring  | October | 9              | 300.6 b                  |
|         | November| 10             | 200.4 c                  |
|         | December| 14             | 400.1 a                  |
| Summer  | January | 15             | 200.3 c                  |
|         | February| 14             | 170.2 d                  |

Number of cuts refers to the total number of times trees were pruned before the end date of sample data. Mean with the same letter are not significantly different using Duncan’s multiple range test at the 5% level.

Table 5. Seasonal and cumulative dry matter production in bush tea under field conditions.

| Seasons | Month   | Number of cuts | Mean production (g/tree) |
|---------|---------|----------------|--------------------------|
| Autumn  | March   | 4              | 30.8 e                   |
|         | April   | 4              | 40.9 d                   |
|         | May     | 4              | 49.1 d                   |
|         | June    | 1              | 10.0 f                   |
| Winter  | July    | 2              | 15.0 f                   |
|         | August  | 1              | 10.0 f                   |
|         | September | 5         | 46.9 d                   |
| Spring  | October | 3              | 89.4 e                   |
|         | November| 7              | 90.4 c                   |
|         | December| 8              | 170.4 a                  |
| Summer  | January | 10             | 143.4 b                  |
|         | February| 10             | 169.3 a                  |

Number of cuts refers to the total number of times trees were pruned before the end date of sample data. Mean with the same letter are not significantly different using Duncan’s multiple range test at the 5% level.

Table 6. Seasonal/monthly and cumulative dry matter production from day of planting to harvest of wild and cultivated bush tea.

| Correlation: | Wild bush tea | Cultivated bush tea |
|--------------|---------------|---------------------|
| Number of cuts | Mean production g/tree | Number of cuts | Mean production g/tree |
| Number of cuts | 1              | 1                   |
| Mean production | 0.75           | 0.91                |

Number of cuts refers to the total number of times trees were pruned before the end date of sample data.

White, 1973), and similar results were evident in this study. Previous studies suggest that the rate of photosynthesis in winter is reduced in comparison with that of other seasons, particularly summer and spring (Athanasou et al., 2010). During winter plants tend to store most of the photosynthetic products for rapid recovery during favorable flourishing seasons. The results show this particular trend in the abundance of carbohydrate reserves in the winter pruning at all the different plant locations wild, field condition, and greenhouse plants. Highest reserves of carbohydrates were found at the end of the winter, and this is in line with the source-sink principle as explained by Morgenthal et al. (2006). The authors reported that the most reserves across the seasons are found in the roots (sink) with moderate to the same amounts in stems and leaves for greenhouse and field condition plants, whereas the wild plants had the least amounts in the stem and moderate quantities in the leaves (Ahmad et al., 2014).

Pruning has been reported to have an effect on the quality of tea, with the time and frequency of pruning being standard commercial practice (Ahmad et al., 2014; Ravichandran 2004). Compared with the unpruned plants, in the undomesticated bush tea plants, autumn and winter pruned plants produce higher quantities of both starch and sugar in relation to the spring and summer pruned plants, with plants pruned in winter having the most and those pruned in spring showing the least. The spring pruned plants had the lowest carbohydrate reserves and this was attributed to the higher pruning frequency. Thus, led to lower carbohydrates reserved meant for replenishment of the plant.

Pruning is known to encourage growth, and thus increased photosynthetic rate to provide energy for the new plant flush (Kaur et al., 2014). However, the greenhouse and field-grown plants pruned in all seasons had more overall carbohydrate reserves than unpruned plants, as expected. The winter pruned plants had the highest overall carbohydrate reserves, with the lowest being in autumn pruning instead of the spring pruning, as was seen in the natural-growing bush tea plants. This could be attributed to the fact that undomesticated growing plants will tend to experience harsher conditions and noncontinuous water supply compared with field condition and greenhouse plants.

As expected with an increase in pruning frequency, there is a direct increase in flush growth and carbohydrate increase as the photosynthetic rate is increased. With lesser pruning in winter as plants are in a dormant stage, there is a lower photosynthetic rate. Similar results were reported in Camellia sinensis by Ahmad et al. (2014), who reported that higher shoot density and shoot growth were associated with top pruning of black tea during December and January produced the highest production of fresh leaves in black tea in the southern hemisphere. A gradual increase in flush growth and carbohydrates is observed from winter to the summer pruning season. When comparing field-grown plants to greenhouse plants, cumulative production of dry matter is higher in the field-grown plants. This could be attributed to the number of prunings, as field-grown plants could be pruned more frequently. Under greenhouse conditions the highest dry matter production was observed in December at 170 g per plant, whereas in field-grown plants in the same month 400 g per plant of dry matter was produced, a marked double-fold difference. This reveals a much higher growth rate with an increased number of prunings.

In conclusion, accumulation of carbohydrate reserves in different parts of the plant vary with seasons, and different seasonal trends are observed with variable conditions. Similar findings were observed in bush tea plants grown under glasshouse conditions and in the field conditions, with wild bush tea plants showing slightly different responses. Winter pruning has the highest overall carbohydrate reserves, with the least number of pruning and the least dry matter. Field-grown plants produce the most dry matter content and similar overall carbohydrate reserve abundance to greenhouse plants, thus field-grown plants reproduce the optimal dry matter and carbohydrate reserve conditions. The optimal time to maximize production of bush tea is during the spring and summer seasons since in autumn (fall) the plants are entering the dormancy stage.

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