Response of Plant Growth and Development, and Accumulation of Hydroxyl-cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-grown Bush Tea (*Athrixia phylicoides* DC.)

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Abstract. Horticultural practices and quality of bush tea (*Athrixia phylicoides* DC.) are critical for herbal tea industrialization. The objective of the current study was to determine the effect of selected shade nets and seasonal variation on plant growth and development, and hydroxycinnamic acid content of field-grown bush tea. The trial was laid out in a randomized complete block design consisting of three shade nets (black, green, and white) and control or full sunlight with three different light intensities (40%, 50%, and 80%) replicated three times. Proportion of intercepted radiation by the canopy, chlorophyll content, plant height, and fresh and dry mass were measured, and hydroxycinnamic acid accumulation was determined. In addition, hydroxycinnamic acid composition was determined using liquid chromatography linked to mass spectrometry (LC-MS). The application of shade nets resulted in plant growth and yield reduction as compared with the plants exposed to full sunlight during summer followed by white shade net. The accumulation of hydroxycinnamic acid was higher in 80% white shade net plots compared with unshaded plants (control) and the other shade nets. Therefore, lack of shading provides a conducive environment to enhance plant growth and development of bush tea. The white shade net (80%) was an effective microclimate tool to enhance accumulation of caffeoylquinic acid (*m/z* 353), *p*-coumaric acids (*m/z* 337), dicaffeoylquinic acid (*m/z* 515), and tricaffeoylquinic acids of bush tea. This study is the first to demonstrate light as a determining factor for production of chlorogenates in bush tea plants. Future studies will be conducted to determine the effect of light on extracts of the bush tea using different solvents.

Bush tea (*A. phylicoides* DC.) is a native plant of South Africa and it grows naturally in different climatic conditions of South Africa (Mavundza et al., 2010; Nchabeleng et al., 2012; Van Wyk and Gericke, 2000). The plant grows to a height of ≈ 0.5 m to 1 m, with branches of thin woolly stems (Mudau et al., 2006). It has simple alternate leaves that are described as linear, light gray-green above, and white-woolly below (Mudau et al., 2006). The leaf bases are broadly lanceolate, short stalked, taper to a sharp point, auriculate, smooth on the upper surface, and have margins that are entirely or slightly revolute (Mudau et al., 2006). The head of the inflorescence is sessile or subsessile with terminal axillary in large sub corymbose panicles (Herman et al., 2000).

It is used traditionally as an important herbal and medicinal plant by South Africans to cleanse and purify blood, treat boils, heal wounds, and treat headaches (Fouché et al., 2006; Mudau et al., 2007). In some parts of the country, it is used as an aphrodisiac (Rakuambo, 2011). To date, bush tea is still harvested from the wild for a variety of medicinal purposes (e.g., to treat chronic diseases such as cardiovascular disease and cancer) and hence there is a huge potential for development of *A. phylicoides* as a commercial health benefit herb in South Africa (Mudau et al., 2006). Therefore, domestication of bush tea is critical for commercial exploitation to protect the species from possible extinction.

Previous studies have documented the effects of cultural practices such as mineral nutrition (Mudau et al., 2006), pruning (Mohale et al., 2018; Yilmaz et al., 2004), irrigation (Bandara, 2012) and harvesting methods (Mphangwe, 2012), as well as processing (Hlahla, 2010) and environmental conditions (Tshivhandekano et al., 2013) on chemical compositions of bush tea. The quality, economic value, and health function of bush tea is determined by the content of secondary metabolites such as flavonoids (or phenolic compounds), alkaloids, and amino acids (Tounekti et al., 2013). Zhang et al. (2014) reported that quality parameters of tea are significantly affected by environmental factors and management practices. Application of nitrogen (N) fertilizer increases total phenol concentrations in cultivated medicinal plants by enhancing photosynthetic rate (Haukioja et al., 1998) and accumulation of non-structural carbohydrates (Wanyoka, 1983).

However, it has been reported that carbon-based secondary metabolites increase frequently when environmental conditions enhance the accumulation of nonstructural carbohydrates in *Labisia pumila* plants (Ibrahim et al., 2011). Hydroxycinnamic acids (coumaric acid, ferulic acid, sinapic acid, and caffeic acid) are phenolic compounds that are found in plants either as free compounds or conjugated to other molecules such as quinic acid, tartaric acid, citric acid, and sugars (Ncube et al., 2014). These derivatives are known as chlorogenic acids (CGAs) when they are conjugated/esterified to

Fig. 1. (A) Data denoting minimum and maximum temperatures during 2018. (B) Data denoting minimum and maximum temperatures in 2019. (C) Data denoting temperature >40 °C in 2018. (D) Data denoting temperature >40 °C in 2019. Tx, maximum temperature.

other molecules (Masike et al., 2017; Nobela et al., 2018; Roleira et al., 2018). CGAs are classified into two groups: mono- and dicaffeoylquinic acids (CQAs) (Ncube et al., 2014). Plants naturally produce and use CGAs to aid their protection against abiotic (Lallemand et al., 2012; Taofiq, et al., 2017) and biotic (Ramabulana et al., 2020) stresses, as they have been reported to be effective defense phytochemicals (Kundu and Vadassery, 2019). Several hydroxyl-cinnamic acid derivatives such as 3,5-dicaffeoylquinic acid (3,5-diCQA) have been shown to have anti-HIV-1 INT enzyme activity that is attributed to *trans-cis* isomerization (Masike et al., 2017).

Net shades improve the quality of tea leaves due to an increase in the concentration of amino acids with lower content of catechin in the plant (Ku et al., 2010) and it also prevents the concentration of flavonoids (Wang et al., 2012). Net shade has also been reported to provide 50% to 70% of diffused solar insolation to the tea cultivation area (Lehlohonolo et al., 2013). Shade netting has been used in normal tea plantation in major tea producing areas to enhance optimum growth and productivity (Janendra et al., 2007). In green tea production, the ratio of oxalate content to reduced N content was higher in the flushes exposed to shading environment than the flushes under full sunlight (Morita and Tuji,

2002). Although several studies have documented plant growth and yield, as well as the quality response of bush tea to shade nets, most research has been conducted in pot experiments under controlled environments (de Beer et al., 2011; Mavundza et al., 2010; Mudau et al., 2007; Tshivhandekano et al., 2014). Moreover, information that describes the accumulation of hydroxycinnamic acids (HCAs) in bush tea grown in the field under different shade nets, as a standard agricultural horticultural practice to create conducive microclimate due to climate change, is still lacking. Therefore, the objective of the current study was to determine the effect of selected shade nets and seasons on plant growth and development, and hydroxycinnamic acid accumulation of field-grown bush tea.

Materials and Methods

Experimental site. The field trial was established in Autumn 2018 at the University of Venda's experimental farm, which is situated at Thohoyandou (lat. 22°58.081'S, long. 30°26.411'E, and 595 m asl), Limpopo Province, South Africa. The site is characterized by an annual rainfall of ≈500 mm that falls mainly in summer, and average maximum and minimum temperature of 31 °C and 18 °C, respectively (Tadross et al., 2006). The type of the soils at the experimental site are characterized by deep, well-drained clays with slightly acidic pH (Soil Classification Working Group, 1991). Weather data were supplied by South African weather service for 2018 and 2019 cropping seasons.

Annual and seasonal weather pattern during 2018 and 2019. Thohoyandou had high maximum and minimum average temperatures throughout the year. Monthly average maximum and minimum temperatures ranged from 30 to 40 °C and 10 to 20 °C, respectively, in both years (Fig. 1A and B). Temperature >40 °C was recorded in Dec. 2018 and Sept. and Oct. 2019 (Fig. 1C and D). Rainfall distribution was very poor with hardly any rainfall being recorded between May and October in both years (Fig. 2).

Preparations of stem cuttings. The wild bush tea stem cuttings were collected from Tshivhulani Village (lat. 22°55.331'S, long. 30°18.218'E, altitude 610 m), which is characterized by cold and dry winters. Planting materials of apical cuttings were cut at ≈7 to 8 cm long and dipped in Seradix No. 2 hormone (0.3% IBA) to stimulate rapid and prolific rooting. "True-to-name and type" material that was free of disease and insect damage was selected for planting. Stem cuttings were established on a 25-cm-round plastic pot in a lath house at the University of Venda on 25 Oct. 2017. Plants were irrigated daily except on rainy days. Rooted cuttings were transplanted into 1-L bags and placed into a net shade on 7 Dec. 2017 for a period of 3 months. Planting materials, with ≈25 leaves, were transplanted into four different types of environmental conditions (i.e., control, black, white, and green shade nets) on 12 Mar. 2018. Data collection commenced 3 months after transplanting when the plants were well established. Growth/rooting media was a mixture of pine bark and sand at a ratio of 2:1. The initial media test chemical analyses were determined using Hanlon et al. (1994) procedure. The nitrogen-phosphorous-potassium (N-P-K) fertilizer was applied in two

Fig. 2. The pattern of rainfall of Thohoyandou, South Africa, Limpopo Province during 2018 and 2019.

equal splits (at transplanting and 2 weeks after transplanting) at a rate 300 kg·ha⁻¹ N, 300 kg·ha⁻¹ P, and 200 kg·ha⁻¹ K based on previous studies (Mudau et al., 2007).

Experimental description. The experiment was laid out in a randomized complete block design consisting of three shade nets (black, green, and white) and full sunlight with three different light intensities (40%, 50%, and 80%) replicated three times. The size of the individual plots was 4.8 m × 4.8 m. Each plot consisted of four plant rows, 1.2 m apart and the intrarow spacing was 0.75 m, giving a total of 24 plants per plot. The plots were watered, using a drip irrigation system, when necessary. Weeding was done manually throughout the cropping season to keep the experimental plots weed-free. Methamidophos was applied at a rate of 10 mL per 20 L after each weeding occasion to protect the plants against termites.

Growth parameters. Bush tea is a perennial crop that flowers throughout the year and is able to resprout after being cut. Data were collected only from regenerated plants for each season. Pruning was done after each harvest to avoid mineral resource competition between the previous and regenerated plants. In 2018, data collection began on 12 June for the winter season. Each net plot had four rows excluding the guard rows. Sixteen plants from the second inner rows of each experimental plot were harvested once per season. Plant height (from the soil surface to the tip of the topmost leaf) was determined a day before each harvest using a measuring tape. Fresh weight was recorded after harvest in each season each year using a weighing balance. The harvested plants were air-dried, under room temperature, for 2 months. The proportion of dry matter content was obtained by expressing the dry weight as a percentage of the fresh weight of the sample taken as shown in Eq. 1.

$$\text{Dry matter (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Chlorophyll content. Leaf chlorophyll content was determined, between 0900 and 1200 hr on each occasion, from five plants in each plot using chlorophyll content meter

(CCM-200 Plus; Opti-Sciences, Tyngsboro, MA). Measurements were taken weekly between 26 and 33 weeks (Winter 2018), 38 and 46 weeks (Spring 2018), 49 and 62 weeks (Summer 2018), 71 and 73 weeks (Autumn 2019) and 78 and 87 weeks (Winter 2019), 90 and 98 weeks (Spring 2019), 102 and 112 weeks (Summer 2019), and 118 and 120 weeks (Autumn 2020).

Proportion of intercepted radiation by the canopy cover. Photosynthetically active radiation (PAR) measurements were taken at 7-day intervals, and the dates cover the vegetative and reproductive stages of plant growth in each season. The proportion of intercepted radiation was determined by measuring PAR above and below the canopy on various occasions, mostly at 7-day intervals, between 26 and 33 weeks (Winter 2018), 38 and 46 weeks (Spring 2018), 49 and 62 weeks (Summer 2018) and 71 and 73 weeks (Autumn, 2019) and 78 and 87 weeks (Winter 2019), 90 and 98 weeks (Spring 2019), 102 and 112 weeks (Summer 2019), and 118 and 120 weeks (Autumn, 2020). The measurements were taken between 1100 and 1300 hr on clear, cloudless days using an AccuPar LP-80 Ceptometer and the proportion of intercepted radiation (α) was calculated as shown in Eq. 2.

$$\alpha = 1 - (P_A/P_B)$$

where P_A is the PAR above the canopy; P_B is the PAR below the canopy; and α is the proportion of the intercepted radiation.

Metabolites extraction. Metabolite extraction was accomplished by a method proposed by Makita et al. (2016). In brief, bush tea leaves were manually threshed from dried plants and ground as soon as the dry mass was weighed for the autumn season. They were ground to fine powder using a hammer grinder, and 2 g of fine ground leaves was mixed with 20 mL (1:10 m/v) of 80% aqueous methanol. The homogenate was centrifuged at 5000 g_n for 20 min to remove debris, and finally, the supernatant was transferred to new clean tubes. The extracts were further diluted 1:1 (v:v) to final volume of 20 mL using methanol, followed by transfer of 10 mL of the diluted extracts into cylindrical

quartz glass vials (2 × 10 cm). The samples were filtered into a 2-mL vial fitted with a 0.2-mL conical bottom glass insert using a syringe fitted with a 0.2-μm filter.

LC-MS analysis. Bush tea sample analysis was conducted on an LC–quadrupole time of flight (QTOF)–MS, model LC-MS 9030 instrument with a Shim Pack Velox C18 column (100 mm × 2.1 mm with particle size of 2.7 μm) (Shimadzu, Kyoto, Japan), placed in a column oven set at 40 °C. A binary solvent mixture, consisting of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) was used at a constant flow rate of 0.4 mL/min. A mass spectrometer detector was used for monitoring analyte elations, under the following conditions: ESI (electrospray ionization) negative modes; interface voltage of 3.5 kV; nitrogen gas was used as nebulizer at flow rate 3 L/min, heating gas flow at 10 L/min; heat block temperature at 400 °C, CDL temperature at 250 °C; detector voltage of 1.70 kV and the TOF temperature at 42 °C.

Tandem MS experiments. For tandem MS (MS/MS) experiments, a mass calibration solution of sodium iodide (NaI) was used to obtain typical mass accuracies with a mass error below 1 ppm, and a range of m/z 100 to 1000 was used for high resolution. Argon gas was used as a collision gas for MS/MS experiments along with MS^E mode using collision energy ramp of 12 eV to 25 eV for generation of fragments.

Statistical analysis. All plant growth data were subjected to analysis of variance using SPSS version 27 (IBM Corp., Armonk, NY). Means were separated using the Duncan's multiple range test when the F-test indicated significant differences among the treatments. Correlation analysis was conducted to assess the relationships between the physiological parameters.

Results and Discussion

Proportion of intercepted radiation. Shade nets and seasons affected ($P \leq 0.001$) the proportion of intercepted radiation by the crop canopy at all measurement dates in both 2018 and 2019 (Figs. 3 and 4). On average, the proportion of intercepted radiation was higher in control (57.8%) compared with white color (57.2%), green shade (50.2%), and black shade (52.4%) (Table 1). The higher proportion of intercepted radiation observed in control compared with other treatments could be attributed to the larger canopy size and plant height of the plants that were exposed to full sunlight (Muchow et al., 1990). Our findings are comparable to earlier observations that different shade nets can influence microclimatic parameters that control plant growth and development (Kumar et al., 2013).

Total intercepted radiation. Control had significantly ($P \leq 0.001$) higher total intercepted radiation than the other treatments in both years (Figs. 5 and 6), which is consistent with earlier findings that black shade nets tended to absorb more light compared with other shade nets (Mokoka, 2007). These findings are consistent with those of Anwar et al.

Table 1. Response of plant growth and development of bush tea cultivated under different shade nets color, light intensity, and season.

	Source of variation	IR (%)	Plant ht (m)	Fresh wt (g/plant)	Dry mass (g/plant)	Chlorophyll content (nm)
Color	Control	57.8 a	159.8 a	762.1 a	382.5 a	16.1 a
	Black	52.4 b	155.2 a	391.9 c	196.9 b	13.9 a
	Green	50.2 b	147.6 a	402.3 c	240.6 b	14.4 a
	White	57.2 a	151 a	533.6 b	262.2 b	13.2 a
Light intensity (%)	100	57.7 a	158 a	772.3 a	387.6 a	15.9 a
	40	56.9 a	152.5 ab	499.8 b	288.1 b	14.8 a
	50	55.2 a	157.2 ab	465.6 b	230.9 bc	11.9 a
	80	47.6 b	144.2 c	362.4 c	180.7 c	14.7 a
Season	Autumn	54.9 c	181.8 a	550.1 b	274.7 ab	12.1 c
	Spring	36.7 d	109.4 d	368.5 c	186.6 bc	15.5 bc
	Summer	64.6 a	173.4 b	636 a	314.5 a	11.4 a
	Winter	58.7 b	143.9 c	343.6 c	216.9 c	17.1 b

Means within a column followed by the same letter indicate no significant difference. Means within a column followed by different letter indicate significant difference.

(2003), who found that total intercepted radiation varies between treatments due to differences in days to physiological maturity. The interaction of shade nets and season also had a significant ($P \leq 0.001$) effect on total intercepted radiation in all the seasons.

The effect of shade nets on plant growth and development of bush tea. There was a significant difference among plant growth parameters (proportion of intercepted radiation, fresh weight, and dry mass) on bush tea plants grown under different shade nets. However, chlorophyll content and plant height did not vary with shade nets (Table 1).

Fresh and dry mass. Plants from unshaded plots recorded the highest fresh biomass (762.1 g/plant) followed by white shade net treatments (533.6 g/plant), with the lowest fresh biomass (391.9 g/plant) being recorded in plants grown under black shade nets (Table 1). The complex interaction

between quantity and quality of incident radiation determines the response of plant to shading conditions (Lee et al., 1997). The significant decrease of fresh weight on plants grown under black shade net might be due to insufficient light the plants had received, which resulted in stunting growth that clearly demonstrates the importance of a favorable environment in determining plant growth and yield of bush tea in any region. Our results concur with earlier observations (Bell et al., 2000; Rao and Mittra, 1998) that tea grown under shade nets had an ability to reduce *PAR*, change spectral quality, and affect plant photosynthesis, dry matter production, and yield of the crop. Similarly, Marchese and Figueira (2005) reported that plant growth and development was significantly influenced by environmental factors such as radiation, temperature, and photoperiod, and that increased biomass and essential oil was

associated with higher photosynthetic rate of plants and higher radiation. Although plants exposed to low light intensity at the vegetative stage tend to increase their capacity to trap light by increasing the leaf area, this did not significantly contribute toward biomass accumulation in our study. Similar results were reported by Mokoka (2007), who observed that 55% black shade nets significantly reduced fresh shoot mass relative to plants grown under 18% white shade nets in fever tea, which is in line with reports that yield reduction due to shading was determined by crop species and degree of shading (Kumar et al., 2013).

Effect of light intensity on plant growth and development of bush tea. There was a significant difference in plant growth parameters (i.e., intercepted radiation, plant height, fresh weight, and dry weight) of bush tea plants grown under different light intensities of

Fig. 3. The effect of treatments on the proportion of intercepted radiation in 2018. (A) Winter 2018. (B) Spring 2018. (C) Summer 2018. (D) Autumn 2019.

Fig. 4. The effect of treatments on the proportion of intercepted radiation in 2019. (A) Winter 2019. (B) Spring 2019. (C) Summer 2019. (D) Autumn 2020.

shade nets. However, light intensity did not affect chlorophyll content (Table 1).

The unshaded control exhibited the highest proportion of intercepted radiation (57.7%), with the lowest (47.6%) being recorded in 80% shade net plots

(Table 1). The response of plant height and fresh biomass to light intensity followed a similar trend, with the highest (158 m and 772.3 g/plant) and lowest (144.2 m and 362.4 g/plant) plant height and fresh biomass recorded in unshaded

control and 80% shade net plots, respectively (Table 1). The results of the current study are consistent with previous reports that changes in microclimate caused by spacing and shade levels result in significant differences in growth and development, with

Fig. 5. The effect of treatments on the total radiation in 2018. (A) Winter 2018. (B) Spring 2018. (C) Summer 2018. (D) Autumn 2019.

Fig. 6. The effect of treatments on the total radiation in 2019. (A) Winter 2019. (B) Spring 2019. (C) Summer 2019. (D) Autumn 2020.

faster growth and development exhibited in plants exposed to full sunlight compared with those grown under shade (Kumar et al., 2013). More recently, Thakur et al.

(2019) concluded that higher shade level reduced *PAR*, altered light intensities, and affected photosynthetic rate and yield production.

In contrast to our results, Kumar et al. (2013) reported that shaded plants were taller than those exposed to full sunlight, probably due to long internodal lengths

Fig. 7. Representative ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS) base peak intensity (BPI) chromatograms showing the separation of secondary metabolites in extracts of bush tea plants exposed to (A) control, (B) black, (C) green, and (D) white, respectively.

Table 2. Interactive effect of color, light intensity, season, and year on plant growth and development of bush tea cultivated under different conditions.

	df	IR (%)			Plant ht (cm)			Fresh wt (g)			Dry mass (g)			Chlorophyll content (nm)		
		SS	MS	F-value	F-value	SS	MS	SS	MS	F-value	SS	MS	F-value	SS	MS	F-value
Color	3	2,286.3	762.1	19	0.1 NS	3,679.9	1,226.6	3,103,307.1	1,035,535.7	11784	64,065.3	2,135,514	4	164.8	54.9	0.5
Light intensity	4	4,032.3	1,008.1	2.5	0.041*	9,737.6	2,434.4	29,998,834	749,708.5	8461	911,068.7	227,767.2	4.4	499.8	124.9	1.01
Season	3	26,024.5	8,674.8	28.7	0.000***	193,529	64,510	3,609,893.9	1,203,297.9	14051	592,542.6	197,514.2	3.7	1,340.6	446.9	3.9
Year	1	282.6	282.6	7	0.406 NS	33,725	33,725	2,406,997.1	2,406,997.1	26.6	338,873.5	338,873.5	6.3	1,028.2	1028	8.9
L × C	4	1,423.3	355.8	3.6	0.008**	14,489	3,622.2	1,626,428.8	406,607.2	9.8	714,233.3	178,558.3	4.3	196.3	49.1	0.4
L × S	6	1,772.4	295.4	2.9	0.009**	3,558.9	593.1	574,197.6	95,699.6	2.3	221,508.5	36,918.1	0.9	88.7	14.8	0.1
L × Y	2	1,010.5	505.3	5.1	0.007**	3,742.7	1,871.4	19,503.6	9,751.8	0.3	74,335.4	37,167.7	0.9	6	3.7	0
C × S	6	252.8	42.1	0.4	0.863 NS	943.5	157.2	480,211.7	80,035.3	1.945	414,569.3	69,094.9	1.7	611.4	101.9	0.9
C × Y	2	2,792.6	1,396.3	14	0.000***	1,078.2	539.3	111,308.2	55,654.1	1352	97,192.3	48,596.2	1.2	145.6	72.1	0.6
S × Y	3	2,9840.7	9,946.9	99.7	0.000***	165,766	55,255	2,612,234.9	870,744.9	21.2	857,282.3	285,760.8	6.8	2,198.8	732.9	6.4
L × C × S	12	1,647.0	137.3	1.4	0.182 NS	6,977.6	581.5	522,686.8	43,557.2	1.1	573,990.7	47,832.6	1.1	388.1	32.3	0.3
L × C × Y	4	1,299	324.8	3.3	0.013*	945.3	236.3	101,301.1	25,325.3	0.61	72,485.6	18,121.4	0.4	1,314.6	328.6	2.9
L × S × Y	6	1,385.3	230.9	2.3	0.036*	6,093.9	1,015.6	77,875.6	12,979.3	0.3	302,745.3	50,457.6	1.2	584.7	97.5	0.8
C × S × Y	6	1,464.1	244	2.4	0.27*	1,590.8	265.1	121,491	20,248.5	0.5	246,825.6	41,137.6	0.9	492.6	82.1	0.71
L × C × S × Y	12	1,656.2	138	1.4	0.178 NS	1,866.8	155.6	150,966.6	12,580.6	0.3	579,910.8	48,325.9	1.2	851.5	70.9	0.71
Error	160	15,956.6				168,466.0	1,052.9	6,584,500.2	41,153.1		6,701,283.9	41,883.0		18,426.0	115.2	
Total	240	789,637.7				6,179,559.0		77,870,534.3			27,978,801.1			75,781.1		

IR = intercepted radiation; SS = sum of squares; MS = mean of squares.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

and thinner stems in the plants grown under shade.

The lower fresh and dry mass of bush tea plants in the shaded treatments compared with plants from unshaded plots that we observed in the current study may be attributed partly to a combination of low light and high air temperature, which likely reduced available stored energy rapidly (Svenson, 2002). Similarly, Gregoriou et al. (2007) and Wei et al. (2005) reported that growth and productivity of plants were usually inhibited by lower light intensities through imbalances in gaseous exchange. In contrast, Barua and Gogoi (1979) reported that the yield in tea, especially tea shoots, subjected to light intensity less than 35% was higher than in tea plants that were exposed to direct full sunlight, which may suggest that unsuitable light intensities were usually capable of causing damage to the plant's photosynthetic system by interfering with photosynthesis and development (Szymborska-Sandhu et al., 2020). Clearly, the effect of light intensity on growth, development, and yield varies greatly and may need further investigation.

The effect of season on plant growth and development. Bush tea plant growth parameters (intercepted radiation, plant height, fresh weight, dry weight, and chlorophyll content) varied with seasons (Table 1). On average, the proportion of intercepted radiation (%) was higher in summer (64.6%) compared with winter (58.7%), autumn (54.9%), and spring (36.7%) (Table 1), perhaps partly due to the larger canopy size that was exhibited in summer. Similarly, fresh biomass was higher in summer (636 g/plant) compared with the rest of the seasons (343.6–550.1 g/plant) (Table 1). For plant height in contrast, the seasons were ranked as follows: autumn > summer > winter > spring (Table 1). The higher biomass accumulation in summer was associated with the greater proportion of intercepted radiation recorded in summer. The amount of solar radiation received during the cropping season influences plant growth, development, and yield (Challa and Bakker, 1999; Cockshull et al., 1992). Bush tea yield drops significantly under cloudy conditions, with heavy and continuous rainfall, just like it does when the weather is hot, dry, and sunny.

In contrast to our findings, CTIFL (1995) reported that plant growth and yield was restricted during the summer season because of the effects of higher summer temperatures on photosynthesis and respiration.

Chlorophyll content. There was a significant seasonal variation in chlorophyll content, with the highest recorded in winter (17.1 nm) and lowest in summer (11.4 nm) (Table 1). The results are in line with those of MacAlister et al. (2020), who reported that chlorophyll content was higher in winter compared with the summer season.

Interactive effect of color, light intensity, season, and year on plant growth and development of bush tea cultivated under different conditions. There are no distinct research findings that have been reported and discussed on interactive effect of color, light intensity, season,

Table 3. Characterization of chlorogenic acids (CGAs) of bush tea plants exposed to different net shade and light intensities.

No.	m/z	Rt (min)	Molecular formula	Abbr	Con	B40%	B50%	B80%	G40%	G50%	G80%	W40%	W50%	W80%
1	353	3.279	C ₁₆ H ₁₈ O ₉	3-O-Caffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	353	6.453	C ₁₆ H ₁₈ O ₉	4-O-Caffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	353	6.985	C ₁₆ H ₁₈ O ₉	5-O-Caffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	353	13.405	C ₁₆ H ₁₈ O ₉	Cis-3-O-Caffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	337	9.135	C ₁₆ H ₁₈ O ₈	5-O-P-Coumaroylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	337	10.844	C ₁₆ H ₁₈ O ₈	Cis-5-O-P-Coumaroylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	337	5.208	C ₁₆ H ₁₈ O ₈	3-O-P-Coumaroylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	337	14.297	C ₁₆ H ₁₈ O ₈	Cis-3-O-P-Coumaroylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	337	9.154	C ₁₆ H ₁₈ O ₈	4-O-P-Coumaroylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
10	515	13.113	C ₂₂ H ₂₄ O ₁₂	3,4-Dicaffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	515	15.998	C ₂₂ H ₂₄ O ₁₂	4,5-Dicaffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
13	677	15.775	C ₃₄ H ₃₆ O ₁₅	1,3,5-Tricaffeoylquinic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Con = control; B40% = black 40%; B50% = black 50%; B80% = black 80%; G40% = green 40%; G50% = green 50%; G80% = green 80%; W40% = white 40%; W50% = white 50% and W80% = white 80%.

led to greater accumulation of chlorogenic acid as compared with the plants exposed to full sunlight. Therefore, a combination of white shade net and 80% light intensity may be the best microclimatic tools to enhance CGAs in bush tea production. However, we recommend further studies to investigate the response of bush tea extracts exposed to different colors of the ultraviolet light.

Literature Cited

- Anwar, M.R., B.A. McKenzie, and G.D. Hill. 2003. The effect of irrigation and sowing date on crop yield and yield components of Kabuli chickpea (*Cicer arietinum* L.) in a cool-temperate sub humid climate. *J. Agr.* 141:259–271, <https://doi.org/10.1017/S0021859603003617>.
- Bandara, S.N. 2012. Agronomy of irrigated tea in low elevation growing areas of Sri Lanka. Univ. Adelaide School Agr., Food Wine, Adelaide, Australia, PhD Diss.
- Barua, D.N. and B.N. Gogoi. 1979. Effects of shade removal. Two and a Bud. 26:40–42.
- Bell, G.E., T.K. Danneberger, and M.J. McMahon. 2000. Spectral irradiance available for turfgrass growth in sun and shade. *Crop Sci.* 40: 189–195, <https://doi.org/10.2135/cropsci2000.401189x>.
- Challa, H. and M.J. Bakker. 1999. Potential production within the greenhouse environment, p. 333–348. In: G. Stanhill and Z.H. Enoch (eds.). *Ecosystems in the world: Vol. 20: Chapter 15: Greenhouse Ecosystems*. Elsevier, Amsterdam, The Netherlands.
- Clifford, M.N., S. Knight, and N. Kuhnert. 2005. Discriminating between the six isomers of dicaffeoylquinic acid by LC–MSn. *J. Agr. Food Chem.* 53:3821–3832, <https://doi.org/10.1021/jf050046h>.
- Cockshull, K.E., C.J. Graves, and C.R. Cave. 1992. The influence of shading on yield of glasshouse tomatoes. *J. Hort. Sci.* 67:11–24, <https://doi.org/10.1080/00221589.1992.11516215>.
- CTIFL, Centre Technique Interprofessionnel des Fruits et des Legumes. 1995. *Maîtrise de la conduite climatique*. Paris. CTIFL, 127.
- de Beer, D., E. Joubert, C.J. Malherbe, and D.J. Brand. 2011. Use of counter current chromatography during isolation of 6-hydroxyluteolin-7-O-B-glucoside, a major antioxidant of *Athrixia phylicoides*. *J. Chromatography* 1218: 6179–6186, <https://doi.org/10.1016/j.chroma.2010.12.096>.
- Fouché, G., E. Khorombi, N. Kolesnikova, V.J. Maharaj, R. Nthambeleni, and M. Van der Merwe. 2006. Investigation of South African plants for anticancer properties. *Pharmacologyonline* 3:494–500.
- Gregoriou, K., K. Pontikis, and S. Vemmos. 2007. Effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.). *Photosynthetica* 45:172–181, <https://doi.org/10.1007/s11099-007-0029-x>.
- Hanlon, E.A., J.S. Gonzales, and J.M. Bartos. 1994. IFAS extension soil testing laboratory chemical procedures and training manual (Circular 812). Fla. Coop. Ext. Serv. Inst. Food and Agri. Sci., Univ. of Fla.
- Hanudin, E., H. Wismarini, T. Hertiani, and B. Hendro Sunarminto. 2012. Effect of shading, nitrogen and magnesium fertilizer on phyllanthin and total flavonoid yield of *Phyllanthus niruri* in Indonesia soil. *J. Med. Plants Res.* 6:4586–4592, <https://doi.org/10.5897/JMPR12.591>.
- Haukioja, E., V. Ossipov, J. Koricheva, T. Honkanen, S. Larsson, and K. Lempa. 1998. Biosynthetic origin of carbon-based secondary compounds: Cause of variable responses of woody plants to fertilization. *Chemoecology* 8:133–139, <https://doi.org/10.1007/s000490050018>.
- Herman, P.P.J., E. Retief, M. Koekemoer, and W.G. Welman. 2000. *Seed plants of Southern Africa*. O.A. Leister Editions, National Botanical Institute, Pretoria, South Africa.
- Hlahla, L.N. 2010. Effect of fermentation temperature and duration on chemical composition of Bush tea (*Athrixia phylicoides* DC.). Univ. of Limpopo, Turfloop Campus, Mankweng, South Africa, PhD Diss.
- Ibrahim, M.H., H.Z.E. Jaafar, A. Rahmat, and Z.A. Rahman. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip fatimah (*Labisia pumila* Blume). *Int. J. Mol. Sci.* 12:5238–5254, <https://doi.org/10.3390/ijms12085238>.
- Janendra, W.A., M. Costa, J.A. Mohotti, and M.A. Wijeratne. 2007. *Ecophysiology of tea*. Braz. J. Plant Physiol. 19:299–332.
- Karimi, E., H.Z. Jaafar, A. Ghasemzadeh, and M.H. Ibrahim. 2013. Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. *Aust. J. Crop Sci.* 7:1016–1024.
- Ku, K.M., J.N. Choi, J.K. Kim, L.G. Yoo, S.J. Lee, Y.S. Hong, and C.H. Lee. 2010. Metabolomics analysis reveals the compositional differences of shade grown tea (*Camellia sinensis* L.). *J. Agr. Food Chem.* 58:418–426, <https://doi.org/10.1021/jf902929h>.
- Kumar, R., S. Sharma, and V. Pathania. 2013. Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea* L.) in north western Himalaya. *J. Essent. Oil Res.* 25:23–32, <https://doi.org/10.1080/10412905.2012.742467>.
- Kundu, A. and J. Vadassery. 2019. Chlorogenic acid-mediated chemical defence of plants against insect herbivores. *Plant Biol.* 21:185–189, <https://doi.org/10.1111/plb.12947>.
- Lallemant, L.A., C. Zubieta, S.G. Lee, Y. Wang, S. Acajaoui, J. Timmins, S. McSweeney, J.M. Jez, J.G. McCarthy, and A.A. McCarthy. 2012. A structural basis for the biosynthesis of the major chlorogenic acids found in coffee. *Plant Physiol.* 160:249–260, <https://doi.org/10.1104/pp.112.202051>.
- Lee, D.W., S.F. Oberbauer, B. Krishnapilay, M. Mansor, H. Mohamad, and S.K. Yap. 1997. Effects of irradiance and spectral quality on seedling development of two Southeast Asian *Hopea* species. *Oecologia* 110:1–9.
- Lehlohonolo, N., I.K. Mariga, W. Ngezimana, and F.N. Mudau. 2013. Bush tea (*Athrixia phylicoides* dc.) Success stories in South Africa. A review. *J. Crop Prod.* 2:37–43.
- MacAlister, D., A.M. Muasya, O. Crespo, J.B.O. Ogola, S. Maseko, and A.J. Valentine. 2020. Effect of temperature on plant growth and stress tolerant traits in rooibos in the Western Cape South Africa. *Scientia Hort.* 263:109137, <https://doi.org/10.1016/j.scientia.2019.109137>.
- Makita, C., L. Chimuka, P. Steenkamp, E. Cukrowska, and E. Madala. 2016. Comparative analyses of flavonoid content in *Moringa oleifera* and *Moringa ovalifolia* with the aid of UHPLC–qTOFMS fingerprinting. *S. Afr. J. Bot.* 105:116–122, <https://doi.org/10.1016/j.sajb.2015.12.007>.

- Marchese, J.A. and G.M. Figueira. 2005. Use of technologies before and after harvest and good agricultural practices in the production of medicinal and aromatic plants. *Rev. Brasil. Planta Med.* 7:86–96.
- Masiike, K., F. Tugizimana, N. Ndlovu, E. Smit, L. du Preez, I. Dubery, and N.E. Madala. 2017. Deciphering the influence of column chemistry and mass spectrometry settings for the analyses of geometrical isomers of L-chlorogenic acid. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 1052:73–81, <https://doi.org/10.1016/j.jchromb.2017.03.023>.
- Masiike, K., I. Dubery, P. Steenkamp, E. Smit, and E. Madala. 2018. Reversing reverse-phase chromatographic behavior for efficient differentiation of both positional and geometrical isomers of dicaffeoylquinic acids. *J. Anal. Methods Chem.*, <https://doi.org/10.1155/2018/8694579>.
- Mavundza, E.J., T.E. Tshikalange, N. Lall, A.A. Hussein, F.N. Mudau, and J.J.M. Meyer. 2010. Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Athrixia phylicoides*. *J. Medicinal Plant Res.* 4(23):2584–2587, <https://doi.org/10.5897/JMPR10.823>.
- Mohale, K.C., A.T. Hints, M.A. Emanuel, and F.N. Mudau. 2018. Metabolic profiling of cultivated bush tea (*Athrixia phylicoides* DC.) in response to different pruning types. *Scientia Hort.* 53:993–998, <https://doi.org/10.1016/j.sci.2018.03.023>.
- Mokoka, N.N. 2007. Indigenous knowledge of fever tea (*Lippia javanica*) and effect of shade netting on plant growth, oil yield and compound composition. Univ. of Pretoria, Pretoria, South Africa, PhD Diss.
- Morita, A. and M. Tuji. 2002. Nitrate and oxalate contents of tea plants (*Camellia sinensis* L.) with special reference to types of green tea and effect of shading. *J. Soil Sci. Plant Nutr.* 48:547–553, <https://doi.org/10.1080/00380768.2002.10409237>.
- Mphahlele, N.I.K. 2012. Lung Pruning: A review of practice. Tea Research Foundation of Central Africa (TRFCA) News. 18–23.
- Muchow, R.C., T.R. Sinclair, and J.M. Bennett. 1990. Temperature and solar radiation effects on potential maize yields across locations. *J. Agron.* 82:338–342, <https://doi.org/10.2134/agronj1990.00021962008200020033x>.
- Mudau, F.N., H.T. Araya, E.S. Du Toit, P. Soundy, and J. Olivier. 2007. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in southern Africa: Opportunity for commercialization. *Med. Aromatic Plant Sci. Biotechnol.* 1:74–76.
- Mudau, F.N., P. Soundy, E. Du Toit, and J. Olivier. 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. *S. Afr. J. Bot.* 72:398–402.
- Nchabeleng, L., F.N. Mudau, and I.K. Mariga. 2012. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. *J. Med. Plants Res.* 6:1662–1666, <https://doi.org/10.5897/JMPR11.1453>.
- Ncube, E.N., M.I. Mhlongo, L.A. Pieter, P.A. Steenkamp, I.A. Dubery, and N.E. Madala. 2014. Analyses of chlorogenic acids and related cinnamic acid derivatives from *Nicotiana glauca* tissues with the aid of UPLC-QTOF-MS/MS based on the in-source collision-induced dissociation method. *Chem. Cent. J.* 8:1–10.
- Nobela, O., R.S. Renslow, D.G. Thomas, S.M. Colby, S. Sitha, P.B. Njobeh, L. du Preez, F. Tugizimana, and N.E. Madala. 2018. Efficient discrimination of natural stereoisomers of chlorogenic acid, an HIV-1 integrase inhibitor. *J. Photochem. Photobiol. B* 189:258–266, <https://doi.org/10.1016/j.jphotobiol.2018.10.025>.
- Rakumbo, Z.J. 2011. Indigenous knowledge of bush tea (*Athrixia phylicoides*) and effect of fertigation frequency and growing medium on plant growth. Univ. of Pretoria, Pretoria, South Africa, PhD Diss.
- Ramabulana, A.T., P. Steenkamp, N. Madala, and I.A. Dubery. 2020. Profiling of chlorogenic acids from *Bidens pilosa* and differentiation of closely related positional isomers with the aid of UHPLC-QTOF-MS/MS-based in-source collision-induced dissociation. *Metabolites* 10:178, <https://doi.org/10.3390/metabo10050178>.
- Rao, L.J. and B.N. Mittra. 1998. Growth and yield of peanut as influenced by degree and duration of shading. *J. Agron. Crop Sci.* 160:260–265, <https://doi.org/10.1111/j.1439-037X.1998.tb00327.x>.
- Roleira, F.M., C.L. Varela, S.C. Costa, and E.J. Tavares-da-Silva. 2018. Phenolic derivatives from medicinal herbs and plant extracts: Anticancer effects and synthetic approaches to modulate biological activity. *Stud. Nat. Prod. Chem.* 57:115–156, <https://doi.org/10.1016/B978-0-444-64057-4.00004-1>.
- Soil Classification Working Group. 1991. A Taxonomic System for South Africa. Department of Agricultural Development, Pretoria, South Africa, 257.
- Svensson, S.E. 2002. Shady business. Growers should understand how light levels and photosynthesis are linked in crop production. *Amer. Nurseryman* 19:23–28.
- Szymborska-Sandhu, I., J.L. Przybyl, E. Pióro-Jabucka, A. Jędrzejuk, Z. Węglarz, and K. Bączek. 2020. Effect of shading on development, yield and quality of bastard balm herb (*Melittis melisophyllum* L.). *Molecules* 25:2142, <https://doi.org/10.3390/molecules25092142>.
- Tadross, M.A., W.J. Gutowski, B.C. Hewitson, C.J. Jack, and M. New. 2006. MM5 simulations of interannual change and the diurnal cycle of southern African regional climate. *Theor. Appl. Climatol.* 86:63–80.
- Taofiq, O., A.M. González-Paramás, M.F. Barreiro, and L.C.F.R. Ferreira. 2017. Hydroxycinnamic acids and their derivatives: Cosmeceutical significance, challenges and future perspectives, a review. *Molecules* 22:281, <https://doi.org/10.3390/molecules22020281>.
- Thakur, M., V. Bhatt, and R. Kumar. 2019. Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. *PLoS One* (14):e0214672.
- Tounkete, T., E. Joubert, I. Hernández, and S. Munné-Bosch. 2013. Improving the polyphenol content of tea. *Crit. Rev. Plant Sci.* 32:192–215, <https://doi.org/10.1080/07352689.2012.747384>.
- Tshivhandekano, I., K. Ntshelo, W. Ngezimana, T.E. Tshikalange, and F.N. Mudau. 2014. Chemical compositions and antimicrobial activities of *Athrixia phylicoides* DC. (bush tea), *Monsonia burkeana* (special tea) and synergistic effects of both combined herbal teas. *Asian Pac. J. Trop. Med.* 7:S448–S453, [https://doi.org/10.1016/S1995-7645\(14\)60273-X](https://doi.org/10.1016/S1995-7645(14)60273-X).
- Tshivhandekano, I., F.N. Mudau, P. Soundy, and W. Ngezimana. 2013. Effect of cultural practices and environmental conditions on yield and quality of herbal plants: Prospects leading to research on influence of nitrogen fertilization, planting density and eco-physiological parameters on yield and quality of field-grown bush tea (*Athrixia phylicoides* DC.). *J. Med. Plants Res.* 7:2489–2493, <https://doi.org/10.5897/JMPR2013.4466>.
- Van Wyk, B.E. and N. Gericke. 2000. People's plants: A guide to useful plants of Southern Africa. 1st edition. Briza publications. 102.
- Wang, Y., L. Gao, Y. Shan, Y. Liu, Y. Tian, and T. Xia. 2012. Influence of shade on flavonoid biosynthesis in tea [*Camellia sinensis* (L.) O. Kuntze]. *Scientia Hort.* 141:7–16, <https://doi.org/10.1016/j.scienta.2012.04.013>.
- Wanyoka, J.K. 1983. Fertilizer on tea: Nitrogen – a review. *Tea (Nairobi)* 4:28–35.
- Wei, S.L., W.Q. Wang, X.H. Chen, S.Y. Qin, and X.T. Chen. 2005. Studies on the shade-endurance capacity of *Glycyrrhiza uralensis*. *China J. Chinese Materia Medica* 30:100–104.
- Yilmaz, G., N. Kandemir, and K. Kinalioglu. 2004. Effects of different pruning intervals on fresh shoot yield and some quality properties of tea (*Camellia sinensis* L. Kuntze) in Turkey. *Pak. J. Biol. Sci.* 7:1208–1212, <https://doi.org/10.3923/pjbs.2004.1208.1212>.
- Zhang, Q., Y. Shi, L. Ma, X. Yi, and J. Ruan. 2014. Metabolomic analysis using ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF MS) uncovers the effects of light intensity and temperature under shading treatments on the metabolites in tea. *PLoS One* (9):112572, <https://doi.org/10.1371/journal.pone.0112572>.