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# IMPACT OF SEASON AND HARVEST FREQUENCY ON BIOMASS AND ESSENTIAL OIL YIELDS OF *ARTEMISIA HERBA-ALBA* CULTIVATED IN SOUTHERN TUNISIA

*By* HEDI MIGHRI†, AHMED AKROUT, JOSEPH CASANOVA‡, FELIX TOMI *and* MOHAMED NEFFATI

*Laboratoire d'Ecologie Pastorale, Institut des Régions Arides, Km 22.5, Route du Djorf-4119 Médenine, Tunisie and* ‡*Université de Corse, Equipe chimie et biomasse, UMR CNRS 6134, Route des Sanguinaires, 20000 Ajaccio, France*

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SUMMARY

*Artemisia herba-alba* Asso has been successfully cultivated in the Tunisian arid zone. However, information regarding the effects of the harvest frequency on its biomass and essential oil yields is very limited. In this study, the effects of three different frequencies of harvesting the upper half of the *A. herba-alba* plant tuft were compared. The harvest treatments were: harvesting the same individual plants at the flowering stage annually; harvesting the same individual plants at the full vegetative growth stage annually and harvesting the same individual plants every six months. Statistical analyses indicated that all properties studied were affected by the harvest frequency. Essential oil yield, depended both on the dry biomass and its essential oil content, and was significantly higher from plants harvested annually at the flowering stage than the other two treatments. The composition of the $\beta$- and $\alpha$-thujone-rich oils did not vary throughout the experimental period.

## INTRODUCTION

The genus *Artemisia* is widely distributed in arid areas of the Mediterranean regions. It belongs to family Asteraceae and consists of approximately 400 species of small evergreen shrubs having aromatic foliage and flowers (Willis, 1966). Of these, five species are represented in Tunisia: *A. arborescens*, *A. atlantica*, *A. campestris*, *A. vulgaris* and *A. herba-alba* (Ferchichi *et al.*, 1997). *Artemisia herba-alba* Asso is widespread in southern Tunisia and is largely used in folk medicine, particularly for treatment of bronchitis, diarrhoea, hypertension and aerophagia (Said *et al.*, 2002). Its essential oils (EO) are known for their therapeutic, disinfectant, anthelminthic and antispasmodic virtues (Houmani *et al.*, 2004). Previous investigations carried out on EO yield and quality of this species did not give homogeneous results and confirmed the existence of several chemotypes of the *A. herba-alba* oil (Akrout, 2004; Boutekedjiret *et al.*, 1992; Feuerstein *et al.*, 1987; Lawrence, 1993; 1995; Salido *et al.*, 2004). However, the authors worked on plant material from various locations corresponding to many environmental variables and, in some of these studies, the parts of the plant used were not clearly specified. Furthermore, in most studies, the phenological stage was not established. Moreover,

†Corresponding author: mighrih@yahoo.fr

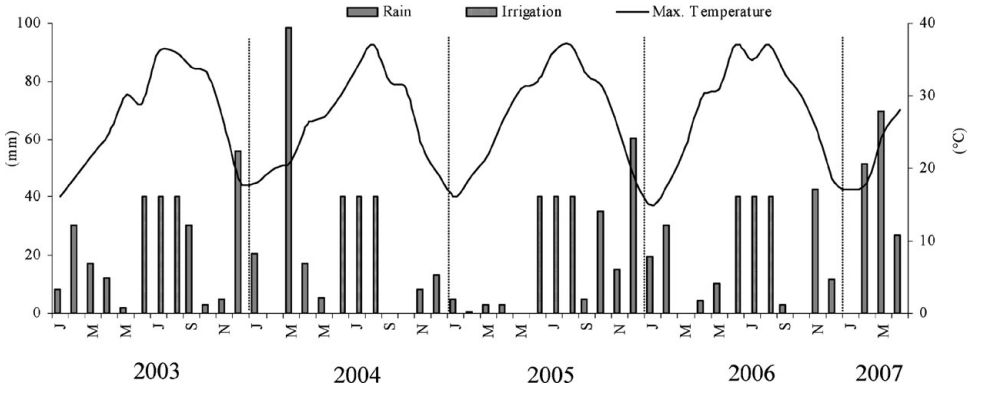


Figure 1. Weather data for the experimental site.

variation in the EO quality may occur in the regrown aerial parts of *A. herba-alba* tuft after harvesting at various phenological stages, and this was not specified in all of the previous studies.

In southern Tunisia, the harsh climatic conditions, overgrazing and the extension of agriculture at the expense of the natural ranges of *A. herba-alba*, contribute to the decreasing abundance of this species. A previous study about the EO isolated from the aerial parts (without stems) of cultivated *A. herba-alba* in southern Tunisia showed that the highest EO yield was obtained from the upper half of the plant tuft with almost the same chemical composition as the oil isolated from plants growing wild in the same area (Mighri *et al.*, in press). Continuing our investigation of these oils with the aim of ensuring successful long-lasting production from cultivated species in arid regions (without manure, and irrigation only during the highest dry months), in this paper we describe a study of the effects of season and frequency of harvest on biomass production, and the content and composition of EO isolated from the regrown aerial parts of the *A. herba-alba* tuft.

## MATERIALS AND METHODS

### *Field studies*

An experimental field was established and followed during the period 2002–2007 at the Institut des Régions Arides Médenine (33°30′N, 10°40′E; southeast Tunisia). The study site has an arid Mediterranean climate. The mean annual rainfall recorded during the period 1992–2001, was about $150 \pm 46$ mm. The mean temperature was 21 °C, the highest (35 °C) being in August and the lowest (5 °C) in January. Meteorological data for the study period (average maximum temperature and rainfall at monthly intervals) are shown in Figure 1. The soil of the experimental site was sandy, the calcium carbonate content (total $CaCO_3$) was 10%, the organic matter was less than 1% and pH 7.2.

The experiment was laid out in a randomized block design with three treatments harvesting the upper half of *A. herba-alba* tufts (see below for treatment details) replicated

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four times on individual plots with an area of 9 m × 9.5 m. Each plot contained 36 plants brought from the natural range lands and transplanted in November 2002 at 150 cm row-to-row and plant-to-plant spacing. The experimental field was irrigated immediately after planting for early establishment of plants. Thereafter, the field was irrigated only during the driest months (June, July and August) throughout the experimental period and the crop was weeded manually in April each year.

The first treatment (T1) consisted of harvesting the aerial parts (stems, leaves and flower buds) from the same plants four times at the flowering stage (the first harvest performed 12 months after transplanting in November 2003 and subsequently in November 2004, 2005 and 2006). The second treatment (T2) consisted of harvesting the aerial parts from the same plants (stems and leaves) four times at the full vegetative growth stage (the first harvest performed 18 months after transplanting in May 2004, and the others three harvests in May 2005, 2006 and 2007). In November 2004, two years after transplanting, a third treatment (T3) consisting of harvesting the aerial parts from the same plants every six months, was applied in November and May.

Before each harvest, the height and diameter of plants were recorded on four randomly selected plants in each plot. The fresh biomass was air-dried in the shade at ambient temperature until weight stabilization. The dry matter ($DM_1$) and its corresponding recovered matter ($DM_2$) including flower buds and/or leaves used for distillation (after removing stems that are virtually devoid of oil) were determined. $DM_1$ and $DM_2$ were expressed in g $m^{-2}$ or evaluated in t $h^{-1}$. EO of pooled $DM_2$ was distilled (100 g were mixed with 600 ml of distilled water) in 2-l round bottom flasks in a modified Clevenger-type apparatus for 4 h. The EO yield (g 100 $g^{-1}$ $DM_2$) was expressed as a mean value of six repetitions. The oil obtained was then separated from the remaining distillate, dried by anhydrous sodium sulphate and stored at 0 °C in tight vials until analysis.

*Analysis of the essential oil*

The chemical composition of *A. herba-alba* EO was determined by gas chromatography (GC) combined with the retention indices (RIs) on an apolar column and by $^{13}$C-nuclear magnetic resonance ($^{13}$C-NMR).

Analytical GC was carried out using a Perichrom-2100 gas chromatograph fitted with flame ionization detector (FID) and an electronic integrator, using capillary columns (30 m × 0.22 mm internal diameter, film thickness 0.20 μm) SE-52 (5% diphenyl 95% dimethylsiloxane). The oven temperature was programmed to increase from 40 to 220 °C at 5 °C/min; injector temperature: 220 °C; detector temperature: 240 °C; carrier gas: nitrogen (1.0 ml/min); sample manually injected: 0.2 μl of 1% diluted solution in hexane. The relative proportions of the EO constituents were expressed as percentage obtained by peak area normalization, without using correcting factors. RIs were determined relative to the retention times of a series of n-alkanes ($C_8$–$C_{22}$) with linear interpolation.

NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.13 MHz for $^{13}$C–NMR, equipped with a 5-mm

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Table 1. Morphology and regrowth of *Artemisia herba-alba* at different phenological stages as influenced by harvest frequency.

| | Treatment | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T1 (N: November) | | | | T2 (M: May) | | | | T3 (N: November and M: May) | | | | | |
| Harvest dates | N.03 | N.04 | N.05 | N.06 | M.04 | M.05 | M.06 | M.07 | N.04 | M.05 | N.05 | M.06 | N.06 | M.07 |
| Tuft height (cm) | $50^{a}$ | $56^{b}$ | $66^{c}$ | $57^{b}$ | $43^{a}$ | $58^{c}$ | $51^{b}$ | $63^{c}$ | $41^{a}$ | $53^{b}$ | $35^{a}$ | $38^{a}$ | $34^{a}$ | $49^{b}$ |
| Tuft diameter (cm) | $99^{a}$ | $133^{b}$ | $143^{c}$ | $126^{b}$ | $102^{a}$ | $123^{ab}$ | $117^{a}$ | $141^{b}$ | $95^{c}$ | $105^{c}$ | $49^{a}$ | $78^{b}$ | $60^{a}$ | $96^{c}$ |
| $DM_1$ ($gm^{-2}$) | $236^{a}$ | $445^{b}$ | $440^{b}$ | $246^{a}$ | $203^{a}$ | $292^{b}$ | $246^{ab}$ | $248^{b}$ | $219^{d}$ | $168^{c}$ | $99^{ab}$ | $61^{a}$ | $42^{a}$ | $106^{bc}$ |

For each treatment (T1, T2 and T3), means with the same letters are not significantly different at the 5% level.
T1: Harvest annually at flowering stage; T2: Harvest annually at full vegetative growth stage; T3: Harvest every six months; DM1: Dry matter of upper half of *A. herba-alba* tuft.

probe, in $CDCl_3$ (deuterochloroform), with all shifts referred to internal TMS (tetramethylsilane). $^{13}C$–NMR spectra of the EOs were recorded with the following parameters: pulse width = 4 μs (flip angle 45°); acquisition time = 2.7 s for 128K data table with a spectral width of 25 000 Hz (250 ppm); complex pulse mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3000–5000 for each sample depending on the available amount of oil (when available, 40 mg of oil in 0.5 ml of $CDCl_3$).

Identification of the individual components was (i) by comparison of their GC RIs on an SE–52 apolar column, determined relative to the retention times of a series of *n*-alkanes with linear interpolation, with those of reference compounds determined in our laboratory under the same conditions, NIST library data base and literature data (Adams, 2001); (ii) $^{13}C$ NMR spectroscopy, following the method developed and computerized in our laboratories, using in-house software, by comparison with spectral data of reference compounds compiled in a laboratory-built library (Tomi *et al.*, 1995).

### *Statistical analysis*

The data on plant tuft diameter, plant tuft height and biomass production were subjected to analysis of variance (ANOVA) (SPSS Program, 2002) using randomized block design. Multivariate analysis was carried out to study the interaction effect (harvest frequency × phenological stage of plant) in respect of biomass and EO yields. Mean values were considered significantly different when $p < 0.05$.

## RESULTS AND DISCUSSION

### *Crop growth and regenerate biomass*

Harvesting at different phenological stages influenced significantly crop growth of *A. herba-alba* in respect of plant height, plant diameter and consequently the corresponding quantity of biomass ($DM_1$) (Table 1). Harvesting the upper half of the *A. herba alba* tuft, showed that the maximum plant height, plant diameter and DM1 were recorded during in the second and third harvests (November 2004 and 2005) at the flowering stage (T1) and the second and fourth harvests (May 2005 and

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2006) at the full vegetative growth stage (T2). These parameters decreased significantly in the fourth harvest during T1 and the third harvest during T2. At two years after transplanting, the effect of treatment on harvesting the same plant every six months (T3) on all parameters studied was relatively less pronounced especially at the third, fourth and fifth harvests when compared to the first, second and sixth harvests. From these results, it appears that a period of six months did not allow better recovery of *A. herba-alba* plants than annual harvests in November or May. In all treatments, the harvesting delays after transplanting plants (12, 18 or 24 months corresponding to the first harvests of T1, T2 and T3, respectively) seem to have no effects on biomass yields.

*Biomass yield*

The stems, which are virtually devoid of oil, are easily removed after air drying of the biomass. As shown in Table 2, for T1 the maximum $DM_1$ was produced in correspondence to its recovered $DM_2$ (leaves and flower buds) in the second and third harvests (November 2004 and 2005). At the full vegetative growth stage, the maximum $DM_1$ and its corresponding $DM_2$ (leaves only) obtained in the second harvest were significantly different from the first, third and fourth harvests (Table 2).

When the third treatment was used, the variation in $DM_2$ recovered after different harvests was different between the first, second and subsequent harvests. There was a significant decline in $DM_2$ produced between the first harvest in November 2004 and the second in May 2005 (30% reduction). $DM_2$ was similar for the following six-monthly harvests, although it decreased by 64–77% compared to the first harvest (Table 2).

To prevent withering, plants were irrigated during the driest months (June, July and August). The September/October period is considered the beginning of the rainy season in southern Tunisia, and thus resulted in the highest biomass yield in all harvests performed each year during T1. This increase in the recovered biomass observed especially in the second and third harvests of T1 and in the second and fourth harvests of T2, confirms the previous observations carried out in southern Tunisia by Ammari *et al.* (1996), who suggested that individual *A. herba-alba* plants should be pruned of their old biomass to become more productive.

*Essential oil content*

The *A. herba-alba* oil contents obtained in this study varied seasonally and were relatively higher than the values previously reported for *A. herba-alba* growing in southern Tunisia and neighboring countries (Akrout, 2004; Salido *et al.*, 2004). When plants were harvested annually at the flowering stage, the EO content was shown to vary irregularly. It was higher in the first harvest, decreased in the second harvest to reach a minimum value and increased in the third and fourth harvests.

At the full vegetative growth stage, the EO content reached the highest value (especially in the second and fourth harvests) compared to those obtained at the flowering stage. The lowest EO content obtained in the third harvest corresponded to the minimum biomass production.

Table 2. Evaluation of biomass yield, oil content and oil yield of *Artemisia herba-alba* at different phenological stages as influenced by harvest frequency.

| | Treatment | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | | | | T2 | | | | T3 | | | | | |
| Harvest dates | N.03 | N.04 | N.05 | N.06 | M.04 | M.05 | M.06 | M.07 | N.04 | M.05 | N.05 | M.06 | N.06 | M.07 |
| Biomass yield ($DM_1$ t $ha^{-1}$) | 2.29$^{a}$ | 4.32$^{b}$ | 4.27$^{b}$ | 2.39$^{a}$ | 1.97$^{a}$ | 2.84$^{b}$ | 1.30$^{a}$ | 2.41$^{b}$ | 2.12$^{d}$ | 1.63$^{c}$ | 0.96$^{ab}$ | 0.59$^{a}$ | 0.41$^{a}$ | 1.03$^{bc}$ |
| Biomass yield ($DM_2$ t $ha^{-1}$) | 1.43$^{a}$ | 1.87$^{b}$ | 1.90$^{b}$ | 1.43$^{a}$ | 0.99$^{ab}$ | 1.29$^{b}$ | 0.54$^{a}$ | 0.89$^{ab}$ | 1.13$^{c}$ | 0.80$^{b}$ | 0.26$^{a}$ | 0.32$^{a}$ | 0.29$^{a}$ | 0.41$^{a}$ |
| EO content (% w/w) ± *s.d.* | 1.93 ± 0.01 | 1.29 ± 0.02 | 1.65 ± 0.03 | 1.52 ± 0.04 | 1.78 ± 0.01 | 2.07 ± 0.03 | 1.61 ± 0.05 | 2.33 ± 0.02 | 0.89 ± 0.02 | 1.67 ± 0.05 | 1.25 ± 0.02 | 1.88 ± 0.01 | 2.06 ± 0.04 | 2.42 ± 0.03 |
| EO yield (kg $ha^{-1}$) | 27.5$^{b}$ | 24.3$^{a}$ | 31.4$^{b}$ | 21.7$^{a}$ | 17.7$^{ab}$ | 26.7$^{c}$ | 8.7$^{a}$ | 20.7$^{b}$ | 10.1$^{b}$ | 13.6$^{c}$ | 3.2$^{a}$ | 6.0$^{ab}$ | 6.0$^{ab}$ | 9.9$^{b}$ |

For each treatment, means with the same letters are not significantly different at the level of 5%.

Table 3. Biomass and essential oil yield per year of *Artemisia herba-alba* at different phenological stages as influenced by harvest frequency.

| Treatment | Biomass yield (t $ha^{-1}$ $yr^{-1}$) | | Essential oil yield (kg $ha^{-1}$ $yr^{-1}$) |
|---|---|---|---|
| | $DM_1$ | $DM_2$ | |
| T1 | 3.32[a] | 1.66[a] | 26.2[a] |
| T2 | 2.13[b] | 0.93[b] | 18.5[b] |
| T3 | 2.25[b] | 1.04 [b] | 16.2[b] |

Means with the same letters are not significantly different at the 5% level.

The change of EO content observed in different six-monthly harvests was associated with seasonal variation. Indeed, due to the greater number of flower buds on the parts of the plant that grew from May to November, the EO content increased significantly in the third and fifth harvests compared to the first. In May, the EO content increased significantly in the fourth and sixth harvests compared to the second harvest. This variation may be attributed to the regrown leaves that accumulate a higher oil content.

*Essential oil yield*

EO yield was found to be dependent on both the biomass production and EO content, and was generally higher for the annual harvests at the flowering stage (T1) than the two other treatments (Table 2). At the flowering stage, the highest EO yield was obtained during the first and third harvests (2003 and 2005) and was due to maximum EO content and maximum $DM_2$, respectively. However, at the full vegetative growth stage, the second and fourth harvests (2005 and 2007) showed significantly higher oil yield than for the first and third harvests (2004 and 2006).

Harvesting plants every six months showed significant differences in EO yield between the first, second and the three subsequent harvests: the latter three harvests gave 31% of the total EO yield for all six harvests, and the sixth (final) harvest contributed 20% of the total yield of EO.

Considering mean biomass production (t $ha^{-1}$ $yr^{-1}$) and EO yield (kg $ha^{-1}$ $yr^{-1}$), when harvested each year at the flowering stage, *A. herba-alba* produced a significantly higher biomass yield ($DM_2$ and $DM_1$) and EO yield than the other two treatments (Table 3). Annual biomass production and EO yield were similar when plants were harvested each year at the full vegetative growth stage and every six months. These results are different to those found by Zrira *et al.* (1997), who reported that cultivated rosemary plants in Morocco showed that harvesting the same individuals each year at various phenological stages affected the EO production. This was due to the short period of one year, which was not sufficient time for the regrowth of the aerial parts of this plant.

*Essential oil composition*

Nine major volatile compounds accounting for > 68% of different EO distilled from various biomass samples of *A. herba-alba* were selected to demonstrate the chemical variability between different treatments (Table 4). Qualitatively, the chemical

Table 4. Contents (%) of majority chemical components of *Artemisia herba-alba* essential oils at different phenological stages as influenced by harvest frequency

| | | Peak area% | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T1 | | | | T2 | | | | T3 | | | | | |
| Harvest dates | | N.03 | N.04 | N.05 | N.06 | M.04 | M.05 | M.06 | M.07 | N.04 | M.05 | N.05 | M.06 | N.06 | M.07 |
| Constituents | RI | | | | | | | | | | | | | | |
| Camphene | 940 | 0.8 | 1.3 | 2.1‡ | 1.3 | 1.6 | 2.0 | 0.7 | 0.2† | 1.4 | 1.7 | 1.5 | 1.4 | 0.9 | 2.0 |
| p-cymene | 1008 | 1.5 | 1.7 | 3.3‡ | 1.4 | 2.0 | 1.3 | tr. | tr. | 0.8 | 1.7 | 1.3 | 1.4 | 1.5 | 0.8 |
| 1,8-cineole | 1017 | 6.0† | 11.3 | 9.0 | 7.7 | 10.5 | 11.9 | 10.6 | 12.4 | 11.3 | 11.4 | 9.4 | 22.9‡ | 11.5 | 22.5 |
| α-thujone | 1083 | 25.7‡ | 22.3 | 15.5 | 20 | 12.2† | 16.0 | 14.4 | 17.5 | 23.4 | 19.0 | 12.8 | 17.8 | 21.7 | 19.6 |
| β-thujone | 1095 | 30.0 | 20.2 | 19.5† | 25.5 | 19.8 | 21.2 | 27.0 | 28.4 | 25.3 | 26.4 | 33.8‡ | 22.6 | 29.1 | 22.6 |
| Chrysanthenone | 1103 | 0.5† | 4.1 | 5.6 | 8.6‡ | 1.0 | 4.2 | 4.0 | 2.8 | 1.4 | 1.3 | 2.0 | 2.5 | 2.4 | 1.2 |
| Camphor | 1117 | 4.5† | 9.7 | 10.4 | 8.6 | 10.8 | 10.6 | 10.9 | 12.4 | 11.1‡ | 8.1 | 8.6 | 11.0 | 7.8 | 10.3 |
| Terpinen-4-ol | 1158 | 2.8 | 3.9 | 3.6 | 3.3 | 4.2 | 2.3 | 1.9† | 2.2 | 2.8 | 2.2 | 3.3 | 2.9 | 5.0‡ | 2.1 |
| Trans-sabinyl acetate | 1268 | 5.7 | 5.3 | 6.1 | 5.5 | 6.0 | 6.8‡ | 6.1 | 6.0 | 1.4 | 2.8 | 1.3† | 1.5 | 1.3† | 1.7 |

RI: Retention indice calculated on apolar column; tr.: trace < 0.1%.
†Minimum recorded level; ‡maximum recorded level.

composition did not vary throughout the experiment. All analysed EO samples were dominated by oxygenated monoterpenes; thujone (α/β), 1,8-cineole and camphor were found to be major components (53.3–75.0%), and trans-sabinyl acetate and terpinen-4-ol were present in substantial amounts.

Quantitatively, a high level of variability was recorded within treatments between harvests, whereas no clear treatment effects on EO composition can be identified. It is interesting to note that the delayed first harvests after transplanting could explain this variability. The 1,8-cineole content reached a high value in the fourth and sixth harvests in the third treatment (May 2006 and May 2007, respectively). The chrysanthenone content increased gradually to reach a maximum value in the fourth harvest at the flowering stage. Although, the trans-sabinyl acetate content remained almost stable in all six-monthly harvests, it reached a quite high percentage in all harvests yearly performed at the flowering and the full vegetative growth stages. A little variability was observed among the treatments with respect to the 50 other minor and trace constituents of *A. herba-alba* oil.

## CONCLUSION

The results of the field investigation showed that the highest yields of biomass and essential oil of cultivated *A. herba-alba* grown under arid climate conditions in southern Tunisia were obtained at the flowering stage. This stage can be considered to be the most suitable for harvesting *A. herba-alba*. Hence, the production of oil could be evaluated at 26.2 kg $ha^{-1}$ $yr^{-1}$ for harvests carried out during the four years after transplanting. The composition of the β- and α-thujone-rich oils did not vary throughout the experiment and it was close to that of the EO isolated from plants harvested annually or every six months at the full vegetative growth stage.

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