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Seasonal trends in energy content and storage substances in the mediterranean shrub *Ephedra* (*)

S. Diamantoglou, S. Rhizopoulou

Institute of General Botany,
Univ. of Athens, Greece,
Panepistimiopolis G.R., 15784 Athens, Greece

A. Herbig and U. Kull

Biologisches Institut der Univ. Stuttgart, F.R.G.,
Pfaffenwaldring 57, D-7000 Stuttgart-80

ABSTRACT

Green branches of a shrub of *Ephedra distachya* L., growing in Athens, Greece, were investigated. The crude fibre content rises during the late growth period, levels off during winter, then declines as new shoots emerge in spring. The content of soluble sugars and of starch is high during winter, but rather low during the dry season. The content of total lipids rises during winter, especially during the late growth period until June and declines during the drought period. During the winter months in the total saponifiable lipids the percentage of linoleic and linolenic acids is high; however, during the growth and the drought period the content of palmitic acid is rather high. The contents of total nitrogen and protein nitrogen decrease in late spring and remain low until the end of the summer drought. Thereafter, both values rise markedly. Stored energy is lowest at the end of the summer drought period. The ash-free calorific values vary little throughout the year (but the difference is significant) : the highest value is obtained in November (19782 J/g), the lowest in May (19298 J/g). From our data, the tissue construction cost was estimated to be 1.41-1.47 g glucose/g dry weight.

Ephedra distachya is suited to the mediterranean climate, as seen by its ability to adjust to climatic changes during the year while undergoing periods of growth and quiescence. The accumulation of sugar during the winter months suggests that *Ephedra* is also capable of growing under cold-stress conditions.

KEY WORDS: mediterranean species, climatic stress, carbohydrate storage, lipid storage, N content, calorific value, tissue construction cost.

RÉSUMÉ

La variation saisonnière de la composition chimique chez *Ephedra distachya* L. a été étudiée en prélevant périodiquement des rameaux verts sur un buisson situé près d'Athènes. La teneur en cellulose brute augmente à la fin de la période de croissance, plafonne jusqu'à la fin de l'hiver, pour décliner au printemps lorsque les nouvelles pousses font leur apparition. Les teneurs en sucres et en amidon sont élevées en hiver et plutôt faibles durant la saison sèche. La teneur en lipides augmente au cours de

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l'hiver et jusqu'en juin pour ensuite diminuer au cours de la saison sèche. Pour ce qui concerne la fraction saponifiable des lipides totaux, il est à noter qu'en hiver ce sont les acides linoléique et linolénique qui prédominent, tandis qu'au printemps et en été c'est l'acide palmitique qui prédomine. Les teneurs en azote total et en azote protéique diminuent à la fin du printemps et se maintiennent à un faible niveau jusqu'à la fin de la période de sécheresse estivale. Les valeurs énergétiques varient peu au cours de l'année, mais les variations observées sont significatives: 19,8 kJ/g en novembre et 19,3 kJ/g en mai. En nous basant sur nos résultats nous avons pu estimer le coût de fabrication des tissus à 1,41-1,47 g de glucose par g de matière sèche.

Ephedra distachya est adapté au climat méditerranéen, ainsi qu'il ressort de sa capacité de s'ajuster aux variations climatiques dans le courant de l'année, où alternent périodes de croissance et périodes de repos. L'accumulation de sucres dans le courant de l'hiver suggère que cette espèce est apte à la croissance sous l'influence d'un stress par le froid

MOTS CLÉS : espèces méditerranéennes, contraintes climatiques, glucides, lipides, protéines, valeur énergétique, coût énergétique.

INTRODUCTION

Further to our investigations of seasonal trends of storage substances in mediterranean species (DIAMANTOGLOU & KULL, 1982, 1988), we have now examined the twigs of the leafless shrub *Ephedra distachya*. The content of reserve substances was measured during the course of a year and from these data, the energy content of the storage substances (ECS) was calculated. These values can be compared with energy equivalents of dry matter measured by calorimetry (calorific total energy, CTE). CTE and ECS values are needed as reference values for our biomechanical investigations, preliminary results of which and a short description of the methods used are published elsewhere (KULL, 1987) (*).

MATERIALS AND METHODS

MATERIAL

Green twigs of *Ephedra distachya* L. were taken from a shrub of about 0.5 m height growing near the Institut of General Botany at the University of Athens next to the road from Kaisariani to Moni Kaisariani. The low vegetation is partly shadowed by Eucalyptus trees. Climatic data during the period

(*) Abbreviations: CTE, calorific total energy; ECS, energy content of storage substances.

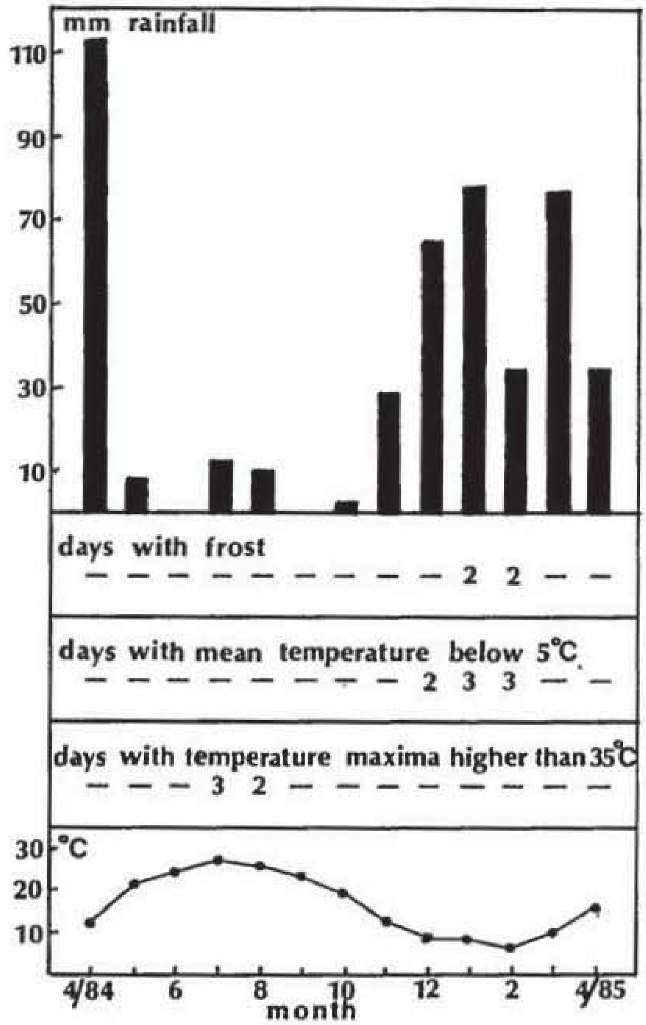

FIG. 1. – Climatic data (Station: Athens, near the airport).
Upper diagram: monthly rainfall; lower diagram: monthly mean temperature.

of harvesting plant material are shown in figure 1. The dry summer period lasted for about 4 1/2 months.

Growth of the *Ephedra* shrub began during early March and lasted until May/June.

Twigs were gathered at monthly intervals throughout the year at 11.00 a. m. While the seeds were ripening, only vegetative twigs were taken. During the period of investigation, the shrub bore few seeds.

METHODS

Stabilization of the plant material was performed immediately after harvesting. Twigs in plastic bags were dipped into boiling water for 10 min. and then dried in a ventilated oven at 60°C (DIAMANTOGLOU and KULL, 1982).

Soluble sugars were analyzed by TLC, total sugar by the anthrone method and mucilage by gravimentry (NAGLSCHMID *et al.*, 1982; DISTELBARTH *et al.*, 1984; DISTELBARTH & KULL, 1985). The starch content was measured by the anthrone-method of MCCREADY *et al.* (1950). The total lipids were quantified according to the gravimetric method of BLIGH and DYER (1959). The gas-chromatographic analysis of fatty acids was performed as described by DIAMANTOGLOU and KULL (1982). The total nitrogen content was measured by the method of KJELDAHL (1883), which also was used for the quantification of the protein-N after precipitation of the proteins with trichloroacetic acid (DIAMANTOGLOU & KULL, 1988). The difference between total-N and protein-N is indicated as soluble N (HÖLLWARTH, 1976).

The content of crude fiber (including lignin) was determined after boiling the plant material with diluted sulfuric acid and diluted potassium hydroxide by the method described by STEUBING (1965).

From the qualitative estimation of the contents of the storage materials the energy contents were calculated as described by DIAMANTOGLOU and KULL (1982) and DISTELBARTH *et al.* (1984).

The CTE-value (total energy content: energy equivalent of dry matter) of the Ephedra twigs was measured by adiabatic calorimetry after a renewed drying of the plant powder in a desiccator. The calibration was made with benzoic acid (26.430 kJ/g). Calorimeter: LGT, Waldkirch. The calorimetry was performed according to LARCHER and THOMASER-THIN [(1988); compare also LIETH (1975); PIPP & LARCHER(1988)]; but 35 bar oxygen pressure was used instead. Three or four replicates gave maximal deviations of 0.39% [coefficient of variation $S_x/\bar{x}$; $S_x=\sqrt{\Sigma(x_i-\bar{x})^2/(n-1)}$].

The ash content was determined after placing the plant material in a muffle furnace for 1.5 hour at 500° C. Based on the values obtained, the calculation of the energy equivalents was corrected.

RESULTS

The water content, the total ash content and the content of crude fibre of the green *Ephedra* twigs are shown in figure 2. From April, the water content declines until the end of the drought period but then rises in the winter months, and especially during the early growth period in March. The crude fibre content starts to rise in April as new shoots become lignified. From July to February crude fibre content remains stable but then declines with new shoot emergence (March/April). The total ash content is highest during the growth period in April and declines in late spring. During the summer drought it remains constant, shows a small increase at the beginning of the wet season and rises remarkably at the onset of growth.

The sesaonal trends of the carbohydrate content (soluble sugars and starch) are shown in figure 3.

One sugar, probably a di-or trisaccharide (according to the R_f-values in TLC and paper chromatography) could not be identified. Its quantification was based on sucrose. Also very small amounts (always <0.2%) of a trisaccharide, most probably raffinose, are present.

The amount of the total soluble sugars measured using the anthrone method was higher by 0.3-0.5% (higher by 0.9% in October) than the sum of the contents of the sugars measured separately by TLC. These total sugar contents were used for the calculation of the energy content of storage substances shown in figure 7.

The content of soluble sugars and starch is high in the winter months but low at the end of the growth period and during the dry season.

The contents of mucilages (figure 4) remains relatively constant. It is somewhat higher during the first half of the drought period and also in March, when the new shoots begin to develop and are still not lignified. The curve of the mucilage content is generally antagonistic to that of the water content of the tissue. The mucilage consists mainly of galactose and arabinose; however, glucose and xylose were also found, but in smaller amounts. Uronic acids and the N-content of the mucilage were not tested.

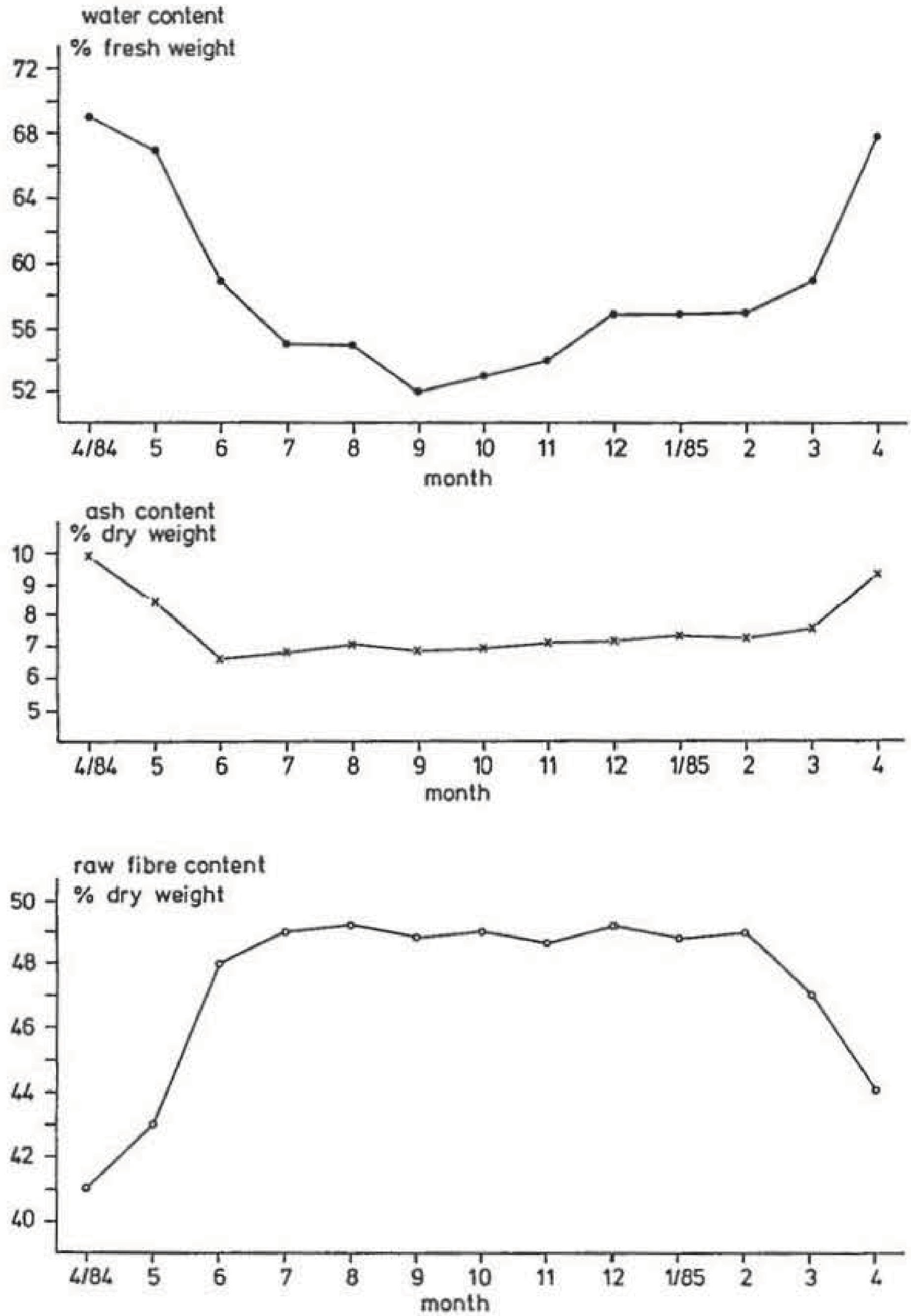

FIG. 2. — Seasoanl variability of water-content (as % of fresh weight). ash content and raw fibre content (as % of dry weight) of twigs of *Ephedra distachya.*

The total nitrogen and protein-nitrogen content (figure 5) show marked and similar changes during the course of the year. Beginning in April, total-N and protein-N-content declines, remains low until the end of the drought period in October, then rises rapidly to a high value, which for total-N remains constant from December to April. The decline in protein-N begins March, when the new shoots develop.

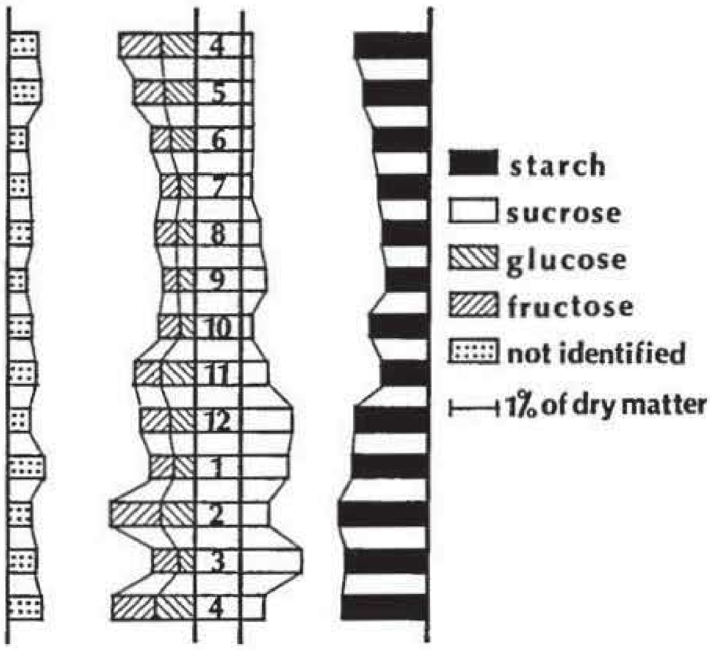

FIG. 3. — Seasonal variability of sugar and starch content in twigs of *Ephedra distachya.*

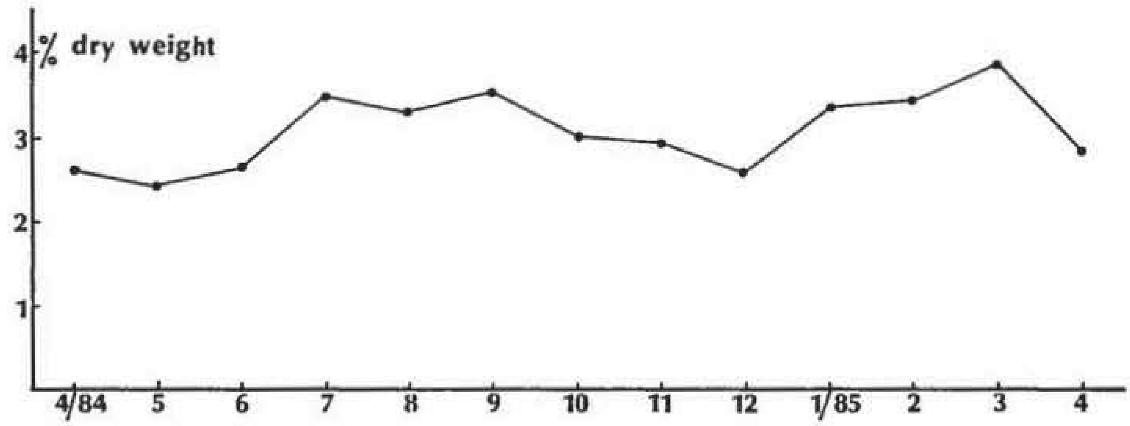

FIG. 4. — Seasonal variability of the mucilage content in twigs of *Ephedra distachya.*

The content of soluble nitrogen remains for the most part of the year at about 0.5%; it is lower in the winter months and higher during the main growth period. Since the content of the protoalkaloids of the ephedrin-group in *Ephedra distachya* is very low (HEGNAUER, 1962, 1986), it does not contribute much to the content of the total soluble N.

If total-N, protein-N and soluble-N were calculated on the basis of the crude fibre content (as in DIAMANTOGLOU & KULL, 1988), then the curves would show essentially the same trend and therefore, they are not shown here.

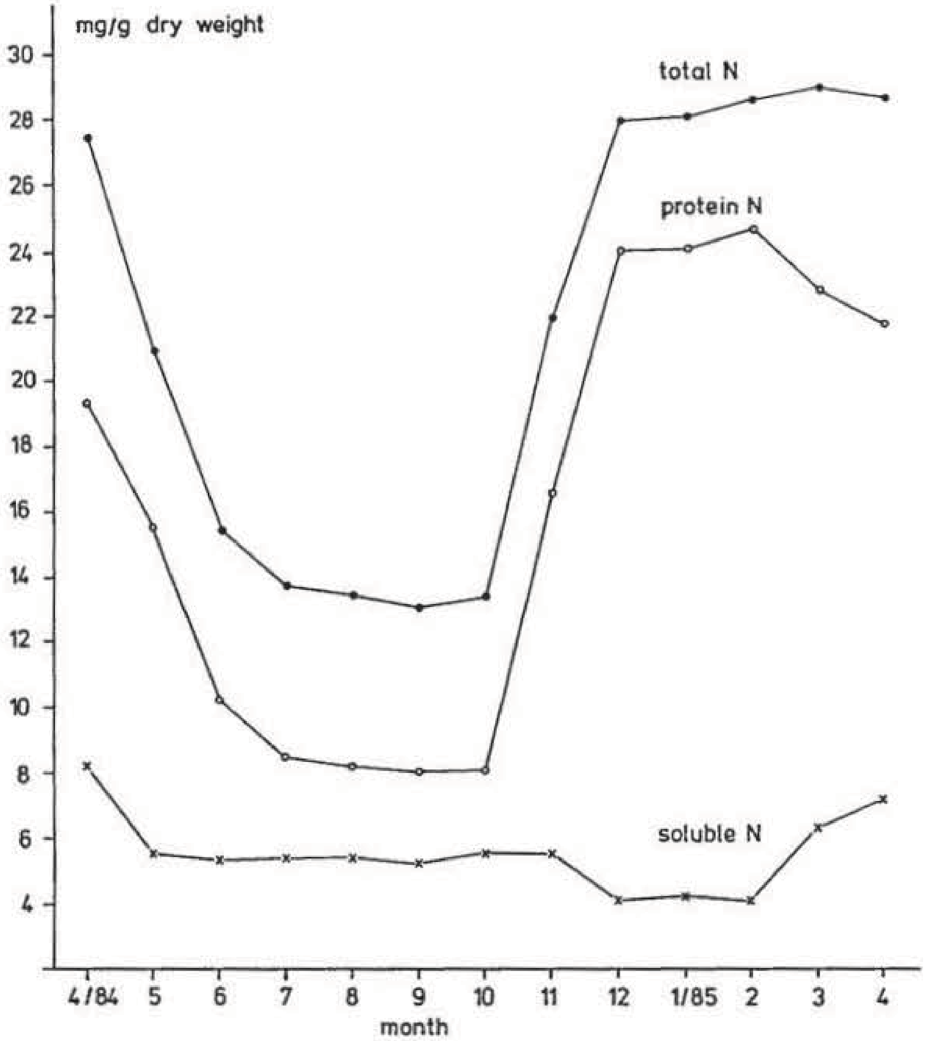

FIG. 5. — Seasonal variability of total N, protein N and soluble N content in twigs of *Ephedra distachya*.

The amount of total lipid (figure 6) rises from October to a maximum (more than 5% per dry weight) in June and then declines during the drought period.

The fatty acid composition of the saponifiable lipids is shown in table I. The content of palmitic acid (16 : 0) is high during both the growth and drought periods but low in winter, whereas the content of oleic acid (18: 1) is highest during the drought period but low at the beginning of the growth period. The percentage of linoleic and linolenic acids is high in the winter months and low in the summer.

By adding up the energy content of the main storage products (lipids, sugars, starch), the ECS values (per g of dry weight) were estimated to range from 1–2.6 kJ/g (figure 7, Table II).

By contrast the CTE-values remain more constant, declining only in spring. The highest value is found in November (19 782 J/g; standard deviation $\pm$57 J/g), the lowest in May (19 298$\pm$27 J/g). When the ECS-values are deducted from the

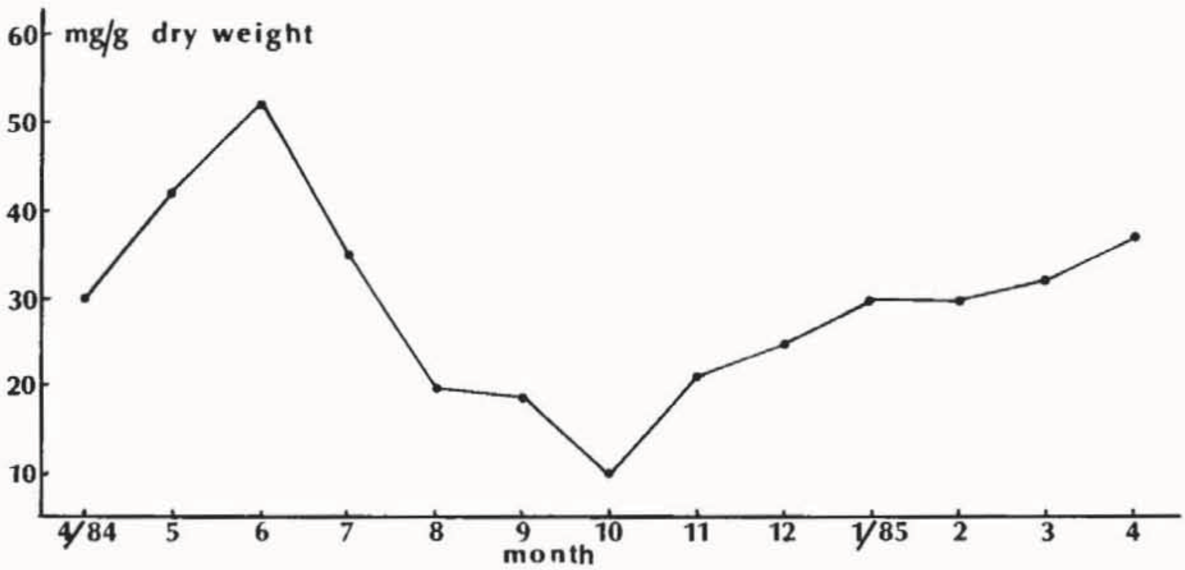

FIG. 6. — Seasonal variability of total lipid content in twigs of *Ephedra distachya*.

TABLE I. — *Fatty acid composition of saponifiable lipids from branches of* Ephedra distachya *(as % of total fatty acids).*

Fatty acid	Month												
	4/84	5	6	7	8	9	10	11	12	1/85	2	3	4
10 : 0	2,0	3,1	3,3	2,0	1,9	4,4	2,4	3,5	2,7	1,4	2,8	3,8	2,9
12 : 0	1,4	1,8	2,6	2,3	2,4	2,2	1,5	1,8	1,1	2,6	1,5	1,0	1,1
14 : 0	1,6	2,1	2,7	1,8	2,3	3,1	1,8	2,1	1,1	2,4	1,9	2,0	2,3
16 : 0	27,2	30,8	32,0	34,2	35,7	32,0	15,1	17,4	13,0	12,5	20,8	25,3	26,1
18 : 0	3,8	1,8	2,7	3,5	2,9	1,2	2,1	2,7	1,4	1,9	2,6	2,2	3,2
18 : 1	7,4	7,4	10,8	19,8	19,5	17,0	16,4	11,4	10,6	10,8	5,9	6,5	6,8
18 : 2	26,5	17,1	18,0	11,8	11,7	14,6	19,6	24,8	28,5	32,0	30,4	28,6	27,3
18 : 3	25,0	29,9	21,3	18,4	19,0	20,9	25,4	28,3	33,9	30,4	25,9	24,8	26,2
Not identified	5,1	6,0	6,6	6,2	4,6	4,6	5,7	8,0	7,7	6,0	8,2	5,8	4,1

CTE-values the highest energy value of what may be considered the "constructive components" of the plant tissue (that means: the plant components less the storage substances) is obtained in October, the lowest value in May during the growth period.

DISCUSSION

The water balance of *Ephedra distachya* is stable during winter, as proposed for mediterranean species by LARCHER (1972), but is stressed in the dry season. The decrease of the contents of total-N, protein-N, soluble-N (especially in April/May) and of the total ash content (from April to June) is at least partially due to a dilution effect, as discussed by DIAMANTOGLOU and KULL (1988). An increase of

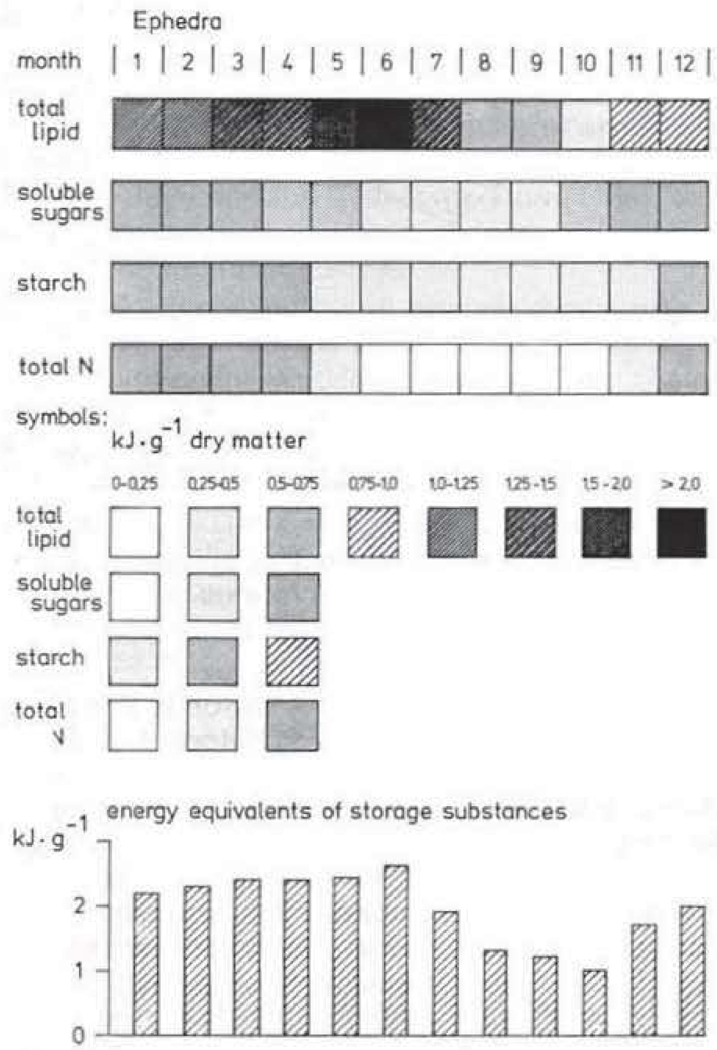

FIG. 7. — Energy content (calculated) of storage substances in twigs of *Ephedra distachya* (as kJ/g dry matter).

TABLE II. — *Calorific values (CTE) and energy content of storage substances (ECS) of Ephedra shoots during different periods of development (in kJ/g dry weight).*

	Period of		
	Growth	Drought	Winter (with episodic frost)
	February-June	July-October	November-January
Total energy content (CTE)	19.3–19.65	19.55–19.75	19.65–19.8
Energy content of storage substances (ECS)	2.3–2.6	1.0–2.0	1.7–2.3

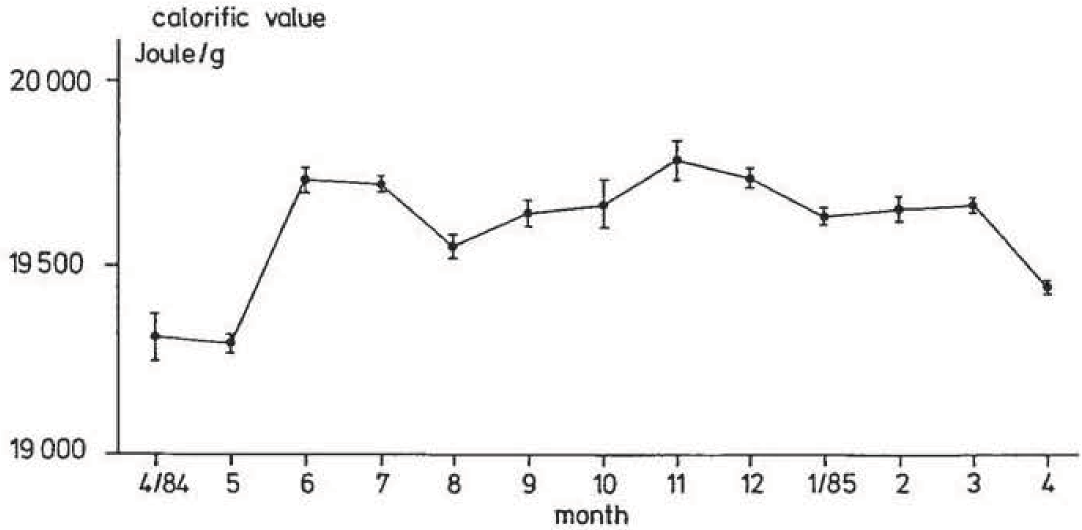

FIG. 8. — Seasonal variability of the calorific values of twigs of *Ephedra distachya.*

the total-N content at the beginning of the wet season in late autumn is also found in the Californian chaparral shrub *Adenostoma* (MOONEY & RUNDEL, 1979) but it is more distinct in *Ephedra.* From our data it may be concluded that root-growth during winter leads to a remarkable rise of the ash-content during spring. This is comparable to the findings of STOCK *et al.* (1987) in the fynbos species *Thamnochortus.* They found that roots developed during winter while the above ground parts developed in the warmer spring period. The similarity to *Ephedra* is striking in spite of the observation that *Ephedra* grows where nutrients are not limiting, in contrast to *Thamnochortus.* Upon termination of growth with summer drought, the N-content, protein-content and ash-content remain low and stable, as seen in other mediterranean species (DIAMANTOGLOU & KULL, 1988). At the start of the wet season, the ECS-value increases and the ash content rises gradually due to lack of growth of the above-ground parts.

In late spring, photosynthetic production surpasses consumption of storage substances resulting in the refilling of the storage pools. ECS is highest at the beginning of the dry season and lowest at the end. This holds true also for the sclerophyllous species of the Greek phrygana (macchia) vegetation (DIAMANTOGLOU & KULL, 1982). The ECS-values suggest that *Ephedra* undergoes climatic stress mainly during summer drought. The variations of these energy values are quantitatively similar to those found in evergreen species in Central Europe during the course of a year (DISTELBARTH *et al.*, 1984).

The decrease in the content of sugars and starch during spring is attributed to growth. During the dry season, no sugar accumulation was found in *Ephedra* twigs. The sclerophyllous species show a different behaviour in this respect. STEWART and BANNISTER (1973), SAUTER and KLOTH (1987) and LARCHER and THOMASER-THIN (1988) found that the ratio of energy content of starch to that of soluble sugars is a good criterion for judging the state of physiological stress of a plant. In *Ephedra,* this ratio remains stable for most of the year, and is lowered only in late autumn.

During this period of low temperature and absence of growth, the excess photosynthetic production is stored primarily as sugars.

The accumulation of lipids is highest when growth stops. The energy stored as lipids by *Ephedra* in Greece seems to be the main source of energy consumed during the drought period. The fatty acid pattern appears to be primarily growth–and temperature–dependent and is little influenced by stress. This confirms the conclusions of LARCHER and THOMASER-THIN (1988), that for sclerophyllous species, lipid accumulation is primarily endogenously controlled and is not normally influenced by environmental stress.

Values for CTE and ash content found here are comparable to those found in an unknown *Ephedra* species from the desert in southwestern U.S.A. (DARLING, 1976). The CTE-values are highly correlated with raw-fibre content. Furthermore, variation in CTE during the year is low and in the same order of magnitude as found for leaves and green above-ground plant parts of different species, respectively (BRZOSKA, 1979 & 1983; GRABHERR *et al.*, 1980).

Using the method of WILLIAMS *et al.* (1987), it was possible to estimate the tissue construction cost from the CTE-values, the organic nitrogen content and the ash content. Based on equation 11 of WILLIAMS *et al.*, (1987) we obtained a construction cost of 1.47 g glucose/g dry weight using our highest calorific value (in November), compared with 1.41 g glucose/g dry weight from our lowest value (in May). According to WILLIAMS *et al.*, the uncertainty is about ± 0.1 g glucose/g. When compared with the data listed by WILLIAMS *et al.*, the value for *Ephedra* is low, although 1.47 g glucose/g dry weight is in the range of the values for leaves of chaparral-shrubs. The low value for *Ephedra* suggests an effective metabolism with regard to the construction of the plant tissue.

Ephedra distachya is well suited to the rather semiarid climate of the Mediterranean, as seen by its ability to adjust to seasonal changes undergoing periods of growth and metabolic dormancy. Sugar accumulation was found in *Ephedra* during the mild mediterranean winter but not in the sclerophyllous species of the same area (DIAMANTOGLOU & KULL, 1982). The latter were found to have only a limited cold resistance (LARCHER, 1980), while *Ephedra* is well known to grow also under more severe cold-stress conditions.

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