YEARLY EVOLUTION OF TOTAL NONSTRUCTURAL CARBOHYDRATES AND PROTEINS IN JOJOBA FOLIAGE.

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RESUMO

EVOLUÇÃO ANUAL DOS CARBOHIDRATOS NÃO-ESTRUTURAIS E PROTEÍNAS TOTAIS NA FOLHAGEM DA JOJOBA

Jojoba, *Simmondsia chinensis* (Link) Schneider, um arbusto do deserto do Arizona e México, foi estudado durante um ano de investigação em três diferentes localidades no estado do Arizona, U.S.A., levandose em consideração o desenvolvimento mensal dos carboidratos não-estruturais e proteínas totais na sua folhagem.

As áreas 1 e 2 estão localizadas nas faces Leste e Oeste, respectivamente, das montanhas de Tucson, enquanto a área 3 está ao sul das montanhas de Santa Catalina.

A técnica descrita por SILVEIRA et alii[10], foi usada para a determinação dos carboidratos não-estruturais totais. Para a determinação das proteinas totais foi usada a técnica BIO-RAD[2] e um extrator convencional de proteína — hidróxido de sódio.

Os níveis mais altos de carboidratos não-estruturais totais na folhagem de jojoba foram alcançados no começo de março e os mais baixos no verão.

Os conteúdos mais altos de proteína coincidiram com as épocas de crescimento ativo da planta.

PALAVRAS-CHAVE: Jojoba, Arizona, Folhagem, Proteina, Carboidratos, Mensal, Ambiente, Crescimento.

SUMMARY

Jojoba, *Simmondsia chinensis (Link)* Schneider, a desert shrub from Arizona and Mexico, was studied during one year investigation at three diferent sites in Arizona considering the monthly development of total nonstructural carbohydrates and proteins in the foliage.

Site 1 is located on the eastern slope of the Tucson Mountains, site 2 is on the west side of the Tucson Mountains, and site 3 is in the Southern foothills of the Santa Catalina Mountains.

Total nonstructural carbohydrates was determined using the technique described by SILVEIRA et alii [10].

Total protein was determined using the BIO-RAD[2] technique and a conventional protein extractor — sodium hydroxide.

Total nonstructural carbohydrates in the foliage reached their highest level in early march and lowest in summer.

Protein content was highest at times of active growth.

KEY-WORDS: Arizona, Jojoba, Foliage, Protein, Carbohydrate, Evolution.

INTRODUCTION

Jojoba is very intensely browsed by cattle and deer. According to DAUGHERTY et alii[6], jojoba is capable of withstanding heavy browsing. DAYTON[7] noted since jojoba is evergreen

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and since its foliage has a relatively high percentage of carbohydrate material, its value as a browse species is considerable. Also, SWANK [11] reported that jojoba is considered to be one of the most important browse plants for deer because of its high protein content in the foliage.

GENTRY[8] noted that where cattle graze, they may browse the bushes severely and prevent any general development of fruit. CLARK[5] reported that jojoba was the key forage species for the Tucson Mountain Mule deer, probably because of jojoba's evergreen characteristics, its abundance, and its year-round availability for browsing. At the Papago Indian Reservation in the summer of 1977 we noted jojoba being preferentially browsed by cattle in comparison to associated shrub species.

It was our impression that female plant is preferred by cattle to male plant. We observed that the browsed plant had practically no seeds. This observation could lead to either two consions: livestock do not graze on heavily seeded plants, or livestock select branches with seeds to feed.

Very few individuals have studied this subject in jojoba. ANI et alii[1] investigated the carbohydrate content of jojoba but only a single mean of leaves and bark carbohydrate values was reported. For Tucson plants these values were 14.6% in winter and 13.1% in summer. In contrast, for San Diego plants, the percentage of carbohydrates were 11.3% in winter and 16.1% in the summer.

SWANK [11] determined the protein content of jojoba clippings for three different years in an Arizona Chaparral community near Prescott. Although the average for the 1953 clippings was 13.5%, he reported peak values of 21.1% in April 1954 and a low value of 8.3% in March 1956. He does not make any assumption about the stage of development of the plants at the time of sampling.

The main objective of this work was to study the behavior of jojoba in three different sites in Arizona, considering the monthly development of total monstructural carbohydrates and proteins in the foliage.

MATERIALS AND METHODS

During an intensive one year investigation (March 23, 1978 to March 26, 1979) the monthly development of total nonstructural carbohydrates and proteins in the jojoba foliage were studied in diferrent areas in Arizona.

Criteria for the selection of the sites were: areas with abundance of jojoba; areas with slopes not exceeding 15 percent; areas topographically suitable for machine operation; areas with elevation above 730 meters more conducive to growth, in Arizona (BURDEN[4]), and areas with ease of accessibility.

Site 1, on the eastern slope of the Tucson Mountains is located on Speedway Blvd. Close to the Painted Hills Road, 10 km west of the University of Arizona Campus. The elevation of the site is 790 m and its topography is undulating.

Site 2, on the westside of the Tucson Mountains, is located at an elevation of 820 m in the Tucson Mountain Park, west of Tucson 24 km from the University of Arizona Campus. Its topography is almost level with 1 to 3 percent slope.

Site 3, in the southern foothills of the Santa Catalina Mountains, north of Tucson, is located 14 km from the University of Arizona Campus. The elevation of this site is 850 m and its topography is almost level with a slope of 1 to 3 percent.

At the beginning of each month, the foliage from the ten selected female plants and five male plants in each site was harvested separately to determine the development of protein and total nonstructural carbohydrates (TNC) in those portions of the plant usually browsed by cattle and other animals and, also, used for asexual propagation. Only those leaves near the terminal points of the branches were used for this purpose. At the time of active growth, only new leaves were picked. The periods during which new growth occurred are indicated as peaks in the curves in Figure 1. Three leaves from each of ten female plants and six leaves from each of five male plants were harvested to make a composite sample of 30 leaves per sex. The number of leaves was limited to thirty to minimize the effects of their removal on the plants studied. The leaves were weighed immediately after harvesting and brought to the laboratory in an ice chest containing dry ice at a temperature of -40° Celsius. At the laboratorry, the samples were dried at -50° Celsius in a freeze dryer made by Labconco Corporation. For the purpose of propagation and browsing studies it would be more valuable to determine the protein and TNC of twigs rather than leaves but as other parameters of the twigs were to be measured it was decided to study only leaves. Picking twigs every month, should presumably cause much higher food stress on the plants than only harvesting leaves. Harvesting of twigs would presumably remove floral bud primordia and thus future fruit. Total nonstructural carbohydrates was determined using the technique described by SILVEIRA et alii [10]. Total protein was determined using the BIORAD[2] technique and a conventional protein extractor – sodium hydroxide as follows:

REAGENTS

Sodium hydroxide (all analytical grade) and Bio-Rad Protein Assay Kit[a]. This provides a 450 ml bottle of dye reagent[b] concentrate and a vial of lyophilized protein standard (bovine gamma globulin).

STANDARD

A standard preparation of the protein being assayed was used following the procedures reported by the Technical Bulletin 1051 issued by BIO-RAD[2] Laboratories.

DIGESTION, EXTRACTION, AND DILUTION

A 100 mg sample (ground to pass a 60 – mesh sieve) was placed in a culture tube. Twenty-five milliters of 0.8 N NaOH was added, the tubes were capped loosely, and were immediately incubated for one hour at 60°C in a shaking water bath. They were removed from incubation and cooled immediately to room temperature in cold tap water for about 5 minutes. The tubes were shaken vigorously and the material was transferred immediately to a centrifuge tube and centrifuged for 5 minutes at 5,000 rpm. One milliliter of the supernatant was diluted by into 2.0 ml of distilled water.

COLORIMETRY

An aliquot of 0.1 ml of the diluted solution was transferred to a test tube. At same time, 0.1 ml of the 0.8 N NaOH was transferred to a different test tube (blank). Five milliliters of the dilute dye reagent (one part dye reagent concentrate with four parts distilled water – filters through Whatman number 1) was added into each tube and swirled (BIO-RAD[2] Laboratory Bulletin 1051). After 20 minutes the samples were read against the blank, containing sodium hydroxide, in a spectrophotometer at 595 nm. The total protein of the unknowns were determined from a standard curve.

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b. This reagent can, also, be prepared by following the procedures reported by BRADFORD[3].

c. Different times of incubation, from one to 20 hours, were tried. One hour was enough to show the highest peak of protein.

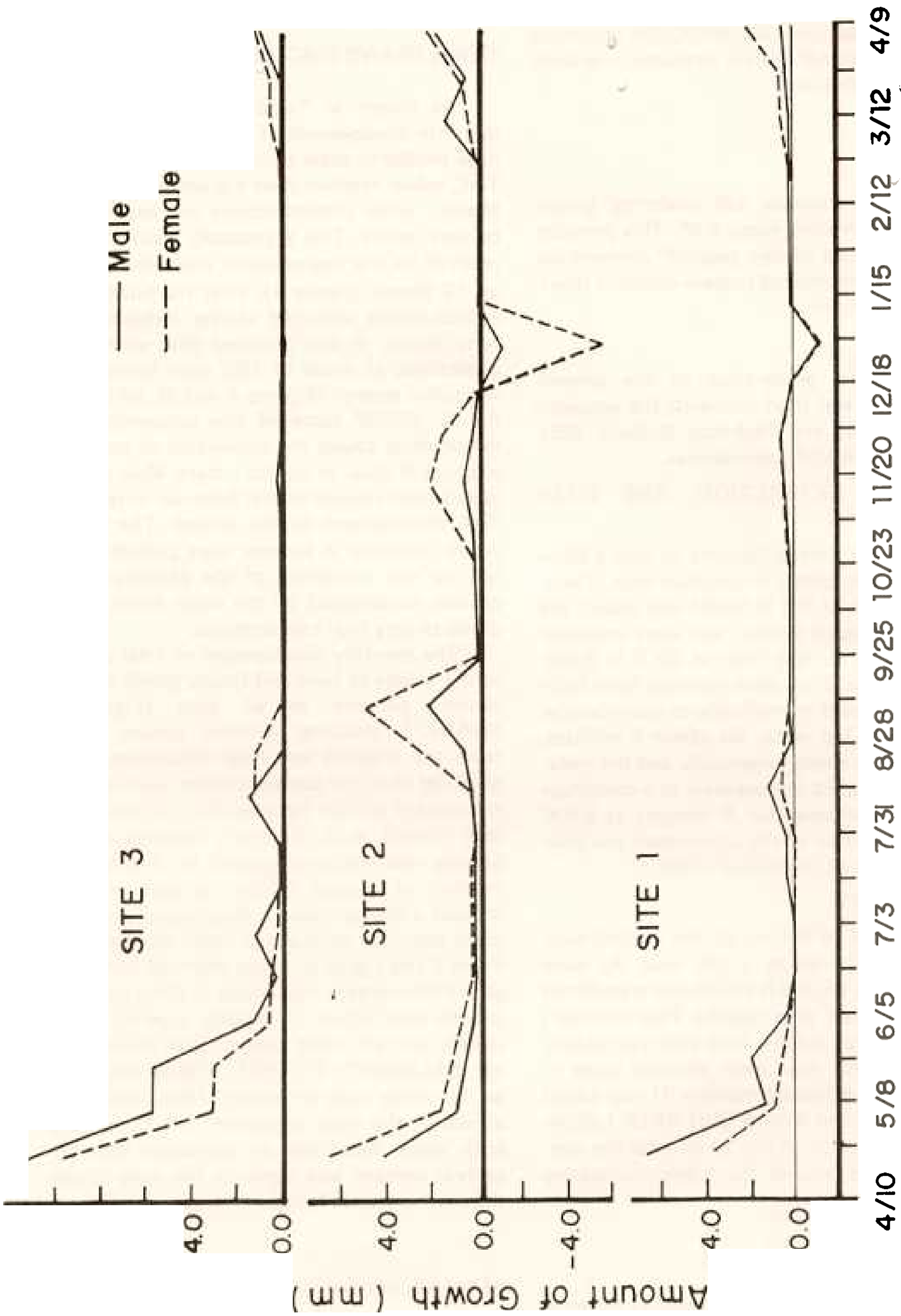
RESULTS AND DISCUSSIONS

As shown in Table 1 and Figure 2 the monthly development of TNC in the jojoba foliage tended to show similar patterns at all sites. TNC values reached their highest peak in early March, when photosynthesis was expected to be very active. This is probably justified when referred to the beginning of vegetative growth on 12 March (Figure 1), after the build up of carbohydrates occurred during February and early March. It was observed that, with some expections, all peaks of TNC were followed by vegetative growth (Figures 1 and 2). SALISBURY & ROSS[9] reported that apparently cool temperature causes the conversion of starch to sugar as it does in potato tubers. Male and female plants showed similar behavior in terms of TNC development in the foliage. The lowest values observed in summer were probably caused by the restriction cf the photosynthetic process conditioned by the water stress in response to very high temperatures.

The monthly development of total protein in the foliage of male and female plants showed similar patterns at all sites (Figure 3). SWANK[11] studying Arizona browse plants from the Prescott and Pinal Mountains areas, observed that the protein content decreased as the current growth became older. In other studies, (Bissell, et al.; Einarsen; Hellmers; Hagen; Stanley and Hodgson, quoted in SWANK[11]), analysis of browse species, as well as grass, showed a similar trend, a drop in protein as the plant becomes dormant or alder. According to Table 1 and Figure 3, it was observed that foliage protein content was higher in times of active growth (see Figure 1). Young, growing leaves usually contain more protein than mature leaves (SALISBURY & ROSS[9]). The annual average, for either male or female plants, was higher at site 2, the most vegetative site. Comparing both sexes, there was an indication that the protein content was higher in the male foliage than in the female foliage.

REFERÊNCIAS BIBLIOGRÁFICAS

1.ANI, H. A. Al – et alii. The physiological ecology of diverse populations of the desert shrub *Simmondsia chinensis*. *Ecology* 60: 41-57. 1972.



IGURE 1. Biweekly amount of growth of male and female jojoba plants at the three sites of study from April 10, 1978 to April 9, 1979.

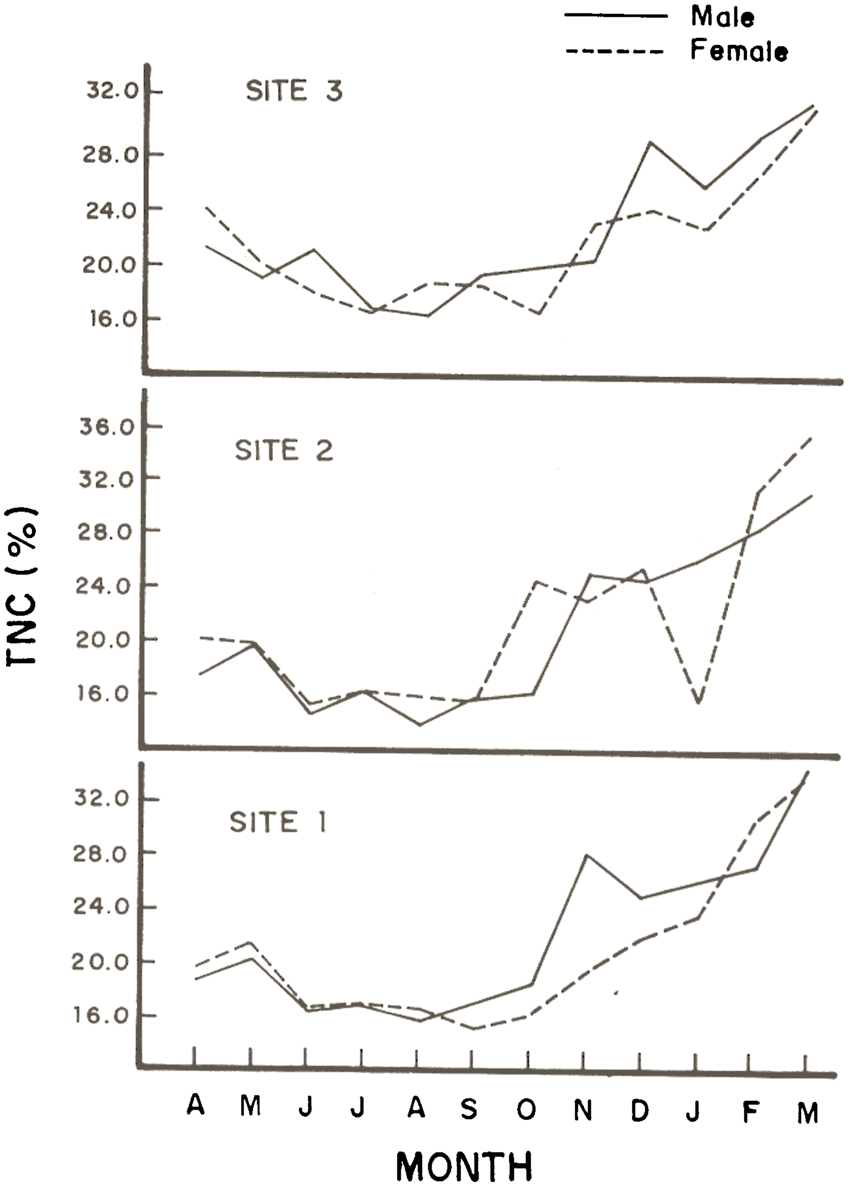


FIGURE 2. Monthly development of total nonstructural carbohydrates (TNC) in the foliage of male and female jojoba plants at three study sites from April, 1978 to March, 1979; leaves harvested at beginning of each month.

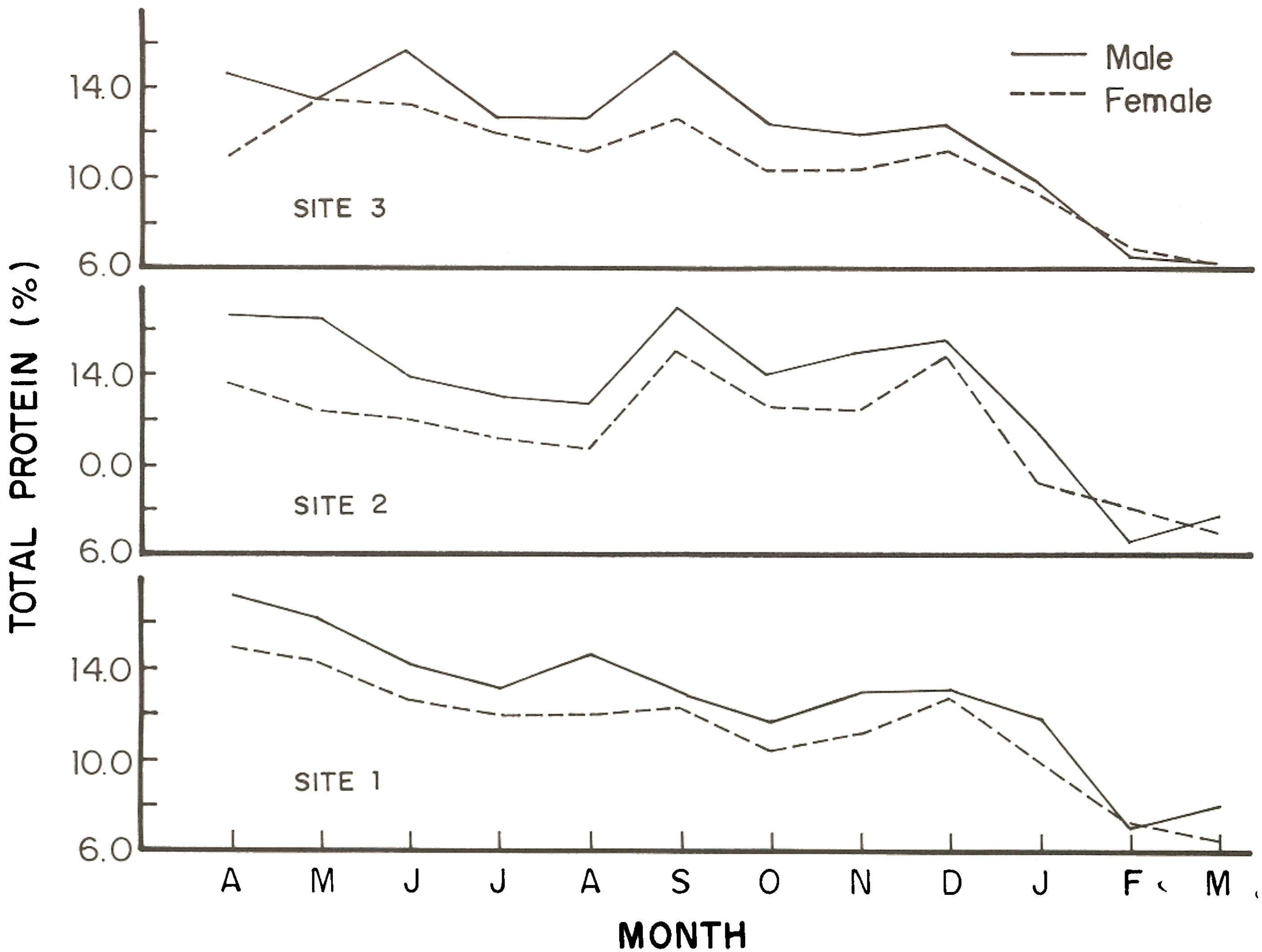


FIGURE 3. Monthly development of total protein in the foliage of male and female jojoba plants at three sites of study from April, 1978 to March, 1979; leaves harvested at beginning of each month.

2.BIO-RAD Laboratories. Bio-Rad protein Assay Tech. Bull. 1051. 1977. 3pp.

3.BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantitaties of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254. 1976.

4.BURDEN, J.D. *Ecology of Simmondsia chinensis (Link) Schneider, at its lower elevational limits.* M.S. Thesis, Arizona State University, Tempe, 1970, 71p.

5.CLARK, E. D. *A study of the behavior and movements of the Tucson Mountain mule deer.* M.S. Thesis, University of Arizona, Tucson, 1953. 111p.

6.DAUGHERTY, P. M. et alii. Industrial raw materials of plant origin. IV. A survey of *Simmondsia chinensis* (Jojoba). Georgia Institute of Technology, Engineering Experiment Station, *Bull.* 17, 1953. 36pp.

7.DAYTON, W.A. *Important western browse plants.* USDA Miscellaneous Publication 101. 1931. 214pp.

8.GENTRY, H.S. The natural history of jojoba (*Simmondsia chinensis*) and its cultural aspects. *Economic Botany 12*(3): 261-295. 1958.

9.SALISBURY, F.B., ana C. Ross. *Plant physiology.* Wadsworth Publishing Company, Inc., Belmont, California, 1969, 747p.

10.SILVEIRA, A.J. et alii. A rapid technique for total nonstructural carbohydrate determination of plant tissue. *Jour. Agric. Food Chem. 26:* 770-772. 1978.

11.SWANK, W.G. The mule deer in Arizona chaparral and an analysis of other important deer herds: A research and management study. Arizona Game and Fish Dept., Phoenix. *Wildlife Bull.* 3. 1958. 104p.

Table 1

Monthly Evolution of Total Protein and Nonstructural Carbohydrates (TNC) in the Foliage of Male and Female Jojoba Plants at Three Sites of Study. Leaves Harvested at the Beginning of the Month.

	Site ≠ 1				Site ≠ 2				Site ≠ 3			
	Protein (%)		TNC (%)		Protein (%)		TNC (%)		Protein (%)		TNC (%)	
Month	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Abril/78	17.1	14.9	18.7	19.7	16.6	13.6	17.5	20.1	14.6	10.9	21.3	24.0
May	16.1	14.2	20.4	21.5	16.5	12.4	19.5	19.8	13.4	13.4	19.0	20.1
June	14.1	12.6	16.3	16.5	13.9	12.0	14.6	15.3	15.6	13.3	21.1	17.8
July	13.1	11.9	16.8	17.0	13.1	11.2	16.4	16.2	12.7	11.9	16.8	16.4
August	14.6	11.9	15.7	16.5	12.7	10.7	14.0	16.0	12.7	11.2	16.3	18.7
September	12.9	12.2	17.0	15.2	17.0	15.1	16.0	15.7	15.6	12.7	19.4	18.4
October	11.6	10.3	18.3	16.1	14.0	12.7	16.3	24.5	12.4	10.5	19.8	16.4
November	12.8	11.0	28.2	19.2	15.0	12.4	25.0	23.1	12.0	10.5	20.4	23.0
December	13.0	12.6	24.8	21.8	15.6	14.8	24.7	25.6	12.4	11.3	29.2	24.0
January/79	11.8	9.9	26.1	23.4	11.5	9.3	26.2	15.9	10.0	9.4	25.7	22.6
February	6.9	7.1	26.9	30.2	6.7	8.1	28.3	31.8	6.8	7.1	29.4	26.8
March	7.8	6.3	34.3	33.9	7.7	7.1	31.4	35.4	6.5	6.0	32.0	31.3