Opuntia ellisiana: Cold Hardiness, Above-Ground Biomass Production and Nutritional Quality in the Mendoza Plain, Argentina♦

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ABSTRACT

Cold damage of *Opuntia ellisiana* plants obtained by micropropagation was estimated visually after 1- and 2-year growth periods in field conditions. Additionally, the effect of irrigation on above-ground biomass production was evaluated after a 2-year growth period and some parameters related to the nutritional quality of 1-year-growth-period cladodes were determined. Frost damage was estimated visually. Above-ground biomass and stem-area index (SAI) were estimated by regression techniques. One-year-growth plants suffered no frost damage when temperatures dropped to -15°C on two occasions in the winter of 2000. Frost damage reached only 0.9% in 2-year-growth-period plants after freezes of -14.5°C and -13.7°C in the winter of 2001. Irrigation did not have a significant effect on above-ground biomass production. Mean biomass reached 170 kg DM ha^{-1} after the 2-year growth period. This low production could be explained mainly by the small size of the plant material used for establishing the plantation and the low SAI after the 2-year growth period (0.03). Cladodes of *O. ellisiana* exhibited crude protein and organic-matter contents of 5.8% and 82.7%, respectively, and an *in vitro* dry-matter digestibility of 78.3%. Plants of *O. ellisiana* obtained by micropropagation appear to be tolerant to freezing temperatures attained in areas with extremely cold winters.

Keywords: *Opuntia ellisiana*, micropropagated plants, cold tolerance, biomass production, stem-area index, crude protein, *in vitro* dry-matter digestibility.

INTRODUCTION

Previous studies (Guevara et al., 1996, 1997) estimated the carrying capacity of the Mendoza plain without considering the incorporation of standing buffer reserves, which could be provided by drought-tolerant and water-efficient fodder shrubs such as *Opuntia* spp.

The experiments with spineless cactus [*Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.] as a fodder crop for drought periods began in the Mendoza plain at the end of 1995 as a consequence of a suggestion from Le Houérou (1995).

Monitoring of the artificially established plantations of this species showed that the major limitation to its cultivation in this area is the cold winter temperatures. In fact, when temperatures in an El Divisadero

♦ Received 12 October 2002

Cattle and Range Experiment Station field trial dropped to -12.3ºC in May 1996, almost all of the 7-month-old plants of *O. ficus-indica* froze to ground level. Similarly, when temperatures dropped to -16ºC and -17ºC on two consecutive days in August 1999, frost damage in the young cladodes from the 9-month-old plants reached 98% and the 3-year-old plants from different *O. ficus-indica* clones exhibited mean frost damage ranging from 19% to 53% (Guevara et al., 2000).

Given winter cold temperatures and water shortage in the Mendoza plain, further attention was given to other cacti species such as *O. ellisiana* Griffiths.

Opuntia ellisiana is the only spineless *Opuntia* fodder species that is completely cold hardy in Texas; it could be a useful forage variety in locations that are too cold for *O. robusta* Wendl. or *O. ficus-indica* (Han and Felker, 1997). *O. ellisiana* was completely tolerant to 20 hours below -7ºC, with a minimum of -16ºC (Felker, 1995). This species was not damaged when temperatures in a Kingsville, Texas field trial dropped to -12ºC in 1989 (Gregory et al., 1993). Furthermore, *O. ellisiana* had no damage from this freeze when temperatures of -20ºC were reached in a site located about 500 km north of Kingsville (Wang et al., 1997).

The average transpiration water-use efficiency of *O. ellisiana* was 162 kg H_2O kg^{-1} dry matter in the fourth year of growth of a plantation (Han and Felker, 1997). This is among the greatest water-use efficiency of any plant species (including C_3 and C_4) that has been measured under long-term field conditions (Han and Felker, 1997).

Unfortunately, *O. ellisiana* is a slower-growing species compared to *O. ficus-indica* (Han and Felker, 1997). In fact, the production of *O. ellisianato* to *O. ficus-indica* ratio ranged from about 0.30 to 0.35 (Barrientos Perez et al., 1992; Han and Felker, 1997) to 0.5 (Le Houérou, 1996., pers. comm.).

There is a lack of information on cold tolerance and biomass production under field conditions for plants of *Opuntia ellisiana* obtained by micropropagation. Our hypothesis was that these plants are cold hardy in areas with extremely cold winters. A field trial was initiated to examine the frost hardiness of *O. ellisiana* plants obtained by micropropagation. Two additional objectives were to evaluate the effect of irrigation on above-ground biomass production and to determine the nutritional quality of the cladodes.

STUDY SITE

This study was conducted on El Divisadero Cattle and Range Experiment Station (33º 45' S, 67º 41' W, elevation 520 m), in the north central Mendoza plain of midwestern Argentina. The mean daily minimum temperature of the coldest month (m) is -3.8ºC (Guevara et al., 2000). Mean annual rainfall (R) (July to June) from 1987-1988 to 2000-2001 was 321.2 mm (SD = 122.3) with nearly 80% occurring during the growing season (October to March). Potential evapotranspiration (PET), estimated according to Le Houérou (1989), is 1057 mm. From the bioclimatic point of view (Le Houérou, 1999), the site was classified as arid ($0.15 < R/PET < 0.33$) with extremely cold winters ($-5 < m < -3$). Soils are Torripsamments with greater silt content in interdunal depressions.

MATERIAL AND METHODS

The field trial with *O. ellisiana* was established on 1 December 1999. This species was planted using 15-month-old plants (Figure 1) with mean height of 5.7 cm (SD = 1.5), obtained by micropropagation (Juárez and Passera. 1998). Material used for micropropagation was of Texas origin and was obtained from Judith

Ochoa's collection in Santiago del Estero, Argentina. The plants were hardened off for 15 months in a greenhouse before transplanting to the field.

In addition, plants of *O. ellisiana* and *O. ficus-indica*, both obtained by micropropagation, were established in Malargüe, located about 260 km southwest of the study site, in 1999. At this site, the mean daily minimum temperature of the coldest month is - 4ºC (Le Houérou, 1999).

The experiment in El Divisadero Cattle and Range Experiment Station used a randomized complete block design with four blocks and three treatments per block (no irrigation; and two irrigation regimes, 30 mm every 30 days and 15 mm every 15 days, totaling 150 mm during the growing season; the two latter treatments are hereafter referred to as 30-30 and 15-15, respectively). Basin irrigation method was used. Thirty-six plants were located in six, six-plant-row plots with 1-m inter-row and 5-m between-row spacing. To avoid a border-row influence on frost damage and above-ground-biomass-production estimations, only the four plants located in the center of each plot were considered.

Frost damage was estimated visually, integrating the individual cladode damage over the entire plant. A 100% frost damage indicated that the plant was dead to the ground level and 0% damage indicated that the plant had suffered no damage. The plants of *O. ellisiana* were evaluated for frost damage in the middle of September 2000 and in the middle of August 2001 when the extent of necrosis from the freezing weather was fully expressed.

Frost damage was also estimated in the middle of August 2001 from adjacent plantations of *O. ficus-indica* and *O. spinulifera* Salm-Dyck f. *nacuniana* Le Houér., f. *nov*. (hereafter referred as *O. spinulifera*), established at 1 m x 5 m spacing in November 1998 and subjected to identical irrigation regimes as *O. ellisiana*.

Temperatures were monitored with a 7-day recording thermograph. This instrument was housed in a weather shelter 1.5 m above the ground located about 200 m from the cacti plantations.

Above-ground biomass production was nondestructively estimated by regression techniques at the end of the 2000-2001 growing season, i.e., after 2-year growth period. For all the cladodes present in each plant, the following parameters were measured: maximum length, maximum width, length of the insertion with the parent cladode, and thickness at the center of the cladode. To estimate regression equations, the same parameters were measured for about 50 randomly selected cladodes that were harvested and oven dried at 70ºC until no further change in weight was observed. The equation: $\log_{10} DW = -1.703 + 2.392 \log_{10} ML$ (Adjusted $R^2 = 0.91$; $p < 0.001$; Standard error of estimate = 0.10), where DW = dry weight of the cladode (g) and ML = maximum length of the cladode (cm) was used to estimate DW of each cladode. The maximum plant height was also measured in the field.

For stem-area index (SAI) estimation, maximum length (ML; cm) of the cladodes was regressed on the area of both sides of the cladodes (A; cm^2). The equation

$$A = -197.46 + 29.48\ ML \text{ (Adjusted } R^2 = 0.95;\ p < 0.001; \text{ Standard error of estimate} = 28.37)$$

was based on paper tracings of 50 randomly selected cladodes.

The nutritional quality of 1-year-growth-period cladodes was determined based on two samples consisting of cladodes collected from randomly selected individuals. Samples were analyzed in duplicate and the results averaged. Crude protein (N x 6.25) was determined by the Kjeldahl method (Müller, 1961), ash was determined following AOAC (1975), and *in vitro* dry matter digestibility was determined using the procedure of Tilley and Terry (1963).

The Kruskal-Wallis single-factor analysis of variance was used to test for frost damage differences among the three species. To determine between which of the species significant differences occur, a nonparametric Tukey-type multiple-comparison test was used ($p < 0.05$) (Zar, 1984). Frost damage data were analyzed at the species level because damage from irrigation treatments was very similar within each species.

One-way ANOVA was used to test for above-ground biomass (g $plant^{-1}$) differences among irrigation regimes. The same procedure was used to test for stem-area (cm^2 $plant^{-1}$) or plant-height (cm) differences. Biomass and stem-area data previously were transformed (log_{10}). Means were separated with Tukey's HSD test ($p < 0.05$).

RESULTS AND DISCUSSION

Temperatures Attained at the Study Site in 2000 and 2001

Temperatures below freezing occurred on 129 occasions from 27 March 2000 to 16 November 2000 and for 53 occasions from 23 April 2001 to 15 August 2001. Freezing temperatures attained after the latter date were not considered. Freezing weather totaled 1423 hours in year 2000 and 497 hours in year 2001. This means that freezing weather occurred at about 25% and 18% of the time in the previously mentioned periods for 2000 and 2001, respectively. The number of hours of freezing weather and the absolute minimum temperatures for events including minimum temperatures of ≤ -10ºC for years 2000 and 2001 are shown in Table 1. Apart from the longest period of freezing weather (127 hours) that began on 8 July 2000, there were two others in year 2000: 44 hours (16 to 18 June) and 55.5 hours (13 to 15 September), but the absolute minimum temperatures were -5.0ºC and -5.5ºC, respectively. Details on the temperatures attained during the four most severe freezes and the freezing periods that occurred in 2000 and 2001 are presented in Table 2. There were more hours with temperatures of ≤ -10ºC in year 2001 than in year 2000.

Frost Damage

One-year-growth-period plants of *O. ellisiana* suffered no frost damage in the winter of 2000. In the winter of 2001, the 2-year-growth-period plants suffered frost damage of about 1% (Table 3). Pads showed only slight necrosis around the margins. Frost damage for *O. ellisiana* was significantly lower than for the 3-year-growth-period plants of *O. ficus-indica* and *O. spinulifera*. We suppose that frost damage differences among *O. ellisiana* and the other two species would have been greater at a comparable plant age. In fact, frost damage proved to be inversely related to the age of the plant (Wang et al., 1997; Guevara et al., 2000). Coincident with our results in the present study, *O. ficus-indica* exhibited frost damage significantly higher than *O. spinulifera* in the winter of 1999 (Guevara et al., 2000).

In Malargüe, the plants of *O. ficus-indica* froze to ground level and the plants of *O. ellisiana* suffered no damage when temperatures dropped to -15ºC in the winter of 2000.

Dry-Matter Production and Stem-Area Index

In August 2001, after a 2-year growth period, *O. ellisiana* plants (Figure 2) had reached 20.9, 22.2, and 24.8 cm in height for no irrigation, 15-15 and 30-30 treatments. Plant height for the 30-30 treatment was significantly higher than that for the no-irrigation treatment.

Irrigation did not significantly affect above-ground biomass production (g DM $plant^{-1}$) and stem area (cm^2 $plant^{-1}$) (Table 4). Nevertheless, dry-matter production and stem area tended to be higher in the irrigated plots than in the nonirrigated ones.

At 5-m x 1-m spacing, total biomass production (kg DM ha^{-1}) would be 146, 157, and 208 for no irrigation, 15-15 and 30-30 treatments, respectively. These dry-matter productions were obtained with a total water input in the growing season of 383 mm in 1999-2000 (150 mm irrigation plus 233 mm rainfall) and 369 mm in 2000-2001 (150 mm irrigation plus 219 mm rainfall).

Our 2-year-growth-period average production (170 kg DM ha^{-1}) represented only 2.8% of the production reported by Han and Felker (1997) for 2-year-growth-period plants of *O. ellisiana* established at 1.5- x 1-m spacing in Texas. Several hypotheses could be advanced to explain this great difference in biomass between the two field trials. First, the Texas plantation was fertilized annually to avoid fertility limitation on water-use efficiency and it received 1691 mm rainfall in the 2-year growth period. In contrast, our plantation was not fertilized and it received 700 mm rainfall plus 300 mm irrigation from the establishment date to the date when the biomass production was estimated. Second, our planting material probably had lower size (in terms of cladode dry weight) than the single cladodes used by Han and Felker (1997) and hence, according to Mondragón-Jacobo and Pimienta-Barrios (1995), the number and size of the shoots produced during the first year of growth in the field were lower in our plantation. Third, the low biomass from our plants after the 2-year growth period seems due to a low stem-area index (SAI) that reached only 0.028, 0.029, and 0.038 for no irrigation, 15-15 and 30-30 treatments, while the Texas plantation had an SAI of 0.39 for the 2-year growth period (Han and Felker, 1997). These authors found that biomass productivity was very low until an SAI of 2 was reached. At an SAI of 4 to 5 productivity of *O. ficus-indica* is maximal (Nobel, 1995).

Nutritional Quality

Some parameters related to the nutritional quality of the 1-year-growth-period cladodes were: crude protein (% DM), 5.8; *in vitro* dry matter digestibility (% DM), 78.3; ash (% DM), 17.3; organic matter (% DM), 82.7.

There is considerable variation in nutritional quality of *Opuntia* forage for various species or clones, growing conditions, and cladode ages (Monjauze and Le Houérou, 1965; Silva, 1987; Gregory and Felker, 1992; Boza et al., 1995; Fuentes-Rodríguez, 1997; Guevara et al., 2000). This fact complicates direct comparisons of our results with other studies.

Crude protein content of *O. ellisiana* cladodes was similar to that of 1-year-growth-period cladodes of *O. ficus-indica* and *O. robusta* clones growing in the same conditions in the study site (Guevara et al., 2000). The IVDMD was high and similar to the overall value reported by Felker (1995) for *Opuntias*. Ash and organic-matter contents were comparable to those found by Boza et al. (1995) for 1-year-growth-period cladodes of *O. ficus-indica*.

In conclusion, our results have demonstrated that plants of *O. ellisiana* obtained by micropropagation appear to be tolerant to freezing temperatures attained in areas with extremely cold winters. In contrast with other *Opuntia* spp., it appears to be not necessary to protect the plants in winter for 1 or 2 years after planting. Further monitoring of our experimental plantation is needed to establish the biomass potential production of this species in the plain of Mendoza.

ACKNOWLEDGEMENT

The technical assistance of Marta N. Paez, Alberto F. Pinna, and Néstor P. Stritzler, and the financial assistance of the Secretaría de Ciencia y Técnica de la U.N. de Cuyo (Project 06/A123) and the ANPCyT (PICT 01-1448-BID 802) are gratefully acknowledged.

REFERENCES

AOAC. 1975. Official Methods of Analysis (12th Edn). Washington, D.C.: Association of Official Analytical Chemists. 1094 pp.

Barrientos Perez, F., F. Borrego Escalante, and P. Felker. 1992. Collaborative Mexico/United States initiative to breed freeze tolerant fruit and forage *Opuntia* varieties. In: Felker, P. (Ed.), Third Annual Prickly Pear Council, pp. 49-55. Kingsville, Texas: Texas A&M University. 65 pp.

Boza, J., J. Fonolla, and J.H. Silva. 1995. Utilización de la chumbera (*Opuntia ficus indica*) en la alimentación animal. Avances en Alimentación y Mejora Animal 35:3-6.

Felker, P. 1995. Forage and fodder production and utilization. In: Barbera, G., P. Inglese, and E. Pimienta-Barrios (Eds.), Agro-ecology, cultivation and uses of cactus pear, pp. 144-154. Rome, Italy: FAO. 216 pp.

Fuentes-Rodriguez, J. 1997. Feeding prickly pear cactus to small ruminants in northern Mexico. I. Goats. J. PACD 2:23-25.

Gregory, R.A., and P. Felker. 1992. Crude protein and phosphorus contents of eight contrasting *Opuntia* forage clones. Journal of Arid Environments 22:323-331.

Gregory, R.A., J.O. Kuti, and P. Felker. 1993. A comparison of *Opuntia* fruit quality and winter hardiness for use in Texas. Journal of Arid Environments 24:37-46.

Guevara, J.C., J.B. Cavagnaro, O.R. Estevez, H.N. Le Houérou, and C.R. Stasi. 1997. Productivity, management and development problems in the arid rangelands of the central Mendoza plains (Argentina). Journal of Arid Environments 35:575-600.

Guevara, J.C., O.R. Estevez, and E.R. Torres. 1996. Utilization of the rain-use efficiency factor for determining potential cattle production in the Mendoza plain, Argentina. Journal of Arid Environments 33:347-353.

Guevara, J.C., J.M. Gonnet, and O.R. Estevez. 2000. Frost hardiness and production of *Opuntia* forage clones in the Mendoza plain, Argentina. Journal of Arid Environments 46:199-207.

Han, H., and P. Felker. 1997. Field validation of water-use efficiency of the CAM plant *Opuntia ellisiana* in south Texas. Journal of Arid Environments 36:133-148.

Juárez, C., and C. Passera. 1998. Micropropagación de *Opuntia ellisiana* Griff. A partir de areolas y su aclimatación a condiciones de campo. In: Resúmenes XXVI Jornadas Argentinas de Botánica, pp. 147. Río Cuarto, Córdoba: Universidad Nacional de Río Cuarto. 367 pp.

Le Houérou, H.N. 1989. Classification écoclimatique des zones arides (s.l.) de l'Afrique du Nord. Ecologia Mediterranea 15:95-144.

Le Houérou, H.N. 1995. Informe de las visitas a la Argentina: Octubre-Noviembre 1992 y Setiembre-Noviembre 1995. Mendoza: IADIZA. 26 pp.

Le Houérou, H.N. (1999). Estudios e investigaciones ecológicas de las zonas áridas y semiáridas de Argentina. Mendoza: IADIZA. 228 pp.

Mondragón-Jacobo, C., and E. Pimienta-Barrios. 1995. Propagation. In: Barbera, G., P. Inglese, and E. Pimienta-Barrios. (Eds.), Agro-ecology, cultivation and uses of cactus pear, pp. 64-70. Rome, Italy: FAO. 216 pp.

Monjauze, A., and H.N. Le Houérou. 1965. Le rôle des Opuntia dans l'Economie Agricole Nord Africaine. Bulletin de l'Ecole Nationale Supérieure d'Agriculture de Tunis 8-9:85-164.

Müller, L. 1961. Un aparato micro-Kjeldahl simple para análisis rutinarios rápidos de materiales vegetales. Turrialba 11:17-25.

Nobel, P.S. 1995. Environmental biology. In: Barbera, G., P. Inglese, and E. Pimienta-Barrios. (Eds.), Agro-ecology, cultivation and uses of cactus pear, pp. 36 48. Rome, Italy: FAO. 216 pp.

Silva, J.H. 1987. Evaluación de los recursos alimenticios de la zona árida del ámbito del proyecto LUCDEME en ganado caprino. Tesis Doctoral. Escuela Técnica Superior de Ingenieros Agrónomos de la Universidad de Córdoba, España. 229 pp.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society 18:104-111.

Wang, X., P. Felker, and A. Paterson. 1997. Environmental influences on cactus pear fruit yield, quality and cold hardiness and development of hybrids with improved cold hardiness. J. PACD 2:48-59.

Zar, J.H. 1984. Biostatistical analysis (2nd Edn). Englewood Cliffs, New Jersey: Prentice-Hall, Inc. 718 pp.

Table 1. Duration of Freezing Temperatures and Absolute Minimum Temperatures (≤ -10ºC) Occurring at the Study Site in Years 2000 and 2001

Date	Number of Hours With Temperatures Below 0ºC	Absolute Minimum Temperature (ºC)
2000		
01 July	13.0	-10.0
08 July	127.0	-12.0
15 July	16.0	-13.0
18 July	16.0	-15.0
21 July	16.0	-11.0
22 July	15.5	-11.5
25 July	14.0	-10.5
03 August	10.5	-10.0
12 August	7.5	-11.0
24 August	14.5	-11.0
27 August	14.0	-11.5
30 August	19.5	-15.0
01 September	14.0	-11.8
02 September	13.0	-10.0
03 September	10.0	-10.5
24 September	12.0	-10.0
2001		
18 June	16.0	-14.5
19 June	14.0	-10.5
20 June	14.5	-10.0
10 July	13.0	-12.0
26 July	15.5	-13.7

Table 2. Temperature Occurrence During the Most Severe Freezes at the Study Site in Years 2000 and 2001

Date	Number of Hours of Occurrence of Temperatures			
	≤ -5°C	≤ -10°C	≤ -12°C	≤ -13°C
18 July 2000	14	7	4	2
30 August 2000	12	5	3.5	2
18 June 2001	14	11	7	3
26 July 2001	12.5	8	4	2

Table 3. Mean Frost Damage for Three *Opuntia* spp. at the Study Site in The Winter of 2001

Species	Frost damage (%)
O. ficus-indica	58.6 a
O. spinulifera	39.8 b
O. ellisiana	0.9 c

Means in the column followed by different letter are significantly different at $p < 0.05$ using Tukey's HSD test.

Table 4. Mean Above-Ground Biomass Production and Stem Area for 2-Year-Growth-Period Plants of *Opuntia Ellisiana* According to the Irrigation Regime at the Study Site

Irrigation Regime	Dry-Matter Production (g $plant^{-1}$)	Stem Area (cm^2 $plant^{-1}$)
No irrigation	72.8 a	1376.3 a
15 mm every 15 days	78.6 a	1447.7 a
30 mm every 30 days	103.8 a	1880.8 a

Means in a column followed by same letter are not significantly different at $p < 0.05$ using Tukey's HSD test.

Figure 1. Fifteen-Month-Old Plants of *Opuntia ellisiana* Obtained by Micropropagation, Which were Used to Establish the Field Trial

Figure 2. Field Trial (left) and Plants of *Opuntia ellisiana*. After 2-Year Growth Period (right) at the Study Site