Productivity, cold hardiness and forage quality of spineless progeny of the *Opuntia ficus−indica* 1281 x *O. lindheimerii* 1250 cross in Mendoza plain, Argentina

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Abstract

The specialized photosynthetic system, Crassulacean Acid Metabolism (CAM) in cactus that provides several fold greater conversion of water to dry matter (DM) than grasses and broadleaf plants makes them especially suited for forage production in arid lands. Unfortunately the spineless fast growing *Opuntia ficus−indica*, that is widely used for forage in Brazil, North Africa and Mexico, is poorly adapted to regions outside the tropics due to lack of tolerance to freezing weather. To overcome this limitation, a wide interspecific cross was made between a fast growing, spineless, frost sensitive *O. ficus−indica* and spiny, cold tolerant, slow growing Texas native *O. lindheimerii* with the objective of combining the high productivity and spinelessness of the *O. ficus−indica* parent with the cold hardiness of the Texas native parent. The first part of this work compared 10 spineless progeny of this cross to the most cold hardy spineless forage species to date, i.e. *O. ellisiana* for forage production and crude protein (CP) content in Mendoza, Argentina (520 m elevation and 33º S latitude), where *O. ficus−indica* does not survive winters. Some of these progeny had 5 times greater productivity than *O. ellisiana* with equivalent cold tolerance. As previous Argentine *Opuntia* productivity trials were N limited, the responsiveness of these progeny to 3 rates of N fertilization was examined. It was found that N application stimulated about a 4 fold increases in dry biomass per plant compared to the treatment in which N was not added (12.7 and 3.2 kg DM plant^{-1}, respectively) and an almost doubling in CP content of the 1−year−old cladodes (7.8 and 4.3% DM, respectively). None of the CP concentrations were high enough to meet the requirements of a 400−kg live weight lactating cow (10%), but the dose/N response did not appear to have reached a plateau. To determine the productivity per unit area, one trial with clone 42 was established. This clone produced a biomass of 40 t DM ha^{-1} in 4 years with a total of 625 mm rainfall. This is the greatest DM production recorded to date for such a low rainfall. This DM production corresponds to a carrying capacity of 0.76 Animal Unit (AU) ha^{-1}. Future trials with the most productive and cold hardy of these spineless hybrid progeny, in combination with higher N fertilization levels that can meet lactating cow requirements are needed in additional test sites with more severe freezing weather regimes. Also, would be important to compare different ways to capture fertilizers such as to apply frequent, low application rates instead of an annual application as we done in the present study, taken into account the characteristics of the root mass of *Opuntia*.

Other interesting alternative to prove for reducing the use of N−fertilizer could be to test if endophytic nitrogen−fixing bacteria such as *Gluconacetobacter diazotrophicus* fixes N with *Opuntia*.

Keywords: CAM, progeny, biomass production, cold hardiness, crude protein content.

Introduction

Opuntia species have the ability to withstand prolonged drought, high temperatures, as well as wind and water erosion (Nefzaoui and El Mourid, 2007). According to these authors, this ability, plus a range of economic uses, makes them ideal for agricultural development in areas affected by the world's two biggest environmental problems: desertification and climate change.

Cacti can produce more dry matter (DM) per millimeter of rainfall than any other type of plant due their Crassulacean Acid Metabolism (CAM) (González, 1989; García de Cortázar and Nobel, 1992; Han and Felker, 1997; Guevara *et al*., 2009). Simulations (García de Cortázar and Nobel, 1990) under natural conditions led to a maximum predicted productivity of about 20 t DM ha^{-1} yr^{-1} worldwide. Measurements with no water limitations gave 40 t DM ha^{-1} yr^{-1} in Chile (García de Cortázar and Nobel, 1991). A very high−density planting (24 plants m^{-2}) with unlimited water and ample nutrients led to 50 t DM ha^{-1} yr^{-1} in Chile (García de Cortázar and Nobel, 1992). When *O. ficus−indica* was fertilized and watered daily, it had a productivity of 47 t DM ha^{-1} yr^{-1} in Mexico (Nobel *et al*., 1992). With high N and P applications to *O. lindheimerii* Engelm. in Texas, González (1989) obtained 62 t DM ha^{-1} yr^{-1}. Above−ground biomass production of 3−year−old plants of *O. robusta* Wend., *O. paraguayensis* K. Sch., *O. spinulifera* Salm−Dyck f. *nacuniana* Le Houér. f. *nov.* and 3 accessions of *O. ficus−indica* established in a dune in the Mendoza plain, Argentina, ranged from 2.5 to 6.8 t DM ha^{-1} in natural conditions. These low yields could be attributable to the fact that plots were not weeded and had no fertilizer additions (Guevara *et al.*, 2000a).

As evidenced by various reviews (Monjauze and Le Houérou, 1965; Felker *et al*., 2006; Suassuna, 2008; Guevara *et al*., 2009), *O. ficus−indica* has been very useful for livestock food in arid lands. As discussed in these reviews, tolerance to temperatures lower than about −7 °C for about half a day is the main limitation to more widespread use of spineless *Opuntia* forage systems. A comparison of the frost sensitivity of *O. ficus−indica* and related species over a 13−year period, found that the age of the plant and the duration of the time below freezing greatly influenced the frost damage (Wang *et al*., 1997). These authors found that neither *O. ficus−indica*, nor *O. robusta* could survive 6 hours below − 6.7 ºC with a minimum of −12 ºC but *O. ellisiana* and *O*. spp. 1233 and *O. lindheimerii* could survive these temperatures without damage.

The major limitation to plantations of *O. ficus−indica* for fodder in Mendoza plain of Argentina is also cold winter temperatures. In fact, when temperature in El Divisadero Cattle and Range Experiment Station field trial dropped to −12.3 ºC in May 1996, almost all the 7−month−old plants of this species froze to ground level (Guevara *et al*., 2000a). Similarly, when temperatures dropped to −16 ºC and −17 ºC 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*., 2000a).

Felker *et al*. (2006) have suggested that spineless *Opuntia* spp. clone 1233 is adapted to USDA cold hardiness zone 8 and O. *ellisiana* Griffiths clone 1364 is adapted to USDA zone 7. Clone 1233 has high productivity even in the first year being able to produce more than 100 cladodes. In Mendoza, Argentina, *O. ellisiana* suffered no frost damage when temperatures dropped to −15 ^{o}C during two brief occasions (2−3 hours) in the winter of 2000 (Guevara *et al*., 2003). While O. *ellisiana* has high productivity 3 years after establishment when it reaches a leaf area index of 2 (Han and Felker, 1997), it would be desirable to have a spineless *Opuntia* with the same cold hardiness of *O. ellisiana* but with much faster growth rate. Valdez−Cepeda *et al*. (2001) evaluated the resistance to the freezing weather in a collection of 73 varieties (five plants each) of *Opuntia* spp. This collection is located near the city of Zacatecas, Mexico. A severe freeze occurred from 11 through 14 December 1997 when the temperatures were about −3, −10, and −10 ºC on three consecutive days. Temperatures below 0 ºC occurred for 14, 24, and 21 hours, respectively. There were two native varieties that were not affected by the freeze (one was spiny and it was nor stated if the other was spiny). The most resistant varieties had 0 to 10% of dead cladodes (DC); other varieties had an intermediate level of resistance to frost (25 to 70% DC), while the most affected varieties had 80 to 100% DC.

The freeze in Texas that killed all of the collection except of *O. ellisiana*, *O*. spp. 1233 and the native *O. lindheimerii* (Wang *et al*., 1997) had 62 consecutive hours below freezing, 16 hours below −6.7ºC and a minimum of −12ºC. Snyman *et al*. (2007) evaluated the cold/frost tolerance of 10 cultivars of *O. ficus−indica* and one cultivar of *O. robusta* over two growing season in a semiarid climate of central South Africa. They found that frost damage only occurred in spring (late−season frost: August to October) after a combination of frequent successive nights of freezing temperatures (between −2.1 and −9.6ºC) when the plants already started sprouting. In winter, during dormancy, no plants suffered any frost damage at freezing temperatures as low as −8.1ºC over several occasions. These authors suggested that it was also clear that a combination of late−season temperatures and soil−water stress could be detrimental to young cactus−pear plants and concluded that optimal plant development, as influenced by soil fertility, could also play a negative role on its frost sensitivity, as well as the phenological stage of the plants.

Attempts to cross *O. ellisiana* with *O. ficus−indica* to combine the cold hardiness of *O. ellisiana* with the much greater productivity of *O. ficus−indica* were not successful (Wang *et al*., 1997). However crosses between the spiny, Texas native *O. lindheimerii* that survived record freezing temperatures in 1989/1990 in Texas when all *O. ficus−indica* and related species suffered 100% mortality, and *O. ficus−indica* were fertile with 32% of the non apomictic seedlings being spineless (Felker *et al*., 2010). Based on productivity and disease resistance, we selected ten of these thornless progeny to compare their biomass and cold hardiness. The evaluation site was selected in the Mendoza plains of Argentina where *O. ficus−indica* cannot survive due to frost damage.

The high productivity of *Opuntias* reported from intensively managed fruit production and research plots have seldom been observed in rangeland systems. Our data for *Opuntia* productivity on unfertilized rangelands only ranged, as mentioned previously, from 2.5 to 6.8 t ha^{-1} (Guevara *et al*., 2000a). We hypothesize that these low productivities are N limited rather than water limited. For example, the approximately 2 kg N ha^{-1} yr^{-1} coming annually into semiarid ecosystems as a result of rainfall and blue green algal lichen crust (Geesing *et al*., 2000) would only support a yearly protein production of 12.5 kg or a DM production of 125 kg at 10% protein. In contrast, the water use efficiency of 162 kg water per kg DM for *O. ellisiana* (Han and Felker, 1997) in combination with a 50% yield of water transpired per total annual rainfall of 350 mm would produce 10,479 kg of DM.

The nutrient content of *Opuntia* spp. depends on many factors such as the age of the cladode, the age of the plant, the number of fruit per cladode and the fertilization program (Monjauze and Le Houérou, 1965; Boza *et al*., 1995; Nefzaoui and Ben Salem, 2001; Gugliuzza *et al*., 2002). The crude protein (CP) content of seven *Opuntia* forage clones was 4.8, 3.9, and 3.3% DM for cladodes of 1, 2 and 3 years of age, respectively (Guevara *et al*., 2004). These authors also found a significant ($p<0.05$) linear relationship between CP content and age classes for all clones.

In addition to a possible benefit of N on DM production, the CP content of *Opuntia* which for unmanaged range systems typically ranges from 3.2 to 5.0% DM^{-1} (Guevara *et al*., 2004) and is thus below the minimal requirements for a beef cow (6−13% CP DM^{-1}; NRC, 2000), has been CP concentration shown to be highly responsive to applied N. In an O. *lindheimerii* trial, González (1989) found that the CP increased from 4.5% for the control (no N and P addition) to 10.5% for additions of 224 kg N ha^{-1} and 112 kg P ha^{-1}. In addition, Nobel (1983) found that the CP concentration of *O. ficus−indica* cladodes in a California plantation near Los Angeles averaged 15.3%. Nitrogen fertilization in *O. ficus−indica* fruit plantation near Salinas, California has resulted in CP concentrations of 15% in 1−year−old cladodes (P. Felker, unpublished observations). For these reasons, we evaluated the effect of various doses of N fertilizer on both the DM production and CP level of these new potentially cold hardy forage types.

When fed as an exclusive diet, cladodes cause diarrhea after about 6 weeks for cattle and 8 weeks for sheep, limiting *Opuntia* use as a single feed to short periods (Le Houérou, 1996). According to this author, diarrhea easily can be prevent and/or cured by adding to the diet approximately 1% dry roughage (straw, hay, browse, grazing) on a body weight basis, in others words by offering a ration with a minimum overall DM content of 25−30%. In agreement with the previous author, Maltsberger (1991) found that the laxative property of cactus was due to the high moisture content of the cactus but this was not a concern to those familiar with its use as livestock forage. An Israeli experiment (Toledano and Katznelson, 1972) concluded that if the amount of cactus in the diet is increased slowly, cattle would adjust to the change with no problems. Texas rancher W.A. Maltsberger has developed a protein and mineral supplement (Felker, 2001) that allows him to feed 800 head herd of Santa Gertrudis cattle with 40 kg of *Opuntia* cladodes per day for up to 300 days per year without any laxative problem (Maltsberger, 1991). The studies of Ben Salem and Nefzaoui (unpublished data) showed that cactus cladodes have high oxalate content. Total oxalate is about 13% of the DM, of which 40% is in a soluble form. These oxalates are probably bound to Ca, making this anion less available to animals. This high amount of oxalates may also explain the laxative effect of cactus cladodes when fed to animals (Nefzaoui and Ben Salem, 2001).

The purpose of this study was to evaluate the productivity, cold hardiness and forage quality of 10 spineless progeny of the *O. ficus−indica* 1281 x *O. lindheimerii* 1250 cross with small bluish looking pads. One clone of *O. ficus−indica* and another of *O. ellisiana* were also included in one of the trials. Another important objective was to measure the productivity of one of these cacti, without the influence of border rows. Two hypotheses in this work were: a) by hybridizing frost tender, spineless but highly productive *O. ficus−indica* with cold hardy, but spiny native *O. lindheimerii*, spineless progeny with more cold hardiness and productivity than *O. ellisiana* could be obtained; and b) N fertilization of these progeny could raise their CP content percentages from very low levels on native rangeland to close to meeting a grazing animal' requirements for protein.

Materials and methods

Genetic materials

In July 1998, 127 progeny of the interspecific cross between two wild, spiny Texas native *O. lindheimerii* Texas A&M University Kingsville (TAMUK) accession 1250 male parents (cold hardy, red fruits, bluish pads) and a spineless commercial *O. ficus−indica* fruit type TAMUK accession 1281 (low cold hardy, spineless, fast growing, red fruits, greenish pads) were transferred from Texas A&M to the University of Santiago del Estero, Argentina and then to our research site in Mendoza. Ten of the segregants that had characteristics of the cold hardy, spiny male parent (small fruits and bluish cladodes) but without spines (Guevara *et al*., 2009), were examined for forage production/freeze hardiness in trials described in this paper.

Study site

The study was conducted on El Divisadero Cattle and Range Experiment Station (33º 45' S, 67º 41' W, elev. 520 m), in the north central Mendoza plain, mid−west Argentina. Daily mean annual minimum and maximum temperatures range from −3.8 to 15.6 ºC and 14.2 to 33.0 ºC, respectively (Guevara *et al*., 2002). Mean annual rainfall for the period 1987−2008 was 293.1 mm (S.D. = 112.8) with nearly 80% occurring during the growing season (October−March) (records of IADIZA, not published). This Mendoza site is similar in the severity of winters and rainfall to other sites such as in Mexico, the USA, South Africa, North Africa and the Middle East (Felker *et al*., 2009).

According to Masotta and Berra (1994), soils are Torripsamments with greater silt in interdunal depressions. These authors reported the following data for these soils: pH, 6.4 −7.6; organic matter (Walkley−Black method), 0.09−0.22%; total N (Macro Kjeldahl method), 336−420 ppm; extractable P (bicarbonate extraction, Arizona method), 9−20 ppm; extractable K (nitric acid extraction, Pratt method), 990−1420 ppm; and EC of soil saturation extract, 0.17−0.38 mS cm^{-1}. Thus, soils have very little amount of organic matter and low total N.

The vegetation is an open xerophytic savanna and shrubland of *Prosopis flexuosa* DC. Eight warm−season perennial grasses dominate the herbaceous layer: *Aristida mendocina* Phil., *A. inversa* Hack., *Chloris castilloniana* Lillo & Parodi, *Digitaria californica* (Benth.) Henrard, *Panicum urvilleanum* Kunth, *Pappophorum philippianum* Roseng., *Setaria leucopila* (Scribn. & Merr.) K. Schum., and *Sporobolus cryptandrus* (Torr.) A. Gray (Guevara *et al*., 2000b).

Trials

Three trials were established with different objectives. The first trial was to rank the 10 clones in order of biomass productivity and CP content. In this case it was not necessary to use border rows. The second trial was established with one clone to accurately determine the biomass production per hectare. The third trial was established to measure the influence of N fertilization on growth and CP content of these ten progeny. The details of each of these experiments follow:

Determination of the ranked order of the hybrid progeny in terms of biomass production per plant and crude protein content

Trail 1 included 10 progeny (42, 46, 64, 80, 83, 85, 89, 94, 97, and 150) and *O. ficus−indica* 1281 and *O. ellisiana*. These entries were arranged in four randomized blocks with 5 plants per repetition on 3 x 5 m spacing. This trial had an initial fertilization of 100 g of 15−15−15 per plant to reduce possible differences in soil fertility. The fertilizer was applied in a circle of about 1 m in diameter around each plant at the beginning of the rainy season. It was broadcast by hand and then incorporated by a rake without disturbing the plant roots.

Determination of biomass productivity per hectare and crude protein content

This trial included only the clone 42 and had 3 replications with each of them having 6 rows of 8 plants per row on 1.5 x 1.5 m spacing since we wanted to achieve complete canopy cover as soon as possible. To obtain valid estimates of productivity per hectare, border effects must be eliminated. We are not aware of the influence of border rows on productivity of *Opuntia* but the influence of border rows on closely spaced tree plots has been intensively studied (Zavitkovski, 1981). To establish valid biomass estimates per ha for this promising *Opuntia* progeny, we eliminated the biomass data of the outer 2 rows (40 plants) thus basing the biomass estimates per hectare on the average of the inner 8 plants. This treatment had annual fertilization of 100 kg N, 50 kg P, and 50 kg K per hectare with the purpose to obtain maximum productivity. The fertilizers were applied in all the area of the trial. Each data point came from a very strong number of plants and for this reason we consider that three replicates are sufficient for giving acceptable results.

Effect of fertilization on the productivity and crude protein content on ten hybrid progeny and controls

The three fertilizer treatment were: a) control without fertilization; b) application a low quantity of fertilizer: 30 kg N, 30 kg P, and 30 kg K per hectare every two year This fertilization level represented a modest fertilizer investment, typical of those faced by a farmer of medium financial resources; and c) annual application of 100 kg N, 50 kg P and 100 kg K per hectare, that represent a high fertilizer investment that could permit the available water and not the fertility to limit the plant growth. This latter treatment should also come close to obtaining the maximum biomass production and forage CP levels. The clones used were the same as in Trial 1. This was a randomized, split−plot design with 5 blocks per fertilizer treatment in which each clone was represented by a single plant per repetition. The spacing used was 5 x 3 m. Fertilizers were applied as was described for Trial 1.

The three trials were installed in September 2005. In all the trials the small fruits were eliminated from the cladodes to just estimate vegetative growth. At the end of the fourth growing season, the plants were destructively harvested to measure fresh and dry matter production per plant, i.e. entire plants were cut and weighed. Representative samples of cladodes were collected from each plant for determining DM and CP content. One−year−old cladodes were used for determining CP content because these are what the livestock mainly eat. The inner portion of the cladodes was sampled as described by Guevara *et al*. (2006) for determining N content. All the samples were oven−dried at 70ºC until no further weight change occurred and weighed to the nearest 0.1 g. Crude protein content was estimated by multiplying the total N, as determined by the Kjeldahl method (Müller, 1961), by 6.25. Samples for determining N content were analyzed in duplicate and the results averaged.

Frost damage was visually estimated by counting the cladodes per plant that suffered frost damage and related this number to the total cladodes of each plant. The number of plants of each clone and species that suffered frost damage was also register. All the trials were non−irrigated.

Statistical analysis

Data were analyzed by a linear mixed ANOVA model with heterogeneous residual variance between parental and progeny clones or between all clones as indicated by Likelihood Ratio Test (LRTs) comparing the heteroscedastic models with the null o classical General Linear Model ($p<0.0001$). Means were evaluated by means of pairwise comparisons from the adjusted means and standard errors according with the selected covariance structure ($\alpha=0.05$). All the statistical analyses were carried out using the Statistic Software InfoStat, Version 2008 (Infostat, 2008).

Results and discussion

Biomass production

Trial 1

The hypothesis of no differences among clones for biomass production was rejected ($p<0.0001$) (Table 1). The results showed that there were a group of clones (85, 89, and 97) that was no different from *O. ellisiana* (the one with lower biomass) and a second group (64, 83, 94, and 80) that did not differ from *O. ficus−indica*. The third group contained 3 clones (42, 46, and 150) that were different from both *O. ficus−indica* and *O. ellisiana* (p=0.041). At a 5 x 3 m spacing (667 plants ha^{-1}), clone 150 produced about 6.7 t DM ha^{-1} at the end of the four year of growth. In this trial, the production of *O. ellisiana* to *O. ficus−indica* ratio was about 0.38, similar to the findings of Barrientos Pérez *et al*. (1992) and Han and Felker (1997) who reported a production ratio ranged from 0.30 to 0.35.

Table 1. Mean values of adjusted biomass (DM) per plant (and standard errors, SE) for clones and species after four growing seasons in the Mendoza plain of Argentina (Trial 1).

Clone and species	Mean value of DM per plant (kg) and SE	
150	10.06−1.15	a
46	7.52−1.15	ab
42	7.25−1.15	ab
80	5.81−1 15	bc
94	5.77−1.15	bc
83	5.74−1.15	bc
64	5.71−1 15	bc
O. ficus−indica	3.86−0.26	c
85	3.80−1.15	cd
89	3.48−1 15	cd
97	2.92−1 15	cd
O. ellisiana	1.45−0.26	d

Means in the column followed by different letters indicate significant differences (p< 0.05) using LSD Fisher.

Trial 2

In the trial to estimate the biomass production devoid of border effects, as estimated by the 95% CI, there were no significant differences (p<0.005) among plots either in biomass per plant or crude protein content for plots 1 and 2 whereas plot 3 had lower biomass per plant and per area than those of plots 1 and 2 (Table 2). The mean N content of the three plots (0.66) was much lower than the value of 0.97 recommended by Magallanes−Quintanar *et al*. (2004) for maximum production in 'nopalitos' and thus this production could be N limited.

Table 2. Mean crude protein content (1−year−old cladodes), mean biomass per plant and per hectare for clone 42 after four growing seasons in the Mendoza plains of Argentina (Trial 2).

Plot	Plant			Area (ha)			CP content		
	DM (kg)	95% CI		DM (t)	95% CI		(% DM)	95% CI	
		UL[1]	LL[1]		UL	LL		UL	LL
1	11.87	14.0	9.8	52.8	62.0	43.5	4.04	4.3	3.8
2	8.68	10.0	7.4	38.6	44.4	32.8	4.00	4.4	3.6
3	6.45	8.4	4.5	28.7	37.4	19.9	4.32	4.6	4.0
Mean	9.00	10.3	7.7	40.0	45.8	34.2	4.12	4.3	4.0

[1]UL: Upper Limit; LL: Lower Limit.

Trial 3

In Table 3 it is shown that there were significant differences among the adjusted means of fertilizer treatments for all the 10 clones combined (p=0.025). Mean yield of DM per plant was significantly higher at the highest doses of fertilizer than those of low doses and control, except for clone 89 that showed no differences among the three treatments. Clone 85 was the most productive under high fertilization (11.2 t DM ha^{-1}). Other productive clones were 83 (9.9 t DM ha^{-1}), 94 (9.8 t DM ha^{-1})

and 150 (9.5 t DM ha^{-1}). The response to fertilization at the highest application rate was 4 fold over the biomass of the zero fertilization treatment.

Table 3. Mean yield of DM per plant (and standard errors, SE) according to fertilizer doses after four growing seasons in the Mendoza plains of Argentina (Trial 3).

Clone	Fertilizer dose	Mean yield per plant (kg DM) and SE
42	C	3.60−0.68 a
42	L	7.48−2.94 a
42	H	13.64−3.55 b
46	C	2.08−0.44 a
46	L	6.04−1.62 a
46	H	10.32−2.95 b
64	C	2.24−0.98 a
64	L	4.89−0.31 a
64	H	10.38−4.16 b
80	C	3.36−0.70 a
80	L	5.98−1.27 a
80	H	10.54−5.65 b
83	C	5.14−1.11 a
83	L	7.12−0.76 a
83	H	14.92−3.02 b
85	C	1.94−0.78 a
85	L	4.42−0.96 a
85	H	16.79−2.34 b
89	C	3.81−0.93 a
89	L	4.43−0.63 a
89	H	7.86−1.17 a
94	C	2.64−1.14 a
94	L	4.62−0.78 a
94	H	14.64−3.08 b
97	C	2.65−0.36 a
97	L	4.32−0.87 a
97	H	13.47−1.85 b
150	C	4.48−1.97 a
150	L	6.52−1.77 a
150	H	14.27−2.93 b

H: high dose of fertilizer; L: low dose of fertilizer: C: control (no fertilizer added). Means in the column followed by different letters indicate significant differences ($p < 0.05$) using LSD Fisher.

Crude protein content

Trial 1

Two clones (97 and 89) had the same crude protein content than *O. ficus−indica* (Table 4). The mean CP concentration of clones 97, 89 and the species *O. ficus−indica* could satisfy the CP requirements of a cow of 400−kg live weight during the last part of the pregnancy (8.0% CP) but not for a lactating cow of the same live weight (10%).

Trial 3

For trial 3 we observed a significant interaction between clones and fertilizer treatments ($p<0.001$). The clone performance depends on the fertilizer doses. With high doses of fertilizer, clone 150 had higher CP content than those of the clones 89, 85, 94, and 46 (Table 5) whereas with low doses of fertilizer there was no significant differences among clones. In the treatment with no addition of fertilizer (C), the clone 85 had significant high CP content than the clone 64. The high doses of fertilizer applied almost doubled the mean CP content of the cladodes when it was compared with the treatment in which no fertilizer was added. (7.8 and 4.3% DM, respectively).

Table 4. Adjusted mean crude protein content (and standard errors, SE) for clones and species after four growing seasons in the Mendoza plain of Argentina (Trial 1).

Clone and species	CP content (% DM) and SE
97	7.92−0.23 a
O. ficus−indica	7.87−0.06 a
89	7.79−0.06 a
O. ellisiana	7.12−0.25 b
64	6.79−0.42 bc
80	6.53−0.02 bc
46	6.39−0.10 bcd
85	6.38−0.32 bcd
150	6.29−0.26 cd
94	6.11−0.08 cd
83	5.92−0.13 cd
42	4.95−0.71 d

Means in the column followed by different letters indicate significant differences ($p < 0.05$) using LSD Fisher.

Frost damage

The temperature data in the period May−September 2009 are from Ñacuñán (30 km south of the study site; 34º 03’ S, 67º 58’ W) where the temperatures were similar to the study site. The number of total hours with temperatures below 0ºC and the absolute minimum temperatures were 6 and −1.8ºC; 77 and −7.1ºC; 146 and −6.1ºC; 37 and −4.7ºC, and 54 and −4.7ºC in May, June, July, August and September, respectively. The total of hours with temperatures below 0ºC was 320.

There were statistical differences in the frost damage among clones and species both in the cladodes ($p<0.0001$) (Table 6) and in the percentage of plants ($p=0.0153$) (Table 7) that were damaged. In both Tables, due to large differences in variances, the standard errors were adjusted by the heterogeneity of variances for both variables. The frost damage in the cladodes of clones 64, 150 and 42 was significantly lower than that of *O. ficus−indica*. Clones 46, 80, 83, 89, 94 and *O. ellisiana* had zero frost damage during the period. This result is in agreement with the frost damage estimations in the winter of 2007, when the minimum temperatures dropped to −9 ºC in two occasions (Felker *et al*., 2009) in which the frost damage in *O. ficus−indica* reached 15.7% and was higher than the other clones. These results are coincident also with those found by Valdez−Cepeda *et al*. (2001) who reported that the most resistant varieties of *Opuntia* spp. had 0 to 10% of dead cladodes, but with more low temperatures than those in our study.

The plants affected by frost (Table 7) were significantly lower in clones 42, 97, 85, 150, and 64 than those of *O. ficus−indica*. The other clones suffered no frost damage. In contrast to the results of Snyman *et al*. (2007), the frost damage in our study site occurred mainly in the winter months when the plants were dormant. These authors did not state the number of hours that these plants were below various temperatures and it is possible that the duration of the below freezing temperatures may be responsible for these differences.

Table 5. Adjusted mean crude protein content (and standard errors, SE) for clones according to the three doses of fertilizer after four growing seasons in the Mendoza plain of Argentina (Trial 3).

Clone	Fertilizer dose	Mean crude protein content (% DM) and SE
150	H	8.70−0.16 a
80	H	8.31−1.01 ab
64	H	8.10−0.06 ab
42	H	7.96−0.08 ab
83	H	7.88−0.06 ab
97	H	7.70−0.72 ab
89	H	7.62−0.27 b
85	H	7.44−0.04 b
94	H	7.32−0.15 b
46	H	7.31−0.18 b
80	L	5.85−1.01 c
85	L	5.81−0.04 c
94	L	5.81−0.19 c
64	L	5.57−0.06 c
89	L	5.50−0.27 c
97	L	5.44−0.72 c
83	L	5.33−0.06 c
150	L	5.17−0.16 c
42	L	5.13−0.08 c
46	L	4.38−0.18 cd
85	C	4.94−0.04 c
80	C	4.53−1.01 cd
150	C	4.52−0.13 cd
42	C	4.49−0.08 cd
89	C	4.46−0.27 cd
97	C	4.34−0.59 cd
83	C	4.19−0.05 cd
46	C	3.94−0.18 cd
94	C	3.91−0.15 cd
64	C	3.67−0.06 d

H: high dose of fertilizer; L: low dose of fertilizer; C: control (no fertilizer added). Means in the column followed by different letters indicate significant differences ($p < 0.05$) using LSD Fisher.

Conclusions

Other intensively managed trials that have reported high productivity for *Opuntias* were all conducted with either irrigation or mean annual rainfalls close to double what occurred in this trial. As far as we are aware, our data for clone 42 yielding 40 t DM ha^{-1} in 4 years with 625 mm over those 4 years is the highest plant productivity recorded at this low annual rainfall. In terms of animal units, at 36 kg consumption per day per AU (AU: one 400−kg live weight cow and a calf or the equivalent), this dry matter yield is equivalent to 0.76 AU ha^{-1}. In the study area, a typical relationship is 0.05 AU ha^{-1} (Guevara *et al*., 1997) and thus this yield represents very dramatic improvement in carrying capacity.

Table 6. Adjusted means of the percentage (and standard errors, SE) of cladodes affected by frost in year 2009 in the Mendoza plain of Argentina (Trial 1).

Clone and species	Frost damage (% of cladodes) and SE	
O. ficus−indica	11.05−2.63	a
97	4.08−2.35	ab
85	3.73−3.72	ab
42	3.03−1.80	b
150	2.23−2.23	b
64	0.50−0.50	b
46	0	c
80	0	c
83	0	c
89	0	c
94	0	c
O. ellisiana	0	c

Means in the column followed by different letters indicate significant differences (p< 0.05) using LSD Fisher.

Table 7. Adjusted mean of the percentage (and standard errors, SE) of plants affected by frost in year 2009 in the Mendoza plain of Argentina (Trial 1).

Clone and species	Mean frost damage (% of plants) and SE	
O. ficus−indica	80.00−11.55	a
42	10.00− 5.77	b
97	10.00− 5.77	b
85	10.00−10.00	b
150	5.00− 5.00	b
64	5.00− 5.00	b
46	0	c
80	0	c
83	0	c
89	0	c
94	0	c
O. ellisiana	0	c

Means in the column followed by different letters indicate significant differences (p< 0.05) using LSD Fisher.

Whereas *O. ellisiana* was a breakthrough in providing virtually 100% security against freezing weather in cold Mendoza plains, this species had low biomass productivity. This trial found some interspecific *O. ficus−indica* x *O. lindheimerii* progeny, i.e. 42 with only minimal frost damage of 10% compared to *O. ficus−indica* 80% but with 5−fold greater biomass productivity than *O. ellisiana*. The high doses of fertilizer applied increased the CP content of 1−year−old cladodes in all the progeny but the CP concentration was not enough to satisfy the lactating cow requirements. However, since the high fertilizer rate was different from the medium rate, it is clear that the fertilizer rates did not reach a plateau and that higher rates might be able to surpass the CP requirement for lactating cows. Since other experiments have found CP contents of 15% in 1−year−old cladodes, higher CP contents are clearly possible to obtain.

In summary, for the first time we have demonstrated that with N fertilization and use of cold hardy *O. ficus−indica* x *O. lindheimerii* progeny, it is possible to obtain high biomass production in regions that are too cold for *O. ficus−indica* with only 156 mm rainfall per year. We have shown that great increases in DM production and CP contents of these hybrid progeny were achieved over non−fertilized rangelands. Future trials with the most productive and cold hardy of these spineless hybrid progeny, in combination with higher N fertilization levels that can meet lactating cow requirements are needed in additional test sites with more severe freezing weather regimes.

Also, further trials are required using these progeny in combination with a different way for increasing the capture of fertilizer because 91 to 100% of *O. ficus−indica* root mass were in the upper 15 cm of the soil at distances between 15 and 150 cm from the plant mother, respectively (Snyman, 2005). Thus, when fertilization is applied, it is important to apply frequent, low application rates instead of an annual application as we done in the present study to avoid leaching the nutrients below the root zone. Other interesting alternative to prove for reducing the use of N−fertilizer could be to test if endophytic nitrogen−fixing bacteria such as *Gluconacetobacter diazotrophicus* (Pedraza, 2008) fixes N with *Opuntia*. It would be possible since *Opuntia* has a lot of acids and sugars in the vascular system.

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