Potential Global Adaptivity of Spinelles, Progeny of *Opuntia ficus-indica* 1281 x *O. lindheimeri* 1250 as Forage Cultivars Adapted to USDA Cold Hardiness Zones 7 and 8

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Abstract

*Opuntia* has a long tradition of use for forage in Mexico, Brazil, South Africa, Tunisia and arid regions of the US. The vast majority of these cacti are spineless as spiny forage types are more difficult to work with. Some spiny *Opuntia* are very cold tolerant existing as far as 56° N in Alberta, Canada. In contrast traditional spineless *Opuntia ficus-indica* species, normally used for fruits, are limited in extension to USDA cold hardiness zones 9 and 10 due to lack of cold hardiness. Discovery of a spineless cold hardy *O. ellisiana* adapted to USDA cold hardiness zone 7 has the potential to greatly expand the range for livestock forage. Additionally new crosses between spineless *O. ficus-indica* and the cold hardy, but spiny Texas native *O. lindheimeri* have produced spineless progeny that are currently being evaluated for forage characteristics in USDA cold hardiness zone 7 and 8 in Argentina. USDA computer models have been used to predict where spineless *Opuntia* types could be used in semi-arid areas world wide in USDA cold hardiness zones 7 and 8.

INTRODUCTION

As described in major reviews, various *Opuntia* species have been important for forage in many semi-arid regions (Felker, 1995, Lopez et al., 1996, Mondragon-Jacobo and Perez-Gonzalez, 2001). Perhaps the 4 most important attributes of *Opuntia* forage species are their (1) drought/heat tolerance, (2) tolerance to freezing weather, (3) growth rate and (4) presence/absence of spines. Fertilization (Gonzalez, 1989), supplementation and management practices that affect cladode age, have profound influences on forage quality (Felker, 1995) and are the simplest methods to impact forage quality. Use of spiny forage varieties avoids the need for fencing and reduce predation from wild animals, however this requires some type of processing (chopping or burning spines) before the cactus are fed. While some *Opuntias* have been useful for livestock forage as far north as Colorado (Shoop et al., 1977) and as feed for wild animals occur as far north as 56° N in Alberta, Canada (Stelfox and Friend, 1977) these species are spiny small plants with low productivity. Development of cold-hardy, spineless cactus has been an objective in the literature for almost 100 years (Griffith, 1915; Uphof, 1916).

Cold hardiness is a difficult issue to quantify since survival depends on acclimatization prior to freezes and various combinations of the duration and absolute minimum temperature of the freeze (Wang et al., 1997) that are required to reach the core critical temperature for tissue mortality (Nobel, 1990). Thus we believe that the USDA cold hardiness zone (http://www.ars-grin.gov/ars/Beltsville/na/hardzone/ushzmap.html) is more appropriate than listing the absolute minimum temperatures. As discussed later, all of the commercial *Opuntia ficus-indica* fruit plantations are located in USDA cold hardiness zones 9 and 10.

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Spineless *Opuntias*, that are primarily used for fruit, are widespread in the subtropics but few spineless *Opuntias* are available for use outside of the subtropics due to lack of cold hardiness. Felker et al., (2006) recently reported on 2 spineless *Opuntias* with more cold hardiness than the common *O. ficus-indica*. One of these spineless accessions, 1233 has produced more than 100 new cladodes in one year and has high productivity and is adaptable to USDA cold hardiness zone 8 to which none of spineless *Opuntia ficus-indica* are adapted. The most cold hardy of the spineless accessions *O. ellisiana*, is well adapted to USDA cold hardiness zone 7. While *O. ellisiana* produced 17 dry metric ton per ha in its 4th year of growth (Han and Felker, 1997), this species is much slower growing than either 1233 or any of the *Opuntia ficus-indica* types. Thus it would be advantageous to find spineless individuals equal in cold hardiness to *O. ellisiana* but with greater productivity.

A wide cross between the spineless, greenish *O. ficus-indica* 1281 and the Texas spiny native bluish *O. lindheimeri* 1250 (which is cold hardy to USDA zone 7) produced many apomict progeny identical to the female *O. ficus-indica* 1281. However there were some thornless progeny with bluish, much smaller cladodes than the female parent that did not have thorns. Since some of these traits are characteristics of the male *O. lindheimeri*, these progeny could not be apomicts. In a 4 year field trial in Argentina, many of these “bluish-spineless, progeny resembling the cold hardy male parent” had many fold greater productivity than *O. ellisiana* growing in an unreplicated trial 5 meters distant (Fig. 1). Thus 10 thornless progeny of the *O. ficus-indica* 1281 x *O. lindheimeri* 1250 cross with small bluish looking pads were selected for productivity, cold hardiness and forage quality trials in replicated trials in the Province of Mendoza, Argentina in a zone where no *O. ficus-indica* survived long term due to freeze damage (Guevara et al., 2000).

To assess the additional locations world wide, where spineless forage *Opuntias* adaptable to USDA cold hardiness zones 7 and 8 might be adapted, we used overlays of annual precipitation and USDA cold hardiness zones from a North Carolina State University APHIS weather based modeling program (NAPPFAST).

**MATERIALS AND METHODS**

The spineless *O. ficus indica* (1281) was used as the female parent, after emasculation and bagging as described by Wang et al., (1996). After removing the perianth covering the stigmas, an entire fruit of the Texas native *O. lindheimeri* (1250) was taken to the emasculated fruit of 1281 (female parent) and brushed on the stigma. The emasculated flower was then rebagged and this repeated for several days. The *O. lindheimeri* (1250) was originally collected on the W.A. Maltzsberger Ranch near Cotulla, Texas. The seedlings were germinated in the greenhouse at Texas A&M –Kingsville and 150 progeny taken to Argentina in June of 1998. These progeny were planted in the field in December of 1998 and evaluated over a 4 year period.

In the spring of 2005, three trials were established with the progeny of the wide *O. ficus-indica* x *O. lindheimeri* cross on the El Divisadero Cattle and Range Experiment Station 33º 45' S 67º 41' W in the Province of Mendoza, Argentina. Those trials were

1. A trial with large number of replications to accurately rank growth of *Opuntia ficus indica* 1281, *O. ellisiana* and 10 spineless progeny of a cross between *Opuntia ficus indica* (1281) x *O. lindheimeri* (1250). This trial contained 4 randomized blocks with 5 plants per repetition on a 3 x 5 m spacing. This trial had an initial fertilization of 100 g of 15-15-15 per plant.

2. A trial with only clone of one progeny N° 42 to accurately estimate biomass production per hectare with extensive border rows. This trial had 3 repetitions with each repetition having 6 rows of 8 plants per row on 1.5 x 1.5 m spacing. Biomass estimates are derived from the inner 8 plants that are each surrounded by 2 border rows. This treatment has no irrigation but annual fertilization of 100 kg/ha N, 50 kg P and 50 kg K.

3. A trial with 10 spineless progeny of the *O ficus-indica* x *O lindheimeri* cross at 3
fertility levels to investigate the influence of fertilization on productivity and N on the protein content of the forage. 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. 5 m x 1.5 m spacing was used. The fertilizer treatments were none, a low level to represent a modest investment (30 kg/ha of N, P and K every 2 years) and a high level 100 kg/ha N, 50 kg/ha P, and 100 kg/ha K per year to find the maximum biomass production and forage crude protein levels.

At the end of the experiment, the trials will be destructively harvested to measure fresh and dry weight production per ha. However during the time course of the experiment, a previously regression equation to estimate biomass production in *O. ellenstiana* was used to estimate fresh weight for all the accessions except for *O. ficus-indica*. For the accessions other than *O. ficus-indica*, total biomass (plant fresh weight - PFW) corresponding to two years of growth, estimated using the regression equation: 

\[
PFW = -1.466 + 0.407 (PCN=Plant cladode number) \quad (\text{Han and Felker, 1997})
\]

Since this equation was based on cladode number and since the cladode sizes of the progeny are different this is only an approximation of the biomass. Since none of the progeny have smaller cladodes than *O. ellenstiana*, this is a minimal estimate of the biomass. Due to the much larger size of *O. ficus-indica* cladodes, we harvested and weighed cladodes of this species to develop and use a unique regression for that species.

Frost damage was visually estimated, integrating the individual cladode damage over the entire plant. A 100% frost damage indicated that all the cladodes that had developed from the parent cladode were dead and 0% damage indicated that the plant had suffered no damage.

Minimum temperature records were obtained by a conventional hygrothermograph located on the Divisadero site.

Overlays of suitable precipitation and USDA cold hardiness zones were made using the NAPPEAST system, a weather based pest prediction system using 30 years of data from 1975-2005 and a resolution of 10 km. While *Opuntia* can survive and grow slowly at 100-200 mm annual rainfall, for substantial economic production, it is our experience that the mean annual precipitation should be at least 350 mm. For the maximum annual rainfall we used 650 mm rainfall. Obviously these precipitation levels are quite arbitrary, but we believe they represent a reasonable estimate.

In the overlays of precipitation and rainfall, USDA cold hardiness zones 9 and above are represented by orange shading, USDA cold hardiness zone 8 by yellow and USDA cold hardiness zone 7 by green.

**RESULTS**

The absolute minimum temperatures for the freezing events between May and August 2007 are presented in Figure 2. There were 48 events with below freezing weather with two minus 9°C absolute minimum temperatures. The maximum duration of time below freezing was less than 6 hours. In contrast the 1989 freeze that killed all *Opuntia ficus-indica* to ground level in Texas in 1989, had 62 consecutive hours below freezing with 16 hours below 6.7°C and a -12°C minimum (Wang et al., 1997). This is the freeze that separated USDA zone 9 from 8 and 7. Thus the survivals reported here are similar to that of USDA zone 8 but not a USDA zone 7.

The comparison of biomass production and frost damage in Table 1 shows that clones 42, 46, 80, 83 and 150 had much greater biomass productivity than the most cold hardy spineless clone to date, i.e. *O. ellenstiana*. All of the progeny had less frost damage than *O. ficus-indica*, one clone (46) had zero damage that was equal to *O. ellenstiana* and various clones were hardly damaged i.e. 83 and 94.

In the plot to measure the biomass per ha with adequate control of border effects, the mean fresh weight of the 8 interior plants was 9.1 kg ± 2.6 kg (95% CI), corresponding to a mean fresh weight per ha of 40,500 ± 11,500 kg/ha and dry weight per ha (assuming 8% moisture content) of 3,240 ± 916 kg/ha. This is less than the approximate 6,000 kg/ha obtained by Han and Felker, 1997 for 2 year old *O. ellenstiana* in
Texas but greater than 0.864 kg fresh weight per plant (assuming 8% dry matter content) for *O. ellisisana* obtained from tissue cultured plants after 2 seasons growth on the same El Divisadero site (Guevara et al., 2000).

A comparison of the growth (estimated from cladode number) and frost damage from the 10 progeny with 3 fertilization regimes is presented in Table 2. The response to fertilization at the highest application rate was dramatic, often with 2 fold and some 3 fold increases over the biomass of the zero fertilization control. The mean frost damage of all progeny was greatly increased by fertilization. However some of the clones 42, 46, 80, and 83 had negligible frost damage at the greatest fertilization application rate and also had the greatest biomass productivities. These four progeny were also among the highest productivity in Table 1. We suspect that the fertilization stimulated a transition from a hardened off state to an actively growing state that was more susceptible to freeze damage. The progeny 42, 46, 80 and 83 seem especially promising as high biomass producing freeze resistant species.

The trial is currently undergoing cladode tissue analyses to examine the effect of fertilization on cladode crude protein levels. We suspect that N fertilization will increase crude protein levels considerably above the values of 5.8 % protein as previously reported for *O. ellisisana* on this site (Guevara et al., 2000).

With the goal to examine potential new areas for forage production using spineless clones with improved cold hardiness, we constructed overlays of our view of optimal annual precipitation 350-650 mm/year with USDA zones 7, 8 and 9 and above. Figure 3 shows a world wide map of these distributions. Existing areas of production for cactus fruits in Mexico (Flores and Aranda, 1997), Brazil (Dos Santos and Albuquerque, 2001), Argentina, Spain, Sicily, Sardinia, Israel, Tunisia (Monjauze and LeHouerou, 1965) and South Africa (De Kock, 2001) are clearly observed for USDA zones 9 and above. There were a surprising number of potential new areas outside of traditional cactus areas for USDA zone 7 in northern Europe, around the Black Sea, Turkey, Iran, Afghanistan and China.

New areas for improved forage production in Africa can be observed for both USDA cold hardiness zones 7 and 8 (Fig. 4). There is a significant new area in South Africa for USDA cold hardiness zone 8 and in the Atlas Mountains in North Africa there are new zones in Algeria and Tunisia for Zone 8 and in Morocco for both zones 7 and 8.

In South America, Argentina has the greatest potential area for new cold hardy clones (Fig. 5). The greatest opportunity for USDA cold hardiness zone 8 from just south of Mendoza all the way to Tierra del Fuego. Opportunities for cold hardiness zone 7 and 350-650 mm in Argentina occur on the eastern slope of the Andes near Mendoza and also in the north in the Province of Jujuy. This USDA zone 7 with adequate precipitation extends from northern Argentina into Bolivia and Southern Peru.

In North America (Fig. 6), the greatest human impact for these new genetic materials would be north of the current cactus pear growing zones in the State of Zacatecas to the western side of the states of Durango and Chihuahua. These new materials would also be adapted to ranching areas of north Texas and many parts of New Mexico.

In areas where *Opuntias* does not currently exist, care must be taken in the introduction of these new materials as there is some potential for weediness. As described earlier (Felker et al., 2006) presence or absence of spines appears to be simply inherited and possibly recessive. When both parents in crosses were spineless, 8% of the progeny (n=155) were spiny (Felker et al., 2006). Cactus is commercially propagated asexually by cladodes, but sexual propagation often occurs from wild animals such as birds, coyotes, rats, etc that eat the fruits. In the wild, spineless progeny resulting from defecation of wild animals, are readily consumed leaving only spiny progeny. This phenomenon evidently has resulted in large stands of spiny *Opuntias* in South Africa from spineless introductions (H. Zimmerman, pers. comm.). Thus forage varieties with low fruit and seed productivity or with sterile fruits, would be desirable where forage varieties are desired for new areas. In an examination of inter species compatibility, Wang et al.,
observed that non-emasculated, bagged flowers control flowers of the spineless clone 1233, (adaptable to USDA zone 8 Felker et al., 2006) absised and did not mature. Further work is necessary to evaluate this clone as a possible sterile forage clone. Possibly several generations of intermating among the spineless progeny could produce varieties that only yield 100% spineless seedlings.

DISCUSSION
Spineless Opuntias have been extensively used for forage in Brazil, South Africa, Tunisia and Mexico (Mondragon-Jacobo and Perez-Gonzalez, 2001). All of the latter regions are in USDA cold hardiness zone 9 and 10 since none of O ficus-indica types are adaptable to regions with more freezing weather. A recent report of spineless Opuntia spp clone 1233 for USDA cold hardiness zone 8 and O. ellisiana clone 1364 for USDA zone 7 has appeared. Clone 1233 has high productivity even in the first year in being able to produce more than 100 cladodes. O. ellisiana has high productivity 3 years after establishment when it reaches a leaf area index of 2, but it would be very useful to have cold hardy clones that would reach a leaf area index of 2 faster than O. ellisiana.

The preliminary data here suggests that selected progeny of a spineless Opuntia ficus-indica x O. lindheimeri have similar cold hardiness to O. lindheimeri, lack of spines of the female parent and much greater productivity than the other spineless variety adapted to USDA cold hardiness zone 7 i.e. O. ellisiana.

Computer modeling for the optimum annual rainfall 350-650 mm combined with data for USDA cold hardiness zones has identified significant new areas in northern Mexico, foothills of the Andes in South America, South Africa, the Atlas Mountains of North Africa (Tunisia, Algeria and Morocco), Spain, Black Sea, Turkey, Iran, Afghanistan, and China to which these genetic materials should be adapted. Care should be taken in introducing hybrids with O lindheimeri as one parent due to recessive alleles for spines and weediness resulting from wild animal propagation of the fruits of these progeny.

Literature Cited

Tables

Table 1. Biomass and frost damage at El Divisadero Cattle and Range Experiment Station, Mendoza, Argentina in a trial with large number of replications to accurately rank growth of Opuntia ficus-indica 1281, O. ellisiana and spineless progeny of a cross between Opuntia ficus-indica(1281) x O. lindheimeri (1250).

<table>
<thead>
<tr>
<th>Clone</th>
<th>Predicted fresh wt /plant (kg)</th>
<th>Predicted frost damage</th>
<th>Mean frost damage (%</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>6.33</td>
<td>1.94</td>
<td>6.15</td>
<td>11.0</td>
</tr>
<tr>
<td>46</td>
<td>7.99</td>
<td>0.81</td>
<td>0.00</td>
<td>0</td>
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<tr>
<td>64</td>
<td>4.37</td>
<td>1.92</td>
<td>8.63</td>
<td>7.8</td>
</tr>
<tr>
<td>80</td>
<td>5.59</td>
<td>0.34</td>
<td>1.73</td>
<td>1.7</td>
</tr>
<tr>
<td>83</td>
<td>6.16</td>
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<td>0.93</td>
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<td>85</td>
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<td>0.84</td>
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<td>89</td>
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<td>94</td>
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<td>O. ficus indica 1281</td>
<td>5.66</td>
<td>0.71</td>
<td>15.73</td>
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</tbody>
</table>

The trial contained 4 randomized blocks with 5 plants per repetition on a 3 x 5 m spacing. In the first year 100 g of 15-15-15 was applied per plant. Total biomass (plant fresh weight - PFW) corresponding to two years of growth, estimated using the regression equation: PFW = -1.466 + 0.407 (PCN) (Han and Felker, 1997). To determine the PFW of O. ficus-indica, additional cladodes were harvested and weighed to develop a separate regression.
Table 2. Influence of N, P, and K fertilization at El Divisadero Cattle and Range Experiment Station, Mendoza, Argentina on biomass and frost damage of *Opuntia ficus-indica* 1281, *O. ellisiana* and spineless progeny of an *Opuntia ficus-indica* (1281) x *O. lindheimerii* (1250) cross. These levels were intended to represent zero, modest and high level investments by a small scale rancher.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Fresh biomass Kg/plt</th>
<th>95% CI</th>
<th>95% CI</th>
<th>Frost damage (%)</th>
<th>Fresh biomass Kg/plt</th>
<th>95% CI</th>
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<th>Frost damage (%)</th>
<th>Fresh biomass Kg/plt</th>
<th>95% CI</th>
<th>95% CI</th>
<th>Frost damage (%)</th>
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<tr>
<td>0 kg/ha N, P, K</td>
<td>30 kg/ha N, P, K every two years</td>
<td>100 kg/ha N, 50 kg/ha P, 100 kg/ha K per year.</td>
<td></td>
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Total biomass (plant fresh weight - PFW) corresponding to two years of growth, estimated using the regression equation: PFW = -1.466 + 0.407 (PCN=Plant cladode number) (Han and Felker, 1997). Randomized, split-plot design with 5 blocks per fertilizer treatment in which each clone was represented by a single plant per repetition. A 5 m x 1.5 m spacing was used.
Figures

Fig. 1. Three years old thornless segregant #80 of cross between *O. ficus indica* 1281 and *O. lindheimerii* 1250 with many characters of cold hardy Texas native male parent with potential for forage production.

Absolute Minimum Temperatures Experienced by Opuntia forage varieties at El Divisadero Cattle and Range Experiment Station, Mendoza, Argentina 2007

Fig. 2. Absolute Minimum Temperatures Experienced by *Opuntia* forage varieties at El Divisadero Cattle and Range Experiment Station, Mendoza, Argentina 2007.
Fig. 3. Global perspective of suitability of *Opuntia* forage clones to zones of 350-650 mm annual precipitation and USDA cold hardiness zones, 7, 8 and 9+.

Fig. 4. African perspective of suitability of *Opuntia* forage clones to zones of 350-650 mm annual precipitation and USDA cold hardiness zones, 7, 8 and 9+. 
Fig. 5. South America perspective of suitability of *Opuntia* forage clones to zones of 350-650 mm annual precipitation and USDA cold hardiness zones, 7, 8 and 9+.

Fig. 6. North American perspective of suitability of *Opuntia* forage clones to zones of 350-650 mm annual precipitation and USDA cold hardiness zones, 7, 8 and 9+. 