A Sampling Procedure to Determine the Nitrogen Content in *Opuntia ficus-indica* Cladodes♦

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ABSTRACT

The objective of this study was to determine which part of the nonfruiting cladodes of *Opuntia ficus-indica* f. *inermis* (Web.) Le Houér. would represent the average nitrogen content of the entire cladode for taking minimally destructive samples. Core samples (2.1-cm diameter) were taken from 91 sites of 1-year old cladodes. Three replicates of 6 cladodes for the whole-cladode mean and 3 replicates of 6 cladodes for the core samples were used. The cladodes or cores (from each location) were bulked for each replicate. Nitrogen was analyzed by the Kjeldahl method. Data were statistically analyzed using the T-test for independent samples. It was found that 40 site-sampling locations that are grouped in a rectangular fashion in the central-basal zone of the cladodes faithfully represent the average N-concentration of the entire cladode (mean ±95% CI = 8.12 ±0.60 mg g^{-1} DW).

Keywords: *Opuntia ficus-indica*; nitrogen content; cladode N-distribution; representative sample

INTRODUCTION

The studies on the nutritional status of plants in relation to the process of growth and to yield capacity are a specialized branch of agricultural science. Currently, the elements more commonly determined for agronomic purposes in soils and plants are nitrogen, phosphorus, and potassium. Of these, N is the element that, in most soils, is found more deficient than the others. Moreover, N in quantitative importance ranks sixth after C, O, H, Ca, and K in the tissues of *Opuntia*.

The N content in plants is extremely variable, from 1% to 10%, according to the organ analyzed and the changes during development. Notwithstanding, most plants contain 1% or 2% on a dry-weight basis (Salisbury and Ross, 1969). As N is the cornerstone of the proteins, and therefore a key element in crop yields, it would be of interest to have available a minimally destructive sampling procedure to analyze N content. Thus, many types of studies about the dynamics of N in *Opuntia* plants may be carried out with more confidence. For example, cacti (*Opuntia*) are usually low in N (Le Houérou, 1994; Guevara et al., 2004), and when used as fodder in arid zones, it needs to be complemented with protein-rich forages like *Atriplex* spp. (Guevara et al., 2003). Therefore, to increase N to an acceptable level, it would be necessary to perform studies of N-nutrition, as well as the set up of fertilization plan for *Opuntias* which requires, among others, knowledge about the evolution of the seasonal demand of the plant in relation to its annual and perennial cycle.

The purpose of this study was to determine an area within the cladode from *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér. in which N content was representative of that for the entire cladode.

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MATERIAL AND METHODS

One-year-old cladodes from six-year-old-plants of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér. ("Cuenca" accession with fruits of green color) were obtained from the Botanical Collection of *Opuntia* species established at the Experimental Field of the Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA) in Mendoza City (32º 52´ S, 68º 49´ W). We selected nonfruiting cladodes, which have more stable values of nutrient content (Gugliuzza et al., 2002). Samples consisted of entire cladodes, as well as core samples (2.1-cm diameter) taken from the cladodes using a sharpened bronze pipe at the intersections of a 3 cm by 3 cm grid laid out on the cladode surface. Cores were separated from each other by 1 cm. Thus, 91 subsample locations were obtained for examining the N distribution in the cladodes. We used three replicates of 6 cladodes for the whole cladode mean and 3 replicates of 6 cladodes for the core samples, i.e. we took cores of 18 cladodes that then were pooled for N analyses into 3 groups of 6 cores per core location. The entire cladodes were also pooled for each replicate. Because of cladode shape, 6 cores per replication were not obtained in all cases. Twenty-five samples that did not fulfill the minimum number of cores established for each replication (6) were excluded from the statistical analysis. The location of each subsample in the grid laid out on the cladode surface is shown in Figure 1. A frequency distribution graph of N-content of all the subsamples included in the statistical analysis (n = 198) versus number of core samples was made.

All samples were oven dried at 70ºC until constant weight, ground, and passed through a sieve of 0.5 mm mesh. Total N was analyzed by the Kjeldahl method (Müller, 1961) and data were expressed in mg N g^{-1} DW. Although many species of *Opuntias* are a source of protein for domestic and wild animals (Nobel, 1994), we prefer to express N-content instead of protein percentage because most fleshy storage organs, like cladodes, may contain more soluble-N (free-N) compounds than insoluble-N (bound-N) compounds, i.e. proteins. For this reason, the old practice of determining total-N and then calculating this as if it were all protein (% N x 6.25) is often subject to large errors (Steward, 1964).

Nitrogen data were statistically analyzed using the T-test for independent samples.

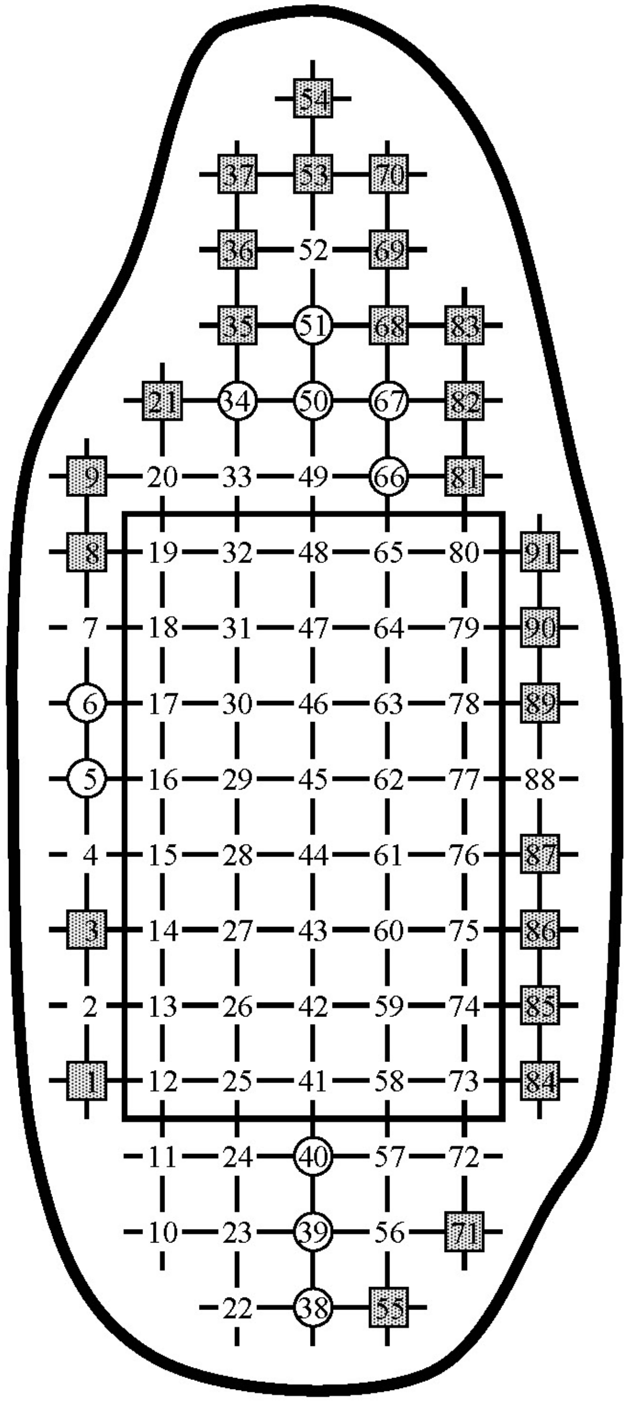

Figure 1. Diagrammatic representation of a typical cladode used in this study, showing the locations of subsamples taken for N-determination. Subsamples within shaded squares were eliminated from the statistical analysis. N-content of the subsamples within circles was significantly different from that for the entire cladode. The remaining subsamples had N-content values that do not differ significantly from that for the entire cladode. The rectangle faithfully represents the average N-content of the entire cladode.

RESULTS

Table 1 shows the mean values of N-content for each one of the 66 samples included in the study. Ten samples showed significant differences in N-content from that of the entire cladode samples, set at mean ±95% CI = 8.12 ±0.60 mg N g^{-1} DW. In contrast, N content of 56 subsamples were not significantly different from that of the entire cladode. Forty of these samples were grouped within a rectangle, but 16 appeared erratically scattered on the cladode surface (Figure 1). The frequency distribution graph (Figure 2) shows that 75% of the core subsamples enclosed within the rectangle (n = 120) fall into the 95% CI for the entire cladode.

According to Figure 1, the representative area of N-content in the whole cladode that may be taken with confidence would be that enclosed within the rectangle. Therefore, to take in a minimally destructive manner a representative sample of cladode tissues for N determination, it is necessary to leave out about 20% of the basal portion of the cladode, 35% of the apical portion, and about 15% of the margins on both sides of the cladodes.

Table 1. N-content (mean ±95% CI) found in the 66 core samples included in the study taken from cladodes of *Opuntia ficus-indica* (see Figure 1).
Entire cladode: 8.12 ±0.60 mg N g^{-1} DW (n = 3)

Core Sample	N-content mg g^{-1} DW Mean ±95% CI	Core Sample	N-content mg g^{-1} DW Mean ±95% CI	Core Sample	N-content mg g^{-1} DW Mean ±95% CI
2	8.21 ±0.40	28	8.21 ±1.06	56	7.61 ±2.32
4	8.49 ±1.06	29	7.47 ±0.40	57	7.65 ±0.80
5	9.11 ±0.63*	30	8.12 ±0.70	58	7.93 ±2.12
6	9.18 ±0.86*	31	7.56 ±2.51	59	7.93 ±2.01
7	8.69 ±2.43	32	8.49 ±0.40	60	7.47 ±1.44
10	7.65 ±2.90	33	8.87 ±1.61	61	7.56 ±1.20
11	7.75 ±1.61	34	8.59 ±0.40*	62	8.12 ±1.20
12	8.21 ±2.63	38	6.07 ±0.80*	63	7.65 ±1.06
13	8.68 ±0.70	39	6.81 ±1.61*	64	8.12 ±1.20
14	7.84 ±2.41	40	6.72 ±1.39*	65	8.59 ±2.24
15	8.31 ±0.40	41	7.47 ±1.75	66	9.05 ±0.40*
16	8.21 ±0.80	42	7.47 ±1.75	67	8.96 ±0.70*
17	8.59 ±1.06	43	7.93 ±1.06	72	7.65 ±1.45
18	8.31 ±1.06	44	8.20 ±1.41	73	7.93 ±1.45
19	8.87 ±2.12	45	8.03 ±2.90	74	7.93 ±2.01
20	8.49 ±2.81	46	7.84 ±0.70	75	7.56 ±3.19
22	6.95 ±1.92	47	8.21 ±1.75	76	8.49 ±0.40
23	7.84 ±1.20	48	8.21 ±0.40	77	8.03 ±1.61
24	7.47 ±1.06	49	8.31 ±0.40	78	8.74 ±2.02
25	8.21 ±0.40	50	8.96 ±0.70*	79	8.10 ±0.44
26	7.93 ±2.01	51	8.77 ±0.80*	80	8.17 ±0.31
27	7.93 ±1.45	52	8.49 ±0.80	88	9.83 ±2.62

* N content of these subsamples was significantly different from the value of 8.12 mg g^{-1} DW for the entire-cladode samples ($p < 0.05$).

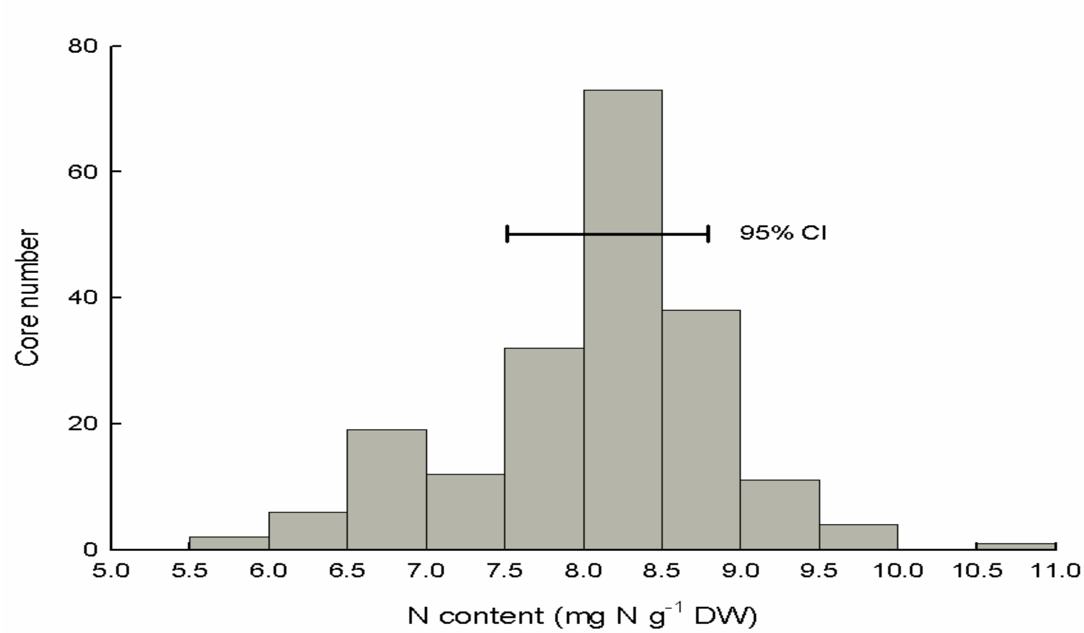

Figure 2. Frequency distribution of the N-content of core subsamples (n=198)
and the 95% confidence interval of the mean of the entire cladode

DISCUSSION

Nitrogen content in cladodes of *Opuntia* species change in relation to cladode age (Nerd and Nobel, 1995; Gugliuzza et al., 2002; Guevara et al., 2004), within chlorenchyma and parenchyma (Nerd and Nobel, 1995; Gugliuzza et al., 2002), number of fruits per cladode (Nerd et al, 1993; Gugliuzza et al., 2002), total biomass produced (Nobel, 1983; Nerd et al., 1993), and cladode sampling location (Gugliuzza et al., 2002). The latter authors found that in one-year-old fertile cladode, during bloom N-content decreased significantly from the basal to the apical area of the cladode; at fruit harvest, the apical part of the cladode still had the lowest N content, while no differences appeared in the other parts of the cladode. Gugliuzza et al. (2002) also measured the within-cladode variation in content of K, Ca, and Mg between flowering and commercial fruit harvest for cladodes with none, 5, 10, and 15 fruits and for defruited cladodes of 8-year-old plants of *O. ficus-indica*. They found that fruiting cladodes showed a higher rate of K depletion and a higher Mg content during the season. Calcium increased throughout the season with no relation with fruiting. Perhaps if we analyzed N content for cladodes with various amounts of fruits we would have found a similar pattern, but because we were only interested in forage measurements this was not done. Future work should examine the kind of study we performed here with cladodes with various amounts of fruits.

Guevara et al. (2004) found that there was a significant negative linear relationship between N content of entire cladodes of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér., "Cuenca" accession, and age class. Thus, N contents were 9.6, 5.9, and 4.4 mg N g^{-1} DW for cladodes of 1, 2, and 3 years old, respectively. The N content for the entire cladode found in this study for 1-year-old cladodes was 18% higher than that reported by Guevara et al. (2004).

In conclusion, this study provides researchers with 40 site sampling locations that are grouped in a rectangular fashion in the central-basal zone of the cladodes, and that faithfully represent the average N-concentration of the entire cladode (8.12 ± 0.60 mg g^{-1} DW) in *Opuntia ficus-indica* L. f. *inermis* (Web.)

Le Houér. Future studies should determine the optimum number of cladodes to sample and the optimum number of cores to take from this middle section in order to determine differences between genotypes or treatments.

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Figure 3. View of fruit of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.
on the plant in Mendoza, Argentina.

Figure 4. Variation in size and external fruit color of *Opuntia ficus-indica* L.
f. *inermis* (Web.) Le Houér.

Figure 5. Internal view of fruit of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.

Figure 6. Overview of cladode of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.

Figure 7. Close up of the aeroles of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.

Figure 8. View one of overall plant morphology of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.

Figure 9. View two of overall plant morphology of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér.