Response to Freezing and High Temperatures of Detached Cladodes from *Opuntia* Species♦

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ABSTRACT

Extreme temperatures are among stress factors than can affect *Opuntia* species. We experimented with detached cladodes, testing their potentiality to endure the actions of freezing and high temperatures. Cladodes of two provenances of *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér. and *O. spinulifera* Salm-Dyck f. *nacuniana* Le Houér., f. *nov.* were treated with temperatures of –5ºC and –10ºC combined with 5 and 10 hours exposure. On the other hand, *O. spinulifera* and *O. robusta* Wendl. were treated with a temperature of –10ºC for 18 hours. In a different trial, the last two species were subjected to high temperature (52ºC) from 3 to 26 days. Each experiment followed a completely randomized design with four replications comprising four cladodes each. At the end of each treatment, cladodes were planted in pots and grown under field conditions during 8 to 9 months. Response to extreme-temperature treatments was evaluated through the number and the dry weight of cladodes produced per plant. Under freezing treatments, although some differences in frost tolerance were found among species, they were tolerant to a temperature of –10ºC for 10 hours. *O. robusta* and *O. spinulifera* resisted –10ºC for 18 hours and appear to be the species most tolerant to freezing treatments. Cladodes of *O. spinulifera* and *O. robusta* from the heat-stress treatment were not affected when they were exposed to continuous heat (52 ±2ºC) for 5 days. Cladodes under more than 8-day heat treatment lost completely the capacity to sprout under field conditions.

Key words: *Opuntia* cladodes; cold and hot stresses; simulated frost and heat; yield after stress

INTRODUCTION

Many plants introduced into new ecosystems may have difficulty thriving successfully owing to their inability to cope with different environmental constrains which may impose limitation to the survival and production by serious injury due to lack of equilibrium in their physiology. This may be applied to some plants of tropical origin such as many *Opuntia* species. Under temperate arid environments that exist in Argentina, plants must endure the intensity and diversity of new biological constrains that are usually greater than those in tropical zones.

Studies on responses to injuries caused by different abiotic agents will be of interest when cultivating plants in areas not previously occupied by them (Berry and Raison, 1981). In this sense, to expand the eco-cultivation (cultivation in nonarable lands) of different species of *Opuntia* into new areas of the arid and semiarid zones of Argentina, it would be advisable, at the outset, to find out how to predict the ability of cladodes to tolerate the action of severe extreme temperatures.

Little precise knowledge about the response of cladodes of the *Opuntia* genus to frost and heat stresses is available. It has been documented that some succulent stems in a hot desert environment may reach

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temperatures 10ºC to 15ºC above the air temperature (Nobel, 1978). *Opuntias* may be subjected to a high thermal stress owing to their Crassulacean acid metabolism because stomata remain closed and transpiring cooling is reduced during the day. Thus, as a photosynthetic and transpiring organ, cladode surface temperature may rise markedly (Kappen, 1981). Moreover, when cladodes are planted under conditions of open or sparse vegetation typical of arid lands, solar radiation can strongly heat the sandy soil surface, causing extreme thermal stress before cladodes initiate the sprouting process. Because temperatures are expected to rise as part of global climate change, with specific effects predicted for cultivated cacti (Nobel, 1996), determination of high-temperature sensitivity is important ecologically and agronomically.

With regard to freezing stress, temperatures in arid environments may drop unexpectedly below 0ºC during winter, causing damage whose magnitude may range from yield reduction to total loss of plants, depending on plant age, physiological stage of the plants or their parts, and duration of temperature below 0ºC (Mendoza and Estrada, 1979; Le Houérou, 1994, 1996, 2002; Guevara et al., 2000).

We hypothesize that exposing detached cladodes of *Opuntia* species to temperatures well beneath or well above the normal physiological range of tissues for different lengths of time that we may predict, by their subsequent yield capacity, the cladode ability to withstand both types of stress temperatures.

The purpose of this work was to evaluate the ability of the cladodes of different species of *Opuntia* to tolerate low and high temperatures during the period of establishment and growth under field conditions.

MATERIALS AND METHODS

Two-year-old cladodes of *Opuntia. ficus-indica* L. f. *inermis* (Web.) Le Houér. provenance "Cuenca" and provenance "Divisadero"; *O. spinulifera* Salm-Dyck f. *nacuniana* Le Houér., f. *nov.;* and *O. robusta* Wendl. were selected to experiment, in separated trials, the effects of freezing and high temperatures for various lengths of time. Hereafter, for brevity, the two provenances of *O. ficus-indica* will be mentioned as if they were species, i.e., *Opuntia "cuenca*" and *O. "divisadero*". All cladodes used were selected on the basis of the uniformity of size, fresh weight, and other traits. Cladodes were cut on August 20, one month before spring begins. They were maintained about ten days under laboratory conditions (ca. 22/14ºC day/night temperature) previous to initiating both thermic treatments. The mean, maximum, and minimum temperatures for the month before they were used for the experiments were 3.4, 12.8, and –4.3ºC, respectively.

Both experiments (low and high temperatures) were independent assays. In the case of high temperature, we were interested to see to what extent, under the artificial drastic conditions imposed, cladodes were capable to cope with temperature well above the normal physiological range during prolonged time and support the concomitant dehydration.

Trial 1 (a and b). Freezing experiments

In Trial 1a, *Opuntia "cuenca", O. "divisadero",* and *O. spinulifera* were subjected to the following combinations of temperatures/times treatments: –5ºC during 5 hours; –5ºC for 10h; 0ºC for 5h; and –10ºC for 10h.

In Trial 1b, because *O. spinulifera* and *O. robusta* were more tolerant of freezes than *O. ficus-indica* (Guevara et al., 2000), we tested this capacity in a separate assay, exposing cladodes to 10ºC for a longer time (18 hours).

Cladodes under freezing treatments were arranged in a low-temperature incubator (Precision Scientific Model 818, GCA Corp. Chicago, IL). Samples were subjected directly to ca. 2ºC and left to attain the desired freezing temperature in a few hours, which occurred at a cooling rate of ca. –3.5ºC per hour. Controls were kept under laboratory conditions (day/night temperature ca. 22/14ºC).

Trial 2. Heat-stress experiments

Cladodes of *O. spinulifera* and *O. robusta* were exposed to continuous heat within a forced-air oven set at 52 ±2ºC for 3, 5, 8, 11, 16, and 26 days. At the end of each treatment, 10 cladodes of each species were randomly removed to determine their water content.

Frost and heat damage was visually estimated, integrating the portion of the cladode damage over the entire cladode. A 100% damage indicated that the entire cladode was dead and 0% damage indicated that the cladode had suffered no damage. Damage was estimated about 15 days after the end of the treatments, i.e., when the extent of necrosis was fully expressed under laboratory conditions (Guevara et al., 2000). The portions of cladode which showed necrotic areas were cut-out before planting. Subsequently, the cladodes were planted in plastic pots ca. 25-dm^3 capacity containing a sandy soil. One cladode was planted per pot, which was maintained under field conditions. Irrigation was done as required, allowing excess water to drain off.

Each experiment followed a completely randomized design with four replications comprising four cladodes each. The growth period of plants coming from freezing and heat treatments lasted 260 and 235 days, respectively. At harvest time, the potentiality of detached cladodes to endure the action of freezing and high temperatures was assessed by their yield capacity, i.e., number and dry weight of cladodes produced. These cladodes were separated from its mother one, counted, and their dry biomass was determined in an oven at 70ºC until no further change in weight was observed.

Data were submitted to ANOVA and means were separated according to Tukey's test ($p<0.05$). For values expressed in percentage, the analysis of variance was made with data modified according to the angular transformation (arc-sine $\sqrt{x}$).

RESULTS

Trial 1 (a and b). Freezing experiments

Table 1 shows that the percentages of cladodes damaged (those that lost the capacity to sprout) and died by the action of freezing treatments (Trial 1a) were very low in *O. spinulifera* and *O. "divisadero*" reaching, respectively, 9% and 12% at –10ºC/10 hours. In contrast, cladodes of *O. "cuenca*" reached values of 20% damaged at –10ºC/5h and 50% (damaged + dead) at –10ºC/10h. When –10ºC was extended during 18 hours (Trial 1b), only 6% of *O. spinulifera* cladodes died and *O. robusta* cladodes were not affected.

The ANOVA indicated that the species (*O."cuenca", O."divisadero*", and *O. spinulifera*) by treatment interaction was significant ($p<0.05$) for number and dry weight of cladodes. This significant interaction precluded mean separation tests for species when all treatments were combined and for treatments when all species were pooled.

Freezing treatments did not affect the number of cladodes produced compared to their respective controls in any species of Trial 1a (Table 2). Cladode number was significantly higher in *O. spinulifera* than those for *O. "cuenca"* and *O. "divisadero"* for all freezing treatments except for *O. "divisadero"* under –10ºC/5h and the control.

On the other hand, freezing treatments did not affect the dry weight of all cladodes produced compared to the respective control in *O. "cuenca"* and *O. spinulifera*. In *O. "divisadero",* the control produced significantly higher cladode weight than the treatment –10ºC/10h.

For O. spinulifera and O. robusta (Trial 1 b) , there were no significant differences for both the cladodes per plant and the cladode dry weight compared to the controls when the cladodes were exposed to –10ºC for 18 hours.

Trial 2. Heat-stress experiments

The percentage of cladodes damaged and dead by the action of continuous heat (Table 3) was zero for *O. spinulifera* and *O. robusta* until five days under stress. From day 8 of treatment onward, the percentage of cladodes damaged plus dead increased markedly in both species (from ca. 80% to 100%). The water content of *O. spinulifera* cladodes was not significantly different until 8 days under heat, but at days 11 and 16 they lost, respectively, 12% and 37% of water compared to the control. In contrast, *O. robusta* dehydration was not significantly increased until 16 days under stress.

The ANOVA indicated that species by heat treatment interaction was not significant. Table 4 shows that in both species none of the parameters of yield evaluated were significantly reduced until day 5 inclusive, but at day 8 an important decrease in the number and dry weight of individual and all cladodes occurred. Cladodes under more than 8 days treatment lost completely the capacity to sprout although many of them maintained a portion apparently undamaged (data not shown).

DISCUSSION

Freezing temperatures

Results of freezing treatments indicate that mature cladodes have the potential to withstand most of the range of temperature applied. This could be attributed to the age of the used cladodes. Our results are consistent with the findings of Nobel and De la Barrera (2003) who reported that cladodes showed 2.0ºC greater tolerance of low temperature with age up to 10 years, which helps prevent the death of plants, especially during episodic frost events. A similar relationship between frost damage and plant age was found by Wang et al. (1997) in Texas, where clone 1287 (*O. streptacantha* Lem.) had a mean frost damage of ca. 78% when the cladode had been planted for less than one year. However, when clone 1287 was 6 years old, the mature cladodes of the plant exhibited no frost damage and the young cladodes experienced only 10% damage. Consistent with these results, Guevara et al. (2000) showed that after freezes of –16ºC and –17ºC on two consecutive days, frost damage in young cladodes from 9-month-old plants of *O. ficus-indica* was 98.1%, whereas the 3-year-old plants of this species exhibited frost damage ranging from 14.9% (San Rafael accession) to 34.4% (San Juan accession). In contrast, there were slight frost-damage differences between one-year and two-year growth-period plants of O. ellisiana when temperatures dropped to about –15ºC (Guevara et al., 2003).

Russel and Felker (1987) and Le Houérou (1996) reported that clones of *Opuntia* scarcely tolerate absolute minimum temperature of –6ºC to –10ºC for a few hours or even few minutes. Our study showed that *Opuntia "cuenca" and O. spinulifera* tolerated –10ºC for 10 hours but the yield, expressed as dry weight of cladodes produced, was significantly reduced in *O. "divisadero*" when it was exposed to –10ºC for 10 hours compared to the control.

Opuntia robusta appears to be the most tolerant species to freezing treatments because it did not present damaged or dead cladodes at –10ºC/18 hours. This result is consistent with the findings of Le Houérou (1971) and Gregory et al. (1993). Snyman et al. (2007) also found that two-year-old plants of *O. robusta* cv. Monterrey suffered frost damage but to a lesser degree than cultivars of *O. ficus-indica*. However, in our study *O. robusta* did not differ from *O. spinulifera* when the number and dry weight of the cladodes produced were compared.

Heat-stress treatment

High temperature typically accompanies dehydration of tissues. In our case, exposure to continuous heat for 8 days, or even 16 days (according to species), did not promote cladode dehydratation. The unusually high water-containing capacity of *Opuntia* cladodes serves as a buffer to keep the water potential for a prolonged time (Osmond, 1978).

In addition, low stomata density, thicker cuticle and ability of cells to withstand dehydration are characteristic traits of *Opuntia* species (Nobel, 1994) that would be responsible for the low water loss. However, the maintenance of high water content under prolonged heat conditions may be detrimental for cladodes because overheating may induce oxidation reactions (Hegarty, 1978) that affect the ageing of tissues (Porto and Siegel, 1960) that lead to processes of senescence and tissue deterioration.

This study showed that well-hydrated cladodes of *O. spinulifera* and *O. robusta* under more than 8 days heat stress were seriously damaged physiologically, losing their capacity to sprout under field conditions. This indicates that prolonged high temperature, not dehydration, would be responsible for the physiological decay observed, supporting the growing evidence that direct injury by high temperature does occur to well hydrated field-grown plants (Sulllivan and Ross, 1979).

Because most cacti, including *Opuntia*, can tolerate in laboratory tests temperatures of 60 to 70ºC for only one or several hours when appropriately acclimated (Nobel, 1997), detached cladodes of *Opuntia* species tested in this study may be considered heat tolerant, and they might be planted safely in arid sites where sandy soil surfaces become extremely hot (55ºC to 70ºC) during the hours of intense solar radiation (Taiz and Zeiger, 1991). This temperature would be lethal for most temperate plants which typically die when exposed to temperatures of 44ºC to 50ºC, although some can tolerate temperatures above these (Salisbury and Ross, 1969). The maximum thermal limit for plants in the field is little documented, but it has been observed that there is a plantation of *Opuntia ficus-indica* thriving in areas where the recorded maximum temperature reaches or exceeds 50ºC, and even 58ºC (Le Houérou, 1996, time duration not reported).

Climate and weather affect all aspects of biological processes and the occurrence of episodes like frost or high temperature behave as limiting factors in plant production. In this study we have a good example to attempt to explain the failure of treated cladodes to sprout and differentiate roots when they presented 40% or more of their biomass seriously affected either by freeze temperature or by prolonged warm treatment. This incapacity may be attributable to several causes between which it may be considered as more probable the following:

a) loss of the ability of cladodes or their undamaged portion to regenerate the whole plant, i.e., loss of their totipotential property (Steward, 1968);

b) drastic decline, at temperatures above and below the normal physiological range (20ºC to 25ºC), of the stabilizing effect of water on protein structure with their concomitant denaturation (Berry and Raison, 1981) and alteration of cellular membranes (Taiz and Zeiger, 1991);

c) particularly, under prolonged heat temperatures, chloroplasts are damaged while the rate of dark respiration is enhanced (Lawlor, 1979; Woolhouse, 1983; Nobel, 1994) leading to a shortage of oxygen in the tissue with the accumulation of toxic products like ammonia (Sutcliffe, 1977) and to further disruption in the field of the metabolic machinery (Larcher and Bauer, 1981);

d) high temperatures can disrupt cellular metabolism by denaturing proteins and by compromising membrane integrity (Nobel and Zutta, in press).

All arguments here exposed show that there is a lot to be learned about the influence of these extreme temperatures on different plant processes (Nobel, 1983) and, therefore, the physiological line of investigation will be key for understanding the behavior of cacti like *Opuntia* under diverse stress conditions. Also, an essential part of the stress research would be extrapolation from laboratory, greenhouse, and other controlled-environment results to field testing.

Data obtained in this study provide a new contribution to the understanding of the physiology of detached mature cladodes of *Opuntia* species in relation to their capacity to endure different extreme temperatures for variable lengths of time.

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Table 1. Damage on detached cladodes of several species of *Opuntia* subjected to different freezing treatments (SEM is shown between parentheses)

Species	Treatment (ºC/hours)	Cladodes (%)		
		Undamaged*	Damaged (40% or more)	Dead
Trial 1 a. O. "cuenca"	–5/5	100	0	0
	–5/10	100	0	0
	–10/5	80 (2.0)	20 (2.0)	0
	–10/10	50 (1.9)	19 (0.6)	31 (1.5)
O. "divisadero"	–5/5	100	0	0
	–5/10	100	0	0
	–10/5	100	0	0
	–10/10	88 (3.3)	6 (2.2)	6 (1.3)
O. spinulifera	–5/5	100	0	0
	–5/10	100	0	0
	–10/5	100	0	0
	–10/10	91 (1.3)	9 (1.3)	0
Trial 1 b. O. spinulifera	–10/18	94 (2.1)	0	6 (2.1)
O. robusta	–10/18	100	0	0

[*] Cladodes with tissues spoiled less than 40% were considered undamaged because the portion not injured did not lose the capability to sprout and produce when planted under field conditions.

Table 2. Yield parameters as responses to different freezing treatments of mature cladodes from several *Opuntia* species

Species	**Freezing Treatment (ºC/hours)**	**Cladodes per Plant**	
		Number Mean ±95% CI	**Dry Weight (g) Mean ±95% CI**
Trial 1 a *O. "cuenca"*	Control	1.19 ± 0.24 ab	29 ± 9.31 abc
	–5/5	1.70 ± 0.35 abc	37 ± 8.59 abcd
	–5/10	1.93 ± 0.82 abcd	41 ± 6.98 abcd
	–10/5	1.25 ± 0.33 abc	25 ± 4.03 ab
	–10/10	0.69 ± 0.67 a	11 ± 12.21 a
O. "divisadero"	Control	2.63 ± 0.76 cde	78 ± 25.57 ef
	–5/5	1.38 ± 0.32 abc	46 ± 8.65 bcde
	–5/10	1.94 ± 0.76 abcd	55 ± 14.52 bcdef
	–10/5	2.56 ± 0.42 bcde	71 ± 21.51 def
	–10/10	1.69 ± 0.54 abc	39 ± 14.57 abcd
O. spinulifera	Control	3.66 ± 0.34 e	77 ± 6.71 ef
	–5/5	3.75 ± 0.53 e	82 ± 19.87 f
	–5/10	3.56 ± 0.31 e	75 ± 3.78 ef
	–10/5	3.89 ± 0.47 e	83 ± 2.58 f
	–10/10	3.13 ± 0.74 e	63 ± 11.99 cdef
Trial 1 b. *O. spinulifera*	Control	3.66 ± 0.28 ab	76 ± 5.68 a
	–10/18	2.81 ± 0.72 a	57 ± 11.64 a
O. robusta	Control	4.69 ± 0.84 b	67 ± 11.24 a
	–10/18	4.06 ± 0.63 ab	58 ± 7.25 a

Different letters indicate significant differences at $p<0.05$ level.

Table 3. Damage and dehydration provoked to detached cladodes of two *Opuntia* species by the action of heat-stress treatment given for different numbers of days

Species	Treatment (days at 52ºC)	Cladodes (%)			Cladode Water Content (%) Mean ±95% CI
		Undamaged* Mean ±95% CI	Damaged (40% or more) Mean ±95% CI	Dead Mean ±95% CI	
O. spinulifera	0	100	0	0	91.6 (0.2) a
	3	100	0	0	92.7 (0.7) a
	5	100	0	0	93.2 (0.3) a
	8	12 (5.8)	40 (2.9)	48 (2.9)	90.5 (1.1) a
	11	6 (3.4)	45 (2.6)	49 (1.3)	80.2 (9.6) b
	16	0	15 (2.3)	85 (2.3)	58.0 (3.3) c
	26	0	0	100	
O. robusta	0	100	0	0	91.8 (1.6) a
	3	100	0	0	91.7 (1.8) a
	5	100	0	0	91.1 (1.4) a
	8	19 (5.7)	35 (2.9)	46 (2.0)	90.8 (1.7) a
	11	13 (4.3)	40 (2.9)	47 (2.6)	90.1 (1.8) a
	16	2 (2.9)	20 (3.4)	78 (2.9)	87.8 (3.5) a
	26	0	5 (1.3)	95 (1.3)	

* Cladodes with tissues spoiled less than 40% were considered undamaged because the portion not injured did not lose the capacity to sprout and produce when planted under field conditions.

Values followed by the same letter for each species are not significantly different at p<0.05 level.

Table 4. Parameters of yield (average per plant) produced by *Opuntia spinulifera* and *O. robusta* previously subjected to heat-stress treatment for different numbers of days

Treatment (days at 52ºC)	**Cladode Number Mean ±95% CI**	**Individual Cladode Dry Weight (g) Mean ±95% CI**	**All Cladodes Dry Weight (g) Mean ±95% CI**
0 (control)	2.31± 0.31 a	21.75 ± 4.57 a	45.25 ± 9.18 a
3	2.05 ± 0.41 a	24.25 ± 5.99 a	43.63 ± 8.35 a
5	2.44 ± 0.27 a	24.13 ± 5.43 a	46.13 ± 7.76 a
8	0.16 ± 0.16 b	4.38 ± 5.45 b	4.38 ± 5.45 b

Values followed by the same letter in each column are not significantly different at $p<0.05$ level.