Tolerances and acclimation to low and high temperatures for cladodes, fruits and roots of a widely cultivated cactus, *Opuntia ficus-indica*

Park S. Nobel and Erick De la Barrera

Department of Organismic Biology, Ecology, and Evolution, University of California, Los Angeles, California 90095–1606, USA

Summary

Author for correspondence:
Park S. Nobel
Tel: +1 310 2063903
Fax: +1 310 8259433
Email: psnobel@biology.ucla.edu

• *Opuntia ficus-indica*, a cactus widely cultivated for fruits and forage/fodder, has shoots composed of flattened stem segments (cladodes) that are relatively sensitive to freezing temperatures below –6°C but extremely tolerant of high temperatures up to 65°C. Based on the uptake of the vital strain neutral red, fruits and roots were damaged by 60 min below –7°C or above 55°C.
• Young (6 wk old) and mature (1-yr-old) cladodes had 1.2°C greater low-temperature tolerance at day/night air temperatures of 20 : 10°C compared with 30 : 20°C and 2.8°C lower high-temperature tolerance. Fruits and roots (both 6 wk old) showed no such low-temperature acclimation; roots had high-temperature acclimation similar to that of cladodes, but fruits showed no high-temperature acclimation.
• High-temperature tolerance did not change with age for cladodes and fruits up to 10 wk old nor did low-temperature tolerance for cladodes, but fruit low-temperature tolerance decreased by 2.6°C from 4.5 to 10 wk of age, a time when sugars, which can act as cryoprotectants, were increasing.
• Cladodes showed 2.0°C greater tolerance of low temperatures with age up to 10 yr and 6.5°C greater high-temperature tolerance, which helps prevent the death of plants, especially during episodic freezing events.

Key words: climate, *Opuntia ficus-indica*, platyopuntia, prickly pear, sugars, temperature acclimation, temperature tolerances.

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Introduction

Extreme temperatures often determine where plant species occur naturally and where they can be cultivated successfully (Smith *et al.*, 1997; Fitter & Hay, 2002). Sensitivity to extreme temperatures can differ among organs on the same plant, for example leaves are generally more sensitive than stems to freezing temperatures (Beck, 1994; Fitter & Hay, 2002) and roots are often more sensitive than stems to high temperatures (McMichael & Burke, 2001; Nobel & Bobich, 2002). Freezing temperatures can lead to the formation of extracellular ice crystals. Water diffusing out of the cells becomes part of these growing crystals, leading to cellular dehydration, which can disrupt metabolism (Nobel, 1988; Rajashekar, 2000). Also, intracellular ice crystals can puncture the cell membrane, leading to cell death. High temperatures denature proteins and disrupt membrane integrity (Nobel, 1988).

Tolerances of low temperatures can be enhanced if the ambient day/night temperatures gradually decrease over a period of days and likewise the high temperatures tolerated can be increased if the ambient temperatures gradually increase, such 'acclimation' or 'hardening' allowing plants to adjust to seasonal changes in air temperature (Levitt, 1980). Acclimation to low temperatures can involve specific proteins (Hughes & Dunn, 1996; Thomashow, 1998) and cryoprotectants such as polyamines and sugars (Nobel *et al.*, 1995; Rajashekar, 2000) and for cacti can be mediated by abscisic acid (Loik & Nobel, 1993) or higher ice nucleation temperatures (Goldstein & Nobel, 1994). Acclimation to high temperatures can also involve specific proteins in cacti and

other plants (Kee & Nobel, 1986; Ortiz & Cardemil, 2001) or changes in membrane properties (Pike & Berry, 1980; Srinivasan *et al.*, 1996).

The species chosen for the present study, *Opuntia ficus-indica*, is a platyopuntia native to semiarid temperate regions of Mexico with relatively cool nights and moderate daytime temperatures (Pimienta-Barrios, 1990, 1994). It is important commercially and currently is cultivated in about 25 countries for fruits, young cladodes (stem segments) used as a vegetable, and older cladodes used as forage or fodder for cattle and sheep (Nobel, 2002). The country with the greatest production of its fruits, known as 'prickly pears' and increasingly as 'cactus pears', is Mexico with about 70 000 ha under fruit cultivation; other countries that extensively cultivate the fruits include Italy (specifically, Sicily), Chile, South Africa, Argentina, and Israel (Inglese *et al.*, 2002). Mexico is essentially the only country with commercial vegetable production of *O. ficus-indica*, with such 'nopalitos' grown on about 2500 ha (Sáenz-Hernández *et al.*, 2002). In terms of land area, the greatest cultivation of *O. ficus-indica* is for forage or fodder, especially in Tunisia (500 000 ha), Brazil, and Mexico (Nefzaoui & Ben Salem, 2002). *O. ficus-indica* is also the host plant for the cochineal insect from which is prepared the red dye carminic acid, used in both the food and the textile industries (Sáenz-Hernández *et al.*, 2002).

Temperature influences on many processes have been determined for *O. ficus-indica*, including CO_2 uptake and transpiration of this Crassulacean acid metabolism species (Nobel, 1988; Israel & Nobel, 1995) and root growth, which is optimal at 27–30°C (Drennan & Nobel, 1998). Depending somewhat on time of year and particular accessions, mature cladodes can be damaged by air temperatures from −6 to −10°C (Russell & Felker, 1987; Goldstein & Nobel, 1994; Nobel *et al.*, 1995; Valdez-Cepeda *et al.*, 2001), which severely limit the areas for the cultivation of *O. ficus-indica* in the United States and other temperate regions. On the other hand, mature cladodes of *O. ficus-indica* on properly acclimated plants can survive 60 min at temperatures exceeding 65°C (Nobel *et al.*, 1986; Nobel, 1988), which is among the greatest high-temperature tolerances observed for vascular plants (Levitt, 1980; Smith *et al.*, 1984; Nobel, 1988), indicating that high temperatures do not severely limit *O. ficus-indica* in the field. However, little is known about the responses of young cladodes, fruits, or roots of *O. ficus-indica* to extreme temperatures (Gutterman, 1995).

Responses of cacti to freezing episodes in the field can take many years to be manifest. For instance, death of the columnar cactus *Carnegiea gigantea* in response to a freezing episode in 1937 occurred 3 yr later for some plants (Steenbergh & Lowe, 1976) and 9 yr later for plants exposed to a freezing episode in 1971 (Steenbergh & Lowe, 1983). Instead of waiting for tissue necrosis and eventual plant death to assess the viability of cactus cells, a technique based on the uptake of neutral red into the vacuoles of living cells was developed (Onwueme, 1979, De Swain, 1994), which proved more reliable for evaluating damage by extreme temperatures than determining cell plasmolysis, leakage of solutes, or reduction of tetrazolium blue by respiratory activity (Didden-Zopfy & Nobel, 1982). Uptake of neutral red into the central vacuole steadily decreases as cells are exposed to increasingly low or high temperatures, and the temperature that is lethal for 50% of the cells relative to the control, LT_{50}, can be determined graphically (Nobel, 1988; Nobel *et al.*, 1995). Extensive cell death and tissue necrosis for cacti occurs about 3°C below LT_{50} for low-temperature treatments and 4°C above LT_{50} for high-temperature treatments (Didden-Zopfy & Nobel, 1982; Nobel *et al.*, 1986, 1995; Nobel, 1988), so predictions of eventual plant death can be based on measurements of LT_{50}.

Three hypotheses were considered for the low- and the high-temperature tolerances of *O. ficus-indica*: first fruits and young cladodes of the same age should have similar tolerances to extreme temperatures and similar acclimation ability, because both are succulent, initially have similar tissue organization, and develop during the late spring when moderate air temperatures prevail; second the tolerance to extreme temperatures should increase with age of the cladodes, because older cladodes would have experienced a greater range of temperatures in the field than would young cladodes; and third, except near the soil surface, roots face a more constant and moderate temperature environment than do the shoots, and consequently roots should show less tolerance of low and high temperatures, less acclimation ability, and less change in temperature tolerances with age than do shoots.

Materials and Methods

Plant material

Twelve-yr-old plants of *O. ficus-indica* (L.) Miller (Cactaceae; accession number 1279 of Texas A & M University, Kingsville, Texas, USA) c. 2 m tall were studied at the Agricultural Experiment Station, University of California, Riverside, CA, USA (33°58′ W, 117°18′ N, 1020 m elevation), on 25–26 May 2002. The plants were irrigated every 2 wk, leading to moist soil conditions. The age of mature cladodes (≥ 1 years old) was based on the year the basal cladodes were placed in the ground (1990) and the fact that a new cohort of daughter cladodes regularly develops each spring on the existing terminal cladodes; such cladodes attain their full length of about 35 cm in c. 8 months (North *et al.*, 1995). The age of young cladodes and flower buds/fruits up to 10-wk-old was based on field observations in April and May 2002 as well as prior experience with *O. ficus-indica* at the field site (Inglese *et al.*, 1994; North *et al.*, 1995; Nerd & Nobel, 2000; Nobel & De la Barrera, 2000). Flowers abscise c. 5 wk after bud appearance, at which stage fruits of *O. ficus-indica* averaged 52 mm in length, slightly more than half of their final length.

For acclimation studies, 1-yr-old cladodes were detached on 10 April 2002 and planted in tubs of soil from the field site. In addition, 1-yr-old cladodes with young cladodes or fruits 4 wk of age were detached on 9 May 2002 (but not planted). The planted and unplanted detached cladodes were maintained in Conviron E-15 environmental chambers (Controlled Environments, Pembina, ND, USA) with day/night air temperatures of 20 : 10°C or 30 : 20°C. The photosynthetic photon flux (PPF, wavelengths of 400–700 nm; measured with a LI-191SB line quantum sensor, LI-COR, Lincoln, NE, USA) at cladode height was 600 µmol m^{-2} s^{-1} for 13-h daytimes. The planted cladodes were watered weekly with 0.2 strength Hoagland's solution (Epstein, 1972) until the temperature tolerances of the planted cladodes and the newly developed roots were determined after 7 wk in the chambers. Both young cladodes and fruits can continue to develop on detached unplanted cladodes of *O. ficus-indica*, with water relations that are similar to those for cladodes under drought conditions (Nobel, 1996a; Nobel & Castañeda, 1998; Nerd & Nobel, 2000); because of the water stored in the detached cladodes, the young cladodes and fruits were under 'wet' conditions for the 2-wk period in the chambers before their tolerances to extreme temperatures were determined.

To characterize the environment of *O. ficus-indica* in the field, temperatures of cladodes and fruits, their transpiration, and microclimatic parameters were measured every 2 h on 25–26 May 2002. Temperatures were determined using copper-constantan thermocouples 0.51 mm in diameter placed within 1.0 mm of the cladode or fruit surface, at 1.2 m above the soil surface, and at soil depths of 0, 10, and 25 cm; the output was monitored with an HH-21 Digital Thermometer (Omega Engineering, Stamford, CT, USA). Transpiration was determined using a LI-COR LI-1600 steady-state porometer; the acrylic top of the cuvette was replaced with a plate with a cylindrical extension having a diameter of 12 mm lined with a foam-rubber gasket to provide an air-tight seal with the organ surface. Temperatures and transpiration for cladodes and fruits at 1.2 m above the soil surface and temperatures for older cladodes closer to the ground were determined for east-facing surfaces. The stem area index (total area of both sides of all cladodes on a plant per unit ground area) was determined for six plants at 1.5 m intervals along rows spaced 2.5 m apart. Roots were excavated in 2.5-cm-thick layers for the first two layers and then 5-cm-thick soil layers in square 40 × 40 cm pits centered 40 cm east of the plant bases; root dry mass was determined after drying for 72 h at 80°C in an STM 135 mechanical convection (forced draft) oven (Precision Scientific, Chicago, IL, USA).

Temperature tolerances

Low-temperature and high-temperature tolerances were determined for cladodes, fruits, and roots of *O. ficus-indica* using the vacuolar uptake of the vital stain neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) following exposure for 60 min to a particular extreme temperature (Onwueme, 1979; Didden-Zopfy & Nobel, 1982, Swain & De, 1994; Nobel *et al.*, 1995). Low temperatures at 1–2°C intervals decreasing from 0°C were obtained in an ULT-80 ultra-low-temperature freezer (Rheem Manufacturing, West Columbia, SC, USA). High temperatures at 1–2°C intervals increasing from 50°C were obtained in the mechanical convection oven. Approximately 1.5 g of cladodes and fruits or 0.5 g of roots were removed with a scalpel from near the organ surface and then sliced into sections *c.* 200 µm thick using razor blades. The sections (10–12 per plant) were placed in contact with copper-constantan thermocouples (0.51 mm in diameter) and wrapped in aluminum foil adjacent to moistened tissue paper to prevent desiccation for the high-temperature treatments; for the low-temperature treatments, the sections were placed in 2-ml plastic microcentrifuge tubes whose tops had been perforated to allow entry of copper-constantan thermocouples and whose bottoms contained moistened tissue paper.

After exposure to a particular temperature for 60 min, the cladode and fruit samples were placed in 0.2% (w/w) neutral red in 0.25 M potassium phosphate buffer (pH 7.8) for 10 min at 25°C for stain uptake, which occurs for the vacuoles of living cells only and indicates cellular membrane integrity (Onwueme, 1979; Nobel *et al.*, 1995). The same protocol was followed for root samples, except that 70 min was required for adequate staining. The tissue samples were then washed for 10 min in 0.25 M potassium phosphate buffer (pH 7.8) at 25°C (without neutral red), after which 300–350 intact chlorenchyma cells for each cladode and fruit sample or 250–300 parenchyma cells from the regions of the vascular cylinder between the xylem wedges for each root sample were examined at 100 × using a BH-2 phase-contrast light microscope (Olympus, Lake Success, NY, USA) to check for stained cells (showing neutral red uptake into the central vacuole, i.e. living) vs unstained cells. The low temperatures and the high temperatures that halved stain uptake from the maximum occurring for the control at 25°C (LT_{50}), a reliable test for predicting eventual cell death and tissue necrosis (Didden-Zopfy & Nobel, 1982; Smith *et al.*, 1984; Nobel *et al.*, 1995), were determined graphically to 0.1°C for each plant sampled under each condition. Acclimation was quantified by comparing LT_{50} for organs from plants at day/night air temperatures of 20 : 10°C vs 30 : 20°C using a Student's *t*-test. The relationships between organ age and LT_{50} were determined using linear regression analyses performed with SigmaStat (SPSS, San Rafael, CA, USA). Data are presented as mean ± 1 SE (*n* = number of plants).

Results

Field temperatures

The air and soil temperatures measured in the field on 25–26 May 2002 ranged from 13°C to 38°C (Fig. 1a). On those
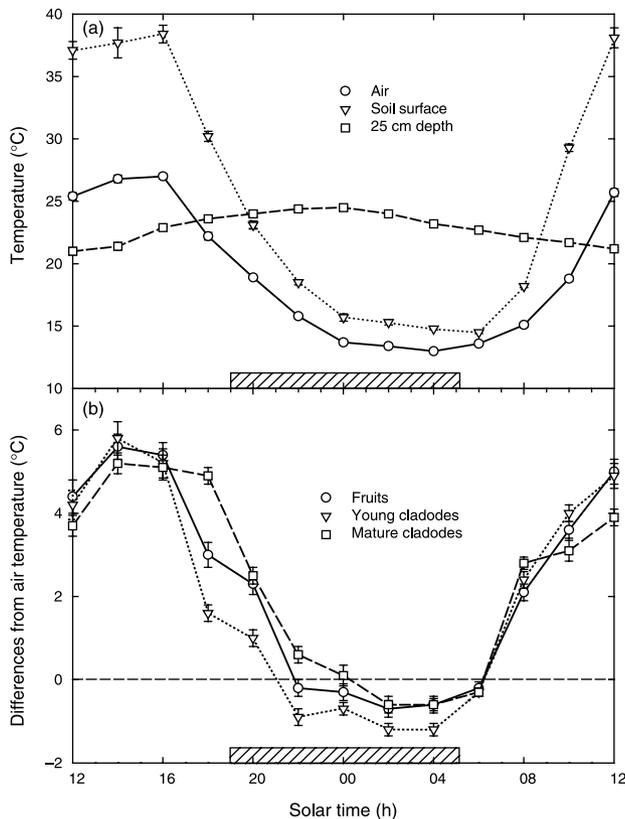
Fig. 1 Temperatures of the air at 1.2 m, the soil surface, and a soil depth of 30 cm (a); and temperatures on the east-facing sides of 6-wk-old cladodes, 6-wk-old fruits, and the 1-yr-old cladodes of *Opuntia ficus-indica* on which they occurred relative to air temperature at 1.2 m (b). Soil temperatures were measured 50 cm east of the plant bases. Data were obtained on 25–26 May 2002 at the Agricultural Experiment Station, University of California, Riverside. Hatched bars indicate nighttime. Data are means ± 1 SE (*n* = 5 plants), except when the error bars are smaller than the symbol.

Fig. 2 Influence of low treatment temperatures on the uptake of the vital stain neutral red into chlorenchyma cells of 6-wk-old cladodes of *Opuntia ficus-indica* (188 mm in length) growing on detached 1-yr-old cladodes (unplanted) collected from the Agricultural Experiment Station on 9 May 2002 and maintained for 2 wk at day/night air temperatures of 20 : 10°C or 30 : 20°C. Samples were held at the indicated cladode temperature for 60 min before the temperature was lowered by 1–2°C. Data are expressed relative to the control at 25°C (for which 87% of the chlorenchyma cells took up stain) and are means ± 1 SE (*n* = 7 plants).

days, the maximum : minimum air temperatures at the University of California, Riverside (CIMIS Weather Station no. 44; Department of Water Resources, 2002) were 25.4 : 12.7°C; for the previous 10 d, the maximum : minimum daily air temperatures at the weather station averaged 23.0 : 13.1°C. The greatest daily temperature variation on 25–26 May occurred at the soil surface (23.2°C), an intermediate value occurred at a soil depth of 10 cm (10.8°C), and the least variation at 25 cm (3.5°C; Fig. 1a). No roots of *O. ficus-indica* were observed in the upper 3 cm of the soil, except in shaded regions near the plant base; their mean depth based on dry mass was 9.8 ± 0.6 cm (*n* = 6 plants) and only 0.3% of the roots occurred below 30 cm. The stem area index was 3.6 ± 0.4 (*n* = 6 plants). At solar times of 12 : 00, 14 : 00, 16 : 00, and 18 : 00 h, PPF increased approximately linearly with height in the canopy, averaging 9.7 times higher at the top of the canopy (2.0 m) than at 0.2 m above the ground.
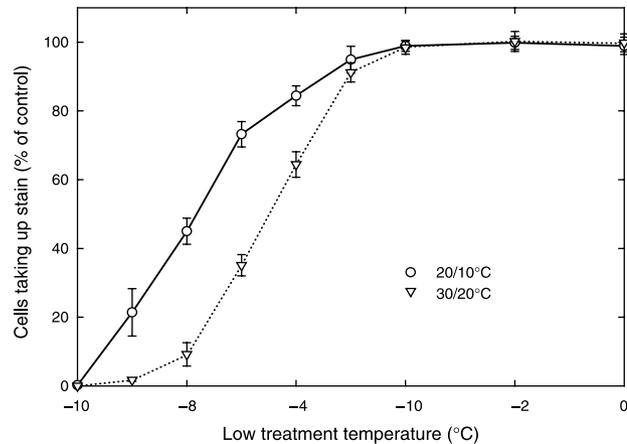
Temperatures of young cladodes (6 wk old), fruits (also 6 wk old), and the 1-yr-old cladodes on which they occurred were similar (Fig. 1b). Also, temperatures for the east-facing sides of 5- and 10-yr-old cladodes were similar to those of 1-yr-old cladodes, generally within 0.7°C (*n* = 5 plants). In the afternoon (solar times of 12 : 00–16 : 00 h), the cladodes and fruits averaged 4.9°C above air temperature; during the late night (solar times of 00 : 00–04 : 00 h), they averaged 0.6°C below air temperature (Fig. 1b). Transpiration rates at night (solar times of 20 : 00–04 : 00 h) averaged 539 ± 20, 752 ± 27, and 234 ± 13 μmol m^{-2} s^{-1} (*n* = 5 plants) for 1-yr-old cladodes, 6-wk-old daughter cladodes, and 6-wk-old fruits, respectively; near midday (solar times of 10 : 00–14 : 00 h), transpiration rates averaged 192 ± 36, 209 ± 10, and 178 ± 16 μmol m^{-2} s^{-1} (*n* = 5 plants) for 1-yr-old cladodes, daughter cladodes, and fruits, respectively.

Temperature tolerances of cladodes

As the temperatures to which the samples of young cladodes of *O. ficus-indica* were exposed for 60 min were lowered, the fraction of chlorenchyma cells taking up the vital stain neutral red into the central vacuoles steadily decreased (Fig. 2). For 6-wk-old cladodes on detached 1-yr-old cladodes (unplanted) maintained for 2 wk at day/night air temperatures of 20 : 10°C, the temperatures at which the fraction of chlorenchyma cells taking up stain was halved (LT$_{50}$) averaged −7.8°C, significantly lower than the value for samples from plants at 30 : 20°C of −6.5°C (Fig. 2, Table 1). Similar responses to

Table 1 Sensitivity to extreme temperatures for organs of *Opuntia ficus-indica* on plants maintained at day/night air temperatures of 20 : 10°C or 30 : 20°C

	LT$_{50}$ (°C)					
	Low temperature responses			High temperature responses		
Organ	20/10°C	30/20°C	P	20/10°C	30/20°C	P
Mature cladodes	-7.6 ± 0.2	-6.7 ± 0.2	< 0.05	56.2 ± 0.3	59.0 ± 0.3	< 0.01
Young cladodes	-7.8 ± 0.2	-6.5 ± 0.1	< 0.05	54.6 ± 0.3	57.4 ± 0.2	< 0.01
Fruits	-6.3 ± 0.3	-6.3 ± 0.2	> 0.9	55.2 ± 0.3	55.4 ± 0.2	> 0.5
Young roots	-6.5 ± 0.2	-6.4 ± 0.2	> 0.5	52.7 ± 0.4	55.6 ± 0.3	< 0.05

LT$_{50}$ for uptake of neutral red for samples treated at low or high temperatures for 60 min for young cladodes (Figs 2, 3), fruits (Fig. 4), 6-wk-old roots (1.7 mm in diameter), and 1-yr-old mature cladodes was obtained graphically to within 0.1°C. Data are means ± 1 SE (*n* = 5–7 plants), and pairwise comparison at 20 : 10°C and 30 : 20°C used a Student's *t*-test.
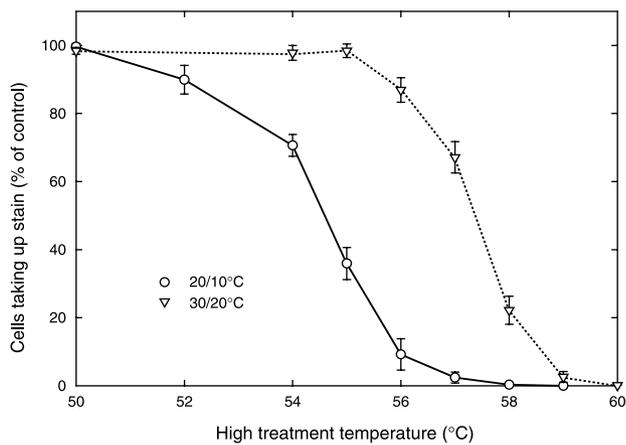

Fig. 3 Influence of high treatment temperatures on the uptake of neutral red into chlorenchyma cells of 6-wk-old cladodes of *Opuntia ficus-indica* growing on detached 1-yr-old cladodes maintained for 2 wk at day/night air temperatures of 20 : 10°C or 30 : 20°C (see Fig. 2). Samples were held at the indicated cladode temperature for 60 min before the temperature was raised by 1–2°C. Data are expressed relative to the control at 25°C and are means ± 1 SE (*n* = 7 plants).
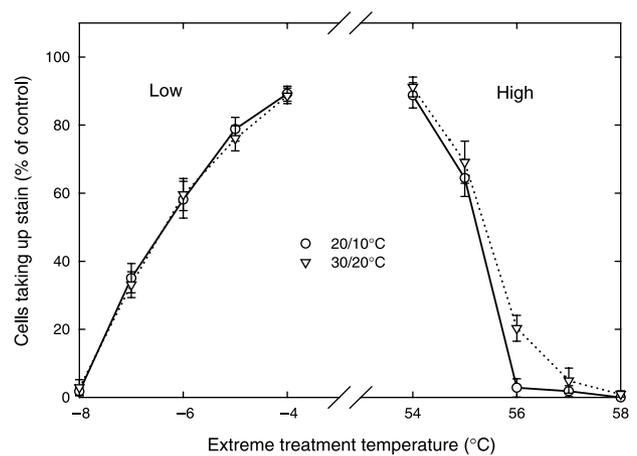

Fig. 4 Influence of low and high treatment temperatures on the uptake of neutral red into chlorenchyma cells of 6-week-old fruits of *Opuntia ficus-indica* (65 mm in length) on detached 1-year-old cladodes (unplanted) collected from the Agricultural Experiment Station on 9 May 2002 and maintained for 2 wk at day/night air temperatures of 20 : 10°C or 30 : 20°C. Samples were held at the indicated fruit temperature for 60 min before the temperature was lowered by 1°C for low treatment temperatures or raised by 1°C for high treatment temperatures. Data are expressed relative to the control at 25°C (for which 88% of the chlorenchyma cells took up stain) and are means ± 1 SE (*n* = 5 plants).

low temperatures occurred for the 1-yr-old cladodes planted 7 wk before measurements (Table 1).

The fraction of chlorenchyma cells taking up neutral red also steadily decreased as the high temperatures to which the samples of young cladodes were exposed for 60 min increased (Fig. 3). The temperature responses for samples from young cladodes at day/night air temperatures of 20 : 10°C vs 30 : 20°C differed more than at low temperatures, LT$_{50}$ being 56.2°C for samples from plants at 20 : 10°C and 2.8°C higher for samples from plants at 30 : 20°C (Table 1). Similarly, LT$_{50}$ was 2.8°C higher for 1-year-old planted cladodes at 30 : 20°C vs 20 : 10°C; as measured by LT$_{50}$, the 1-yr-old cladodes tolerated 1.6°C higher temperatures than did the 6-wk-old cladodes (Table 1, *P* < 0.05).

Temperature tolerances of fruits and roots

By contrast to the case for young (6-wk-old) and 1-yr-old cladodes (Figs 2, 3, Table 1), chlorenchyma cells of 6-wk-old fruits had similar uptake of neutral red at low temperatures for plants (detached cladodes with fruits) maintained for 2 wk at day/night air temperatures of 20 : 10°C vs 30 : 20°C (Fig. 4, Table 1). Also, no differences in responses of chlorenchyma cells of such fruits to high temperatures occurred for plants at 20 : 10°C vs 30 : 20°C (Fig. 4, Table 1). At the higher day/night air temperatures (30 : 20°C), fruits were more sensitive to high temperatures than were 6-wk-old or 1-yr-old cladodes (*P* < 0.05).
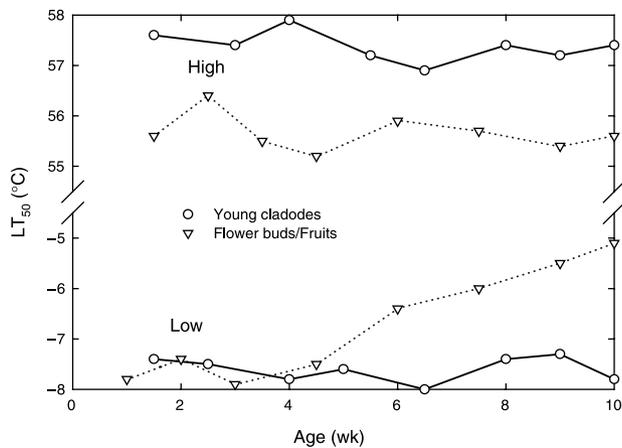
Fig. 5 LT_{50} for low and for high temperatures for chlorenchyma cells of young cladodes and flower buds/fruits of *Opuntia ficus-indica* of various ages. The organs were collected at the Agricultural Experiment Station on 25 May 2002 and were maintained at 22°C until measurement of tolerances to extreme temperatures within 22 h. One or two young cladodes and flower buds/fruits were taken from each of five plants.
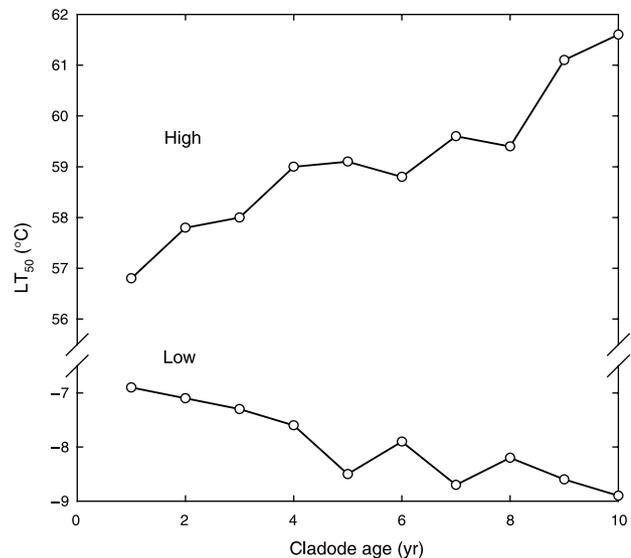

Fig. 6 LT_{50} for low and for high temperatures for chlorenchyma cells of mature cladodes of *Opuntia ficus-indica* of various ages. Cladodes were collected at the Agricultural Experiment Station on 25 May 2002 and were maintained at 22°C until measurement of tolerances to extreme temperatures within 22 h. Two cladodes were taken from each of five plants.

Young roots (6 wk old, as for young cladodes and fruits from the environmental chambers) showed no acclimation to low temperatures, having similar LT_{50}s for samples from plants at 20 : 10°C and 30 : 20°C (Table 1). Although such roots were more sensitive to high temperatures than were the 1-yr-old cladodes on which they developed ($P < 0.01$), the increase in LT_{50} for plants at 30 : 20°C vs 20 : 10°C of 2.9°C was similar (Table 1). Roots ranging in diameter from 0.5 to 9.9 mm were excavated at a depth of 10 ± 2 cm from field plants on 25 May 2002 and maintained at 22°C before measurement of tolerances to extreme temperatures within 22 h; LT_{50} for subzero temperatures was -6.6 ± 0.3°C ($n = 10$), similar to values for the low temperature tolerances for 6-wk-old roots (1.7 mm in diameter) of plants under controlled day/night temperatures (Table 1). The LT_{50} for high-temperature tolerance for such roots was 55.0 ± 0.6°C ($n = 10$), again with no trend with root diameter.

LT_{50} versus organ age

LT_{50} for both high and low temperatures of young cladodes did not vary with age up to 10 wk, averaging 57.4°C and -7.6°C, respectively ($F < 1.4$, $P > 0.3$; Fig. 5). Also, LT_{50} for high temperatures for flower buds/fruits did not vary with age up to 10 wk ($F = 0.5$, $P = 0.5$), averaging 55.7°C (again less than for cladodes, $P < 0.01$). LT_{50} for low temperatures for fruits up to 4.5-wk-old-was similar to that for young cladodes, averaging -7.7°C; LT_{50} increased with fruit age after 4.5 wk, becoming -5.1°C for 10-wk-old fruits ($F = 63.3$, $P < 0.001$; Fig. 5).

LT_{50} for high temperatures steadily increased at about 0.5°C yr^{-1} as cladode age increased from 1 yr to 10 yr ($F =$ 70.6, $P < 0.001$; Fig. 6). Also, LT_{50} for low temperatures steadily decreased at about 0.2°C yr^{-1} over the same range in cladode age. Thus, 10-yr-old cladodes tolerated 4.8°C higher temperatures and 2.0°C lower temperatures than did 1-yr-old cladodes ($F = 40.7$, $P < 0.001$; Fig. 6).

Discussion

Organs of *Opuntia ficus-indica* differed in their tolerances and acclimation to extreme temperatures, but various trends were evident. For example, tolerances and acclimation to low temperatures were similar for the chlorenchyma of the current year's daughter cladodes (6 wk old) and the 1-yr-old terminal cladodes on which they were produced. LT_{50} for 1-yr-old cladodes of *O. ficus-indica* on plants at day/night air temperatures of 20 : 10°C had previously been shown to be -7.0 to -7.7°C with an acclimation of 1.1–1.3°C as the day/night temperatures are lowered by 10°C (Nobel, 1988; Nobel *et al.*, 1995), in close agreement with the present results. The tolerances to low temperatures and to high temperatures steadily increased with cladode age up to 10 yr old. The tolerances to low temperatures for the chlorenchyma of fruits and the parenchyma of young roots (both 6 wk old) were similar for day/night air temperatures of 30 : 20°C and 20 : 10°C; fruits also did not show acclimation for high temperatures, but cladodes and roots did, LT_{50} averaging 2.8°C higher at day/night air temperatures of 30 : 20°C vs 20 : 10°C. In the field, cladodes and fruits could have maximum temperatures 6°C above air temperatures in the

afternoon and, because of transpiration, 1°C below air temperature at night, with the lowest temperatures occurring for young cladodes due to their higher transpiration rates than for fruits, consistent with previous observations (Nerd & Nobel, 2000; Nobel & De la Barrera, 2000).

The first hypothesis proposing that young cladodes and fruits should behave similarly to extreme temperatures received limited support. Fruits of *O. ficus-indica* were less tolerant of freezing temperatures than were young cladodes of the same age (6 wk) on plants maintained at day/night air temperatures of 20 : 10°C and were less tolerant of high temperatures than young cladodes on plants at 30 : 20°C. Essentially, the fruits showed no acclimation to extreme temperatures, whereas young cladodes did, which may be due to metabolic changes leading to the specialization of fruits of *O. ficus-indica* (Gutterman, 1995). Although the basis of acclimation ability for stems of cacti is not understood in molecular terms, it correlates with tolerances of both low and high temperatures for over 20 species (Nobel, 1988); that is, better acclimaters tend to tolerate more extreme temperatures. Fruit damage should be increasingly evident compared with damage to the current year's cladodes as field temperatures increase during the summer, but because LT_{50} was fully 55°C for fruits, extremely high temperatures would be required.

The LT_{50} for high temperatures had no significant trend with organ age up to 10 wk for cladodes or fruits, but the fruits were more susceptible to high temperatures than were the young cladodes. The LT_{50} for low temperatures had no trend with age for young cladodes up to 10 wk old and was similar to that for fruits up to 4.5 wk old, but fruits greater than 4.5 wk of age became steadily more susceptible to low temperatures. By 4–5 wk, anatomy, carbon relations, and water relations are different between cladodes and fruits of *O. ficus-indica* (Luo & Nobel, 1992; Nobel *et al.*, 1994; Gutterman, 1995; North *et al.*, 1995). For instance, daughter cladodes of *O. ficus-indica* begin to become independent of carbon from the underlying mother cladodes at 4 wk of age (Wang *et al.*, 1998), whereas its fruits are always carbon sinks (Inglese *et al.*, 1994; Nobel & De la Barrera, 2000). The lower CO_2 uptake and transpiration rates for 6-wk-old fruits than for young cladodes of that age (Nobel & De la Barrera, 2000) indicate that the physiology of the fruits has changed. Indeed, fruit sugars increase substantially after 6 wk for *O. ficus-indica* (Kuti, 1992). By 10 wk of age, the tolerance of fruits to freezing temperatures had decreased by 2.6°C, as indicated by the increasing LT_{50}. The increase in sugars during the latter phases of fruit development, which also occurs for fruits of other species (Ho, 1988), coincides with a decrease in the freezing tolerance of fruits, whereas accumulation of glucose, fructose, and sucrose in cladodes of *O. ficus-indica* increases their tolerance to freezing temperatures (Goldstein & Nobel, 1994).

The response of cladodes of *O. ficus-indica* to low temperatures, showing 2.0°C greater tolerance from 6 wk to 10 yr of age, is consistent with the second hypothesis. Moreover, this result is consistent with observations for *O. ficus-indica* in the field, as terminal and subterminal cladodes are more susceptible to freezing damage in the winter than are more basal cladodes. For example, freezing episodes in Texas and California can kill terminal and immediately subterminal cladodes of *O. ficus-indica*, while the older cladodes survive (P. Felker, unpublished; P. S. Nobel, unpublished). No change in tolerance to low temperatures was observed for young cladodes from 1.5 to 10 wk of age, during which time many developmental and metabolic changes occur (North *et al.*, 1995; Wang *et al.*, 1998). The change in temperature tolerance for cladodes from 1 to 10 yr of age does not reflect changes in the ambient air temperature, as minimum air temperatures as the University of California, Riverside, weather station have not changed substantially over the last 10 yr, averaging −0.2°C from 1993 to 1997 and 0.0°C from 1998 to 2002 (Department of Water Resources, 2002). Also, minimum nighttime temperatures along a branch of *O. ficus-indica* at the field site did not vary substantially, the variation generally being less than 1.0°C. However, over several seasons the older cladodes may have accumulated cryoprotectants such as sugars and mannitol (Goldstein & Nobel, 1994; Nobel *et al.*, 1995).

Maximum air temperatures at the weather station also have not changed substantially over the last 10 yr, averaging 41.1°C from 1992 to 1996 and 40.9°C from 1997 to 2001 (Department of Water Resources, 2002). Thus, the steady rise in the high temperature tolerated with cladode age from 6 wk to 10 years, amounting to 6.5°C overall, cannot be explained by concomitant changes in air temperature. Although bare soil in arid regions can attain temperatures of over 65°C, leading to similar tissue temperatures for single planted cladodes of *O. ficus-indica* (Nobel *et al.*, 1986), the branching of the 12-yr-old plants considered here caused considerable shading near the plant bases, leading to only moderate daily variations in soil surface temperatures. Also, the soil was kept moist by biweekly irrigation. Consequently, maximum cladode temperature did not vary substantially with position along a branch, although for other cacti, older and hence more massive stems tend to have higher maximum temperatures (Smith *et al.*, 1984). Young 4-wk-old cladodes of *O. ficus-indica* have less epicuticular wax and a thinner cuticle than do 1-yr-old cladodes (Mayeux & Johnson, 1989; North *et al.*, 1995), but this should not substantially affect the thermal relations. Substances such as starch and sugars may build up in the older cladodes, leading to greater tolerance of the membranes to high temperatures, a matter that requires further research.

Roots of *O. ficus-indica* at the field site were shallow, with a mean depth of 10 cm and essentially no roots occurred below 30 cm, in agreement with previous results for platyopuntias and which is advantageous during light rainfall events (Nobel, 1988; Drennan & Nobel, 1998). Roots are expected to be at the same local temperature as the soil. Calculated

from the soil temperatures recorded at different depths, the damping depth (incremental depth at which the daily oscillation in soil temperatures are reduced by 63%; Nobel, 1999) was 13 cm at the field site. Based on the damping depth, the fact that no roots of *O. ficus-indica* occurred in the upper 3 cm (except near the plant base), and the observed soil temperatures, the minimum soil and hence root temperatures are predicted to be above minimum shoot temperatures. However, the maximum root temperatures could be above the maximum shoot temperatures. In this regard, roots of *O. ficus-indica* were less tolerant of extreme temperatures than were cladodes, as hypothesized, and as is also the case for roots and stems of *O. acanthocarpa* (Nobel & Bobich, 2002). Thus the absence of roots of *O. ficus-indica* from the upper 3 cm of the soil most likely reflects their sensitivity to high temperatures, which can occur in such regions.

No change in tolerance to low or to high temperatures was evident with root diameter for *O. ficus-indica* from 0.5 mm up to 10 mm, which probably represented root ages ranging from 2 to 3 wk to multiple years. Young roots (6 weeks old, 1.7 mm in diameter) showed no low-temperature acclimation and similar high-temperature acclimation as for cladodes at day/night air temperatures of 20 : 10°C vs 30 : 20°C. Roots of *Opuntia acanthocarpa* also do not acclimate to low temperatures while its stems do, and both its roots and stems acclimate to high temperatures, with changes in LT_{50} for plants at day/night air temperatures of 35 : 25°C vs 25 : 15°C (Nobel & Bobich, 2002) being over twice as great as for plants of *O. ficus-indica* at 30 : 20°C vs 20 : 10°C. Acclimation of *Opuntia* roots only to high temperatures may reflect the greater variation in high compared with low soil temperatures, especially near the soil surface.

The tolerances of shoots to low and to high temperatures have seldom been quantified for ages of photosynthetic organs from 2 wk to 10 yr for any species, so the substantial adaptive increases observed with age for *O. ficus-indica* are noteworthy. Its roots in a more moderate temperature environment have lower tolerances of extreme temperatures than do its cladodes. Fruits of *O. ficus-indica* were the most susceptible part of the shoots to low temperatures, which occur at a time of the year when fruits generally are not present; fruits did not acclimate to high temperatures, but their high-temperature tolerance is already higher than for the shoots of most vascular plants. The lack of acclimation responses of fruits to low and high temperatures may be related to their metabolic specialization. Rising atmospheric temperatures accompanying global climate change should extend the cultivation of *O. ficus-indica* into regions that are currently too cold, and its substantial high-temperature tolerance should allow it to replace other crops with less tolerance and less acclimation ability for high temperatures (Nobel, 1996b). The different responses to extreme temperatures of roots and shoots, including those of fruits, deserve special consideration in ecological models and plant cultivation.

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