Root contraction helps protect the "living rock" cactus *Ariocarpus fissuratus* from lethal high temperatures when growing in rocky soil[1]

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- *Premise of the study*: We investigated how the "living rock" cactus *Ariocarpus fissuratus*, like other low-growing desert plants, can endure potentially lethal high temperatures at the soil surface. Specifically, we examined how shoot descent by root contraction in the presence or absence of soil rocks influences shoot temperatures and transpiration.
- *Methods*: Root contraction was identified by measuring shoot descent and anatomical analysis. Temperatures and transpiration were measured for plants at two heights in sandy and rocky soil, and temperature tolerances were determined by vital staining.
- *Key results*: Plants embedded in rocky soil survived an extreme heat episode, unlike plants in sandy soil, though rocks did not moderate low temperatures. Root contraction occurred regardless of season and soil moisture. Xylem conduits (wide-band tracheids) formed a compressible lattice that decreased root length as rays enlarged the root base radially. Plant position in the soil did not affect transpiration.
- *Conclusions*: Contractile roots pulled plants of *A. fissuratus* into the soil at rates of 6–30 mm yr^{-1}. Maintaining shoots level with the soil surface kept plant temperatures below the high lethal temperature and improved survivorship in soil shaded by surface rocks.

Key words: *Ariocarpus fissuratus*; contractile roots; desert plants; heat avoidance; LT_{50}; rocky soil; temperature tolerance; wide-band tracheids

Small desert succulents inhabit some of the hottest environments occupied by terrestrial plants. Direct solar radiation is intense, leading to temperatures that can exceed 70°C at the soil surface (Nobel et al., 1986; Geiger et al., 2003). In addition, cooling near the soil surface is limited because of friction and reduced wind speed and may be further limited by rough or irregular terrain (Geiger et al., 2003). For crassulacean acid metabolism plants such as the so-called living rocks in the Aizoaceae (e.g., species of *Lithops*) and Cactaceae (e.g., species of *Ariocarpus*), transpirational cooling is also minimal because of daytime stomatal closure. Embedding of much of the shoot below the soil surface can lead to lower shoot temperatures for small succulents. For example, for *Lithops* species with translucent tissue ("windows") near the shoot surface, the photosynthetic tissue in the leaf bases remains relatively cool because of its position well below the soil surface (Turner and Picker, 1993). The habit of growing level with the soil is problematic, however, for succulent species such as cacti that lack windows because their photosynthetic tissue is surrounded by soil that is potentially much hotter than the air temperature a few centimeters above it or the soil temperature a few centimeters below it (Geiger et al., 2003). Nevertheless, for *Ariocarpus*

fissuratus (Cactaceae), the subject of this study, as well as other living-rock cacti, root or shoot contraction has been hypothesized as a means of protection against lethal temperatures through the positioning of much of the shoot below the soil surface.

Small succulents may benefit from root or shoot contraction in other ways. As the nickname "living rocks" implies, small plants that are flush with the soil surface may be less easily detected by herbivores, particularly when plants are cryptically colored (Klooster et al., 2009). Positioning most of the shoot below the soil surface can also reduce transpirational water loss, as has been shown for two species of *Lithops* (Eller and Ruess, 1982). Water uptake also may be improved if contractile roots position plants lower in the soil where moisture is likely to persist longer than at the surface, though this would require new root initiation deeper in the soil. For a number of desert succulents and arid-land species in the Agavaceae, contractile roots also have higher hydraulic conductivity than otherwise similar roots (North et al., 2008), but this feature may be unique to monocots. For plants with clonal growth, contractile roots can help to separate and establish offshoots away from the parent plant. A final advantage of contractile roots is that they help to anchor plants, particularly seedlings and those of small stature, in the sandy or shifting soils characteristic of deserts.

Contractile roots have been described in a number of succulent plant groups, including the Asphodelaceae and Agavaceae (Pütz, 2002; North et al., 2008), though apparently not in the Aizoaceae (the family of *Lithops*), and have been reported in a few species of cactus, including *Leuchtenbergia principis* (Bobich and North, 2008), *Lophophora williamsii* (Terry and Mauseth, 2006), *Neomammillaria macdougali* (Hemenway and Breazeale, 1935), and *Pediocactus* spp. (Sivinski and McDonald, 2007), though the evidence derives mainly from plant position

in the soil. The presence of contractile roots in *A. fissuratus* can be inferred from a number of descriptions of plant habit (Britton and Rose, 1922; Anderson, 1960, 1999), but conclusive anatomical evidence and measurements of contraction apparently have not been presented for any cactus, including *A. fissuratus*. In the case of small globose succulents, shoot contraction due to loss of water during drought could be mistaken for and have much the same effect as root contraction.

In addition to plant traits, external factors that influence the thermal environment on the soil surface can be critical for shoot temperature. Because direct sunlight is the greatest contributor to soil surface temperatures during the day (Geiger et al., 2003; Nobel and Zutta, 2007), features such as rocks that reflect or block sunlight can reduce daytime temperatures substantially. A number of species of small succulents occur predominantly in rocky soil, including quartz outcroppings that can have surface temperatures that are several degrees lower than the regular soil (Schmiedel and Jürgens, 2004; Nobel and Zutta, 2007). Like most members of its genus, *A. fissuratus* typically occurs in rocky soil (Benson, 1982; Godínez-Álvarez et al., 2003), perhaps benefiting from other factors besides temperature moderation, such as increased soil moisture availability, protection from herbivory, favorable microsites for seed germination, and increased availability of mineral nutrients (Lopez et al., 2009).

In a series of experiments carried out with plants of *A. fissuratus*, we investigated whether, how, and why the living rock cactus pulls down into the soil. Three primary hypotheses to explain the value of root contraction to living rock cacti are that pulling plants further down into the soil (1) positions shoots in a more favorable thermal environment, (2) reduces transpirational water loss, and/or (3) reduces the likelihood of plants' detection by herbivores. The third hypothesis was not tested in this study. Related to the first two hypotheses, we ask the following questions:

1. Do both shoots and roots contract?
2. How is contraction affected by water availability and seasonal temperatures?
3. How does plant vertical position in the soil affect shoot temperature and transpirational water loss?
4. How do rocks on the soil surface affect soil and shoot temperatures?
5. What are the high- and low-temperature tolerances of shoots of *A. fissuratus*?

Low-temperature experiments were conducted on plants in a growth chamber, and high-temperature experiments were conducted in full sunlight on the roof of the biology building at Occidental College in southern California, United States. One experiment occurred during an extremely hot period (daytime air temperature maxima over 37°C for more than 3 d) in 2008.

MATERIALS AND METHODS

Plant material—Plants of *Ariocarpus fissuratus* Schumann K. (Cactaceae), with shoots about 3.0–4.0 cm in diameter, were purchased from a commercial nursery, Mesa Garden (Belen, New Mexico, USA). They were grown in 8.5 × 8.5 × 8.5 cm plastic containers in a 1 : 1 : 1 mixture of commercial cactus potting soil, coarse sand, and volcanic pumice, with 12 g of ground oyster shell lime added. Plants were placed in the soil such that the shoot surface was initially 25–30 mm above the soil. For 2 mo before experiments began, plants were maintained in a glasshouse at Occidental College in Los Angeles, California, which received 80% ambient solar radiation and had an annual maximum/minimum temperature of 45°C/5°C. Depending on seasonal temperatures, the

plants were watered weekly or biweekly and fertilized monthly with 0.1-strength Hoagland's solution supplemented with micronutrients (Hoagland and Arnon, 1950).

Root and shoot contraction—To measure changes in plant depth in the soil, fine plastic-coated wires about 70 mm long were inserted at the base of the stem just above the root–shoot junction (determined by color and texture of external tissues), which was initially level with the soil surface. The soil line was marked on the container with ink, and the height of the wire above the ink mark was measured to the nearest 0.5 mm with calipers. To measure vertical shoot contraction, india ink marks were drawn 10 mm apart on the stem near the root–shoot junction, and changes in the distance between marks also were measured to the nearest 0.01 mm with calipers. Changes in shoot surface area were measured from changes in projected area determined from digital photographs analyzed with the freeware program ImageJ (http://rsbweb.nih.gov/ij/). Contraction measurements were made on plants grown in the greenhouse for 90 d in the fall and winter of 2006–2007 ($N = 5$–6) and 50 d in the summer of 2007 ($N = 11$–12). In the fall and winter, plants received water biweekly (well watered) or bimonthly (dry soil); in the summer, the corresponding groups received water weekly or monthly.

Contraction with respect to time and watering regime was analyzed statistically by using repeated measures ANOVA or Friedman repeated measures analysis of variance on ranks, followed by Tukey pairwise testing when data were not normally distributed (SigmaStat 4; Systat Software, San Jose, California, USA).

Anatomy—Anatomical features were investigated by using fresh sections either cut with a razor blade or made with a Vibratome Series 1000 (Vibratome, Bannockburn, Illinois, USA). Sections about 15 μm thick were stained with toluidine blue O (0.05% wt/vol in phosphate buffer) and examined at 100× to 400× with a Nikon ME 600 compound microscope (Nikon Instruments, Melville, New York, USA). To determine possible xylem compression due to root contraction, the distance between rings of secondary lignified cell wall (annuli) in the conducting elements (wide-band tracheids) was measured on digital micrographs analyzed with ImageJ. For all anatomical measurements, $N \geq 5$ plants and, for cell measurements, ≥ 150 cells. Differences in interannular distance were analyzed statistically with Student's *t* test (SigmaStat).

Temperature responses to soil position and soil type—Four experiments were set up to investigate the effects of shoot position in the soil on plant internal temperatures. In all four experiments, programmable thermal sensors (iButtons; Embedded Data Systems, Lawrenceburg, Kentucky, USA) 15 mm in diameter and 4 mm high were used to monitor temperatures of the surrounding soil and of the internal stem tissue of *A. fissuratus*. For the stems, a 17 × 5 mm incision was made with a scalpel about 10 mm below the epidermis and 10 mm from the outside of the stem, and a section of stem tissue was removed, retaining the outer section of hypodermis and epidermis; an iButton was inserted, the section of hypodermis and epidermis was positioned to cover the insertion, and the cut was sealed with quick-setting cyanoacrylate adhesive. When in place, the iButton was located in the chlorenchyma, parallel to and about 10–15 mm below the plant surface. Little tissue necrosis was observed due to iButton implantation.

For the four experiments, two plastic containers 70 cm long × 40 cm wide × 20 cm deep were used, each large enough to accommodate five plants in their original containers such that each smaller container was surrounded on all sides by at least 5 cm of substrate. In the first experiment, the two large containers were filled with sandy desert soil (collected in the Sonoran Desert, outside Palm Desert, California, USA) that was not watered. Plants in their original containers were embedded with the stem surface either level with the surrounding soil or 25–30 mm above the soil surface ($N = 5$ for each treatment). In June 2007, iButtons were placed at 5 mm and 30 mm below the soil surface ($N = 3$ for each depth), and plant and soil temperatures were recorded hourly and monitored for 8 d.

In June 2008, half the soil surface of each container was covered with flat rocks (chiefly granitic, about 50–60 mm on a side and 10 mm deep) collected in the Sonoran Desert (rocky soil), whereas the other half had no surface rocks (sandy soil). Fresh plants were randomly chosen and placed with the shoot surface level with the soil surface, and iButtons were inserted and monitored as in 2007. In July 2010, to investigate specifically the effect of plant depth in rocky soil, both containers were entirely covered with flat rocks as above, and four plants in their original containers were embedded with the stem surface level with the surrounding rocks and four with the stem surface 30–35 mm above the rocks. Thermal iButtons were inserted and monitored as in 2007. For these

three experiments, containers were placed on benches on the roof of the biology building at Occidental and grown under ambient conditions (full sun, no water for8 –10d).

To investigate the effects of low temperatures on plants in sandy soil vs. rocky soil, the two containers were placed in an EGC growth chamber (Environmental Growth Chamber, Chagrin Falls, Ohio, USA) set at 8°C/3°C day/night temperatures with 12-h lighting (photosynthetically active radiation of 360 μmol m^{-2} s^{-1}). Plant and soil temperatures were monitored and recorded as in high-temperature experiments.

For the large container experiments, differences among soil temperatures and stem temperatures were analyzed by using repeated measures ANOVA or Friedman repeated measures ANOVA on ranks, followed by Tukey pairwise testing when data were not normally distributed (SigmaStat). Potential effects of the two large containers on maximum plant temperatures were tested with nested one-way ANOVAs (BIOMstat, Applied Biostatistics, Port Jefferson, New York, USA).

Temperature tolerances—Stem tissue tolerances to high and low temperatures were determined for acclimated plants by exposing sections of stem tissue to stepped temperature changes followed by staining with a vital stain. For high-temperature tolerance, eight plants in their original containers were removed from the greenhouse and placed in a growth chamber initially set at 24°C/18°C day/night temperatures; temperatures were increased by 2°C d^{-1} until plants were at 30°C/24°C for 48 h. Pieces of stem tissue about 8 mm on a side were excised with a scalpel, placed in 1.5-mL Eppendorf tubes (Eppendorf, Hamburg, Germany), placed in a programmed thermal cycler (EchoTherm Model 1C3dXT, Torrey Pines Scientific, San Marcos, California, USA), and exposed to stepped increases in temperature at the rate of 5°C hr^{-1}. After 60 min at a temperature between 30°C and 60°C, tissue was removed from the cycler and cut into 15-μm-thick sections, which were placed in the vital stain neutral red (1% wt/vol in phosphate buffer; Onwueme, 1979; Nobel et al., 1998) for 10 min, followed by rinsing in buffer and viewing under the microscope at 200×.

Living cells in the chlorenchyma (just beneath the epidermis and hypodermis) were identified as those that took up neutral red in the central vacuole (Repetto et al., 2008). They were scored as a percentage of all cells entirely within a microscopic field and were normalized according to a control percentage established for sections taken from plants grown at 24°C/18°C. In addition to their failure to take up neutral red stain, dead cells were identified by other evidence such as plasmolysis and loss of chlorophyll. Results were graphed and curves were fitted with the use of nonlinear regression (SigmaPlot; Systat Software, San Jose, California, USA), and the temperature at which 50% of the cells were dead (LT_{50}) was determined graphically.

Methods for determining low-temperature tolerance were similar, except that plants in the growth chamber were acclimated to decreasing temperatures at the rate of 2°C d^{-1} until they were held at 10°C/3°C for 48 h. Pieces of stem tissue were removed and exposed to decreasing temperatures in a thermal cycler at the rate of –2°C hr^{-1}. The percentage of living cells in the chlorenchyma was determined at 5°C steps from 10°C to –5°C and at 2°C steps from –5°C to –13°C, and LT_{50} was determined as for high-temperature tolerance.

Transpiration—Diurnal water loss was determined for plants on the roof in containers 8.5 × 8.5 × 8.5 cm filled with sandy desert soil. Eighteen plants of approximately the same size were divided into three groups, one with the shoot surface level with the surface of soil without rocks (sandy soil—level), one with the shoot surface level with soil that was covered with rocks (rocky soil—level), and one with the shoot surface 30 mm above the surface of rocky soil (rocky soil—30 mm). Water loss was determined by hourly weighing for 24 h in early July 2008, and weights were corrected by subtracting the weight of control containers with and without rocks on the soil surface. Water loss on an hourly basis was analyzed by repeated measures ANOVA, and total water loss was analyzed by a one-way ANOVA (SigmaStat).

RESULTS

Root and shoot contraction—As indicated by changes in the height above the soil of wires attached to stem bases, plants of *A. fissuratus* moved down in the soil column during both fall/winter (Fig. 1A) and summer (Fig. 1B), amounting to a decrease between 6 and 30 mm yr^{-1} when factored over 12 mo. The decrease over time was significant for summer (two-way repeated

measures ANOVA, time as main effect, $F_{7,146} = 2.15$, $P = 0.042$) and highly significant for fall/winter (time as main effect, $F_{11,88} = 4.90$, $P < 0.001$), though a significant interaction existed between time and soil moisture for the latter period ($F_{11,88} = 2.68$, $P = 0.005$). Comparing the first 8 wk of measurement in fall/winter with the summer measurement period, significantly greater contraction occurred during fall/winter (one-way repeated measures ANOVA, $F_{7,209} = 4.28$, $P < 0.001$). Differences in contraction due to soil moisture were not significant in either fall/winter or summer over the 8-wk periods (two-way repeated measures ANOVA, moisture as main effect, $F_{1,29} = 1.41$, $P = 0.245$).

Changes in plant height above the soil due to stem contraction, as determined by decreases in the distance between ink marks on the stem measured during the summer, were significant (two-way repeated measures ANOVA, $F_{3,62} = 2.82$, $P = 0.046$), though differences due to soil moisture were not ($F_{1,21} = 0.08$, $P = 0.780$). The mean decreases in distance were 0.03 ± 0.18 mm and 0.41 ± 0.15 mm for plants in well-watered and dry soil, respectively. Projected surface area of plants in both well-watered and dry soil did not change significantly during the summer 8-wk period (two-way repeated measures ANOVA, time as main effect, $F_{7,15} = 0.766$, $P = 0.624$).

Anatomy and morphology—Older regions of taproots of *A. fissuratus*, located beneath the subterranean, nonphotosynthetic stem region (Fig. 2A; arrow indicates root–shoot transition), were radially enlarged and showed transverse wrinkling. A single taproot was usually present, though as many as five or six basally enlarged roots occasionally occurred (Fig. 2B). The taproot could be distinguished from the subterranean stem by the presence of lateral roots (Fig. 2A, B), which did not arise from the stem. Longitudinal sections through older, enlarged root regions showed compression in the external tissues (periderm) and in the older (inner) xylem (Fig. 2C), and both longitudinal and cross sections revealed several well-developed rays of parenchyma cells interspersed between regions of xylem (Fig. 2C, E). Rays consisted of fewer and smaller cells in younger, noncontracted root regions than in older, contracted root regions (Fig. 2D vs. 2E). Specifically, the radial length of ray parenchyma cells (arrows, Fig. 2D, E) was 35.5 ± 1.2 μm in noncontracted root regions and 74.0 ± 6.1 μm in older contracted roots regions (*t* test, $t = 6.21$, df = 8, $P = 0.003$).

In both noncontracted and contracted root regions, xylem conduits were exclusively wide-band tracheids (WBTs; Mauseth, 2004), which had unlignified primary walls with thick lignified bands or annuli (Fig. 2F). In longitudinal sections of young, noncontracted roots, the walls of WBTs were relatively straight between lignified bands (Fig. 2G), whereas primary walls of WBTs in contracted root regions often curved inward, showing signs of longitudinal compression (Fig. 2H). In addition to the sinuous appearance of older xylem in longitudinal sections (Fig. 2C, F), evidence of compression was the greater distance between lignified bands in young, noncontracted regions than in contracted root regions: 22.2 ± 1.2 μm vs. 18.4 ± 0.9 μm, respectively (*t* test, $t = 2.51$, df = 8, $P = 0.036$).

Plant temperatures: large container experiments—During summer 2007, in large containers of sandy desert soil (few rocks on the surface), soil temperatures did not differ significantly at 3-mm vs. 30-mm depths (Friedman repeated measures ANOVA on ranks followed by Tukey pairwise testing, $X^2 = 69.82$, df = 3, $q = 0.45$, $P > 0.05$; Fig. 3). Stem temperatures
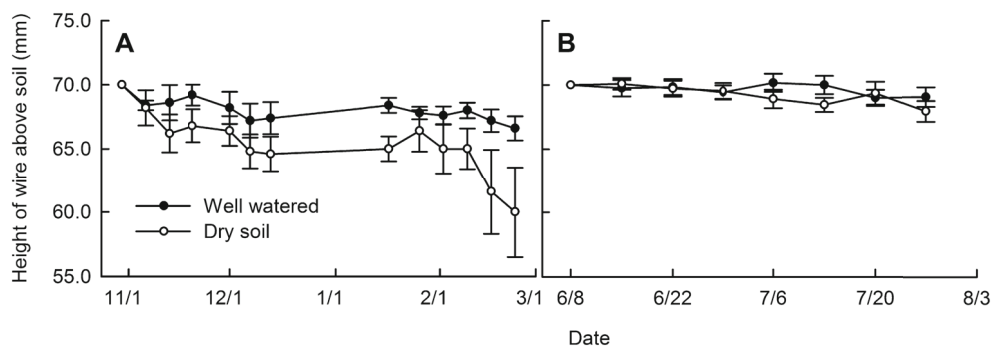
Fig. 1. Shoot descent of *Ariocarpus fissuratus*, as determined by decrease in height above soil of wires attached to stem base, for plants in well-watered soil (filled circles) or dry soil (open circles) during fall/winter 2006–2007 (A) and summer 2007 (B). Data are presented as means ±SE ($N = 5–6$ in fall/wintera nd11 –12i ns ummer).

were lower than soil temperatures, and the internal temperature of stem tissue 30 mm above the soil surface (sensors about 5 mm below soil surface) was lower than that of shoots that were level with the soil surface (sensors about 30 mm below soil surface; $q = 4.35$ for two stem temperatures, $P < 0.05$). The difference in maximum stem temperatures attributable to containers was not significant (nested ANOVA, $F = 3.5270$, df = 2, $P = 0.0798$).

In summer 2008, two large containers, each with half rocky and half sandy soil, were placed outdoors on the roof during a period of high temperatures in Los Angeles. During this time (18–24 June 2008), maximum air temperature exceeded 37°C for 4 d and 41°C for 2 consecutive days. Soil temperatures at two depths (Fig. 4A) and stem tissue temperatures (about 30 mm below soil surface; Fig. 4B) were lower for rocky soil than for sandy soil (Friedman repeated measures ANOVA on ranks, $X^2 = 79.17$, df = 5, $P < 0.001$). The highest temperature reached by plant stems in rocky soil was 56.5°C ± 0.1°C, whereas that for plant stems in sandy soil was 60.0°C ± 0.5°C. The difference in maximum stem temperatures attributable to containers was not significant (nested ANOVA, $F = 0.368$, df = 2, $P = 0.731$). The median temperature of stem tissue for plants in rocky soil was lower than the temperatures for the soil at two depths and for stem tissue in sandy soil, which reached the highest temperature of all. Moreover, the chlorenchyma (photosynthetic tissue) of the five plants in sandy soil was pale brown at the end of 7 d of high temperatures (Fig. 5A), whereas the chlorenchyma of the five plants in rocky soil remained largely dark green (Fig. 5B). After two months of regular watering and moderate temperatures in the glasshouse, all plants from sandy soil were dead (brown, soft stems, easily removed from soil), whereas all plants from the rocky soil were dark green, turgid, and well rooted.

Plants were placed at two heights in large containers of rocky soil in July 2010, with the stem surface either level with the average height of the rocks or 30 mm above the rocks. Stem temperatures did not differ overall during 3 d of measurement (Friedman repeated measures ANOVA on ranks, $X^2 = 0.671$, df = 1, $P = 0.413$; Fig. 6). However, mean maximum temperatures for stems level with rocky soil was lower than for stems 30 mm above the rocks (two-way ANOVA, position as main effect, $F_{1,18} = 7.547$, $P = 0.013$; Fig. 6). Again, no difference existed in maximum plant temperatures that could be attributed to container effects (nested ANOVA, $F = 0.175$, df = 2, $P = 0.841$).

In a growth chamber set to approximate winter temperatures (8°C day/3°C night), large containers of plants in rocky and sandy soil showed significant differences in soil and stem temperatures (Friedman repeated measures ANOVA on ranks, $X^2 = 262.70$, df = 5, $P < 0.001$; Fig. 7). The median temperatures were lower for rocky soil than for sandy soil and were lower for stem tissue of plants in rocky soil than for plants in sandy soil (for both comparisons, $P < 0.05$). The lowest stem temperature for plants in rocky soil was 2.5°C ± 0.6°C compared with 3.0°C ± 0.4°C for plants in sandy soil.

High-/low-temperature tolerances—The high-temperature tolerance for plants previously acclimated at 30°C/24°C was determined by chlorenchyma cell uptake of neutral red stain (Fig. 8A). The high temperature at which 50% of cells were dead (high LT_{50}) as determined graphically, was 56.8°C, and more than 80% of the cells were dead at 60°C. The low-temperature tolerance for plants acclimated at 10°C/4°C was also determined by chlorenchyma stain uptake, and the low LT_{50} was −10.2°C (Fig.8B).

Diurnal water loss—Over a 24-h period (12–13 July 2008), no difference existed in water lost by plants due to soil type or to position in rocky soil, but containers with plants lost significantly more water than did containers of soil only (Fig. 9; Friedman repeated measures ANOVA on ranks, $X^2 = 45.037$, df = 4, $P < 0.001$; $P > 0.05$ for pairwise testing of plants in different soils and for the two soil types). Total water loss per plant (mean weight of containers with plants − mean weight of container with soil only) was 0.323 ± 0.028 g, 0.433 ± 0.040 g, and 0.377 ± 0. 038 g for plants with shoots level with sandy soil, shoots level with rocky soil, and shoots 30 mm above rocky soil, respectively. These differences were not significant (one-way ANOVA, $F_{2,18} = 2.31$, $P = 0.128$). In addition, no difference existed in the amount of water lost by pots of sandy soil or rocky soil without plants ($F_{1,18} = 3.28$, $P = 0.100$).

DISCUSSION

Rates and process of root contraction—Shoots of *A. fissuratus* were pulled deeper into the soil during both fall/winter and summer, primarily by root contraction. The descent occurred at a rate of 6 to 30 mm y^{-1}, which is comparable to the rate of root contraction measured for several species of *Agave* and *Yucca* (North et al., 2008) but is less than that for many mesophytic species (Pütz, 2002). Contractile roots have been inferred from plant position in the soil for a number of cactus species, including *A. fissuratus*, *Neomammillaria* (= *Mammillaria*) *macdougali*
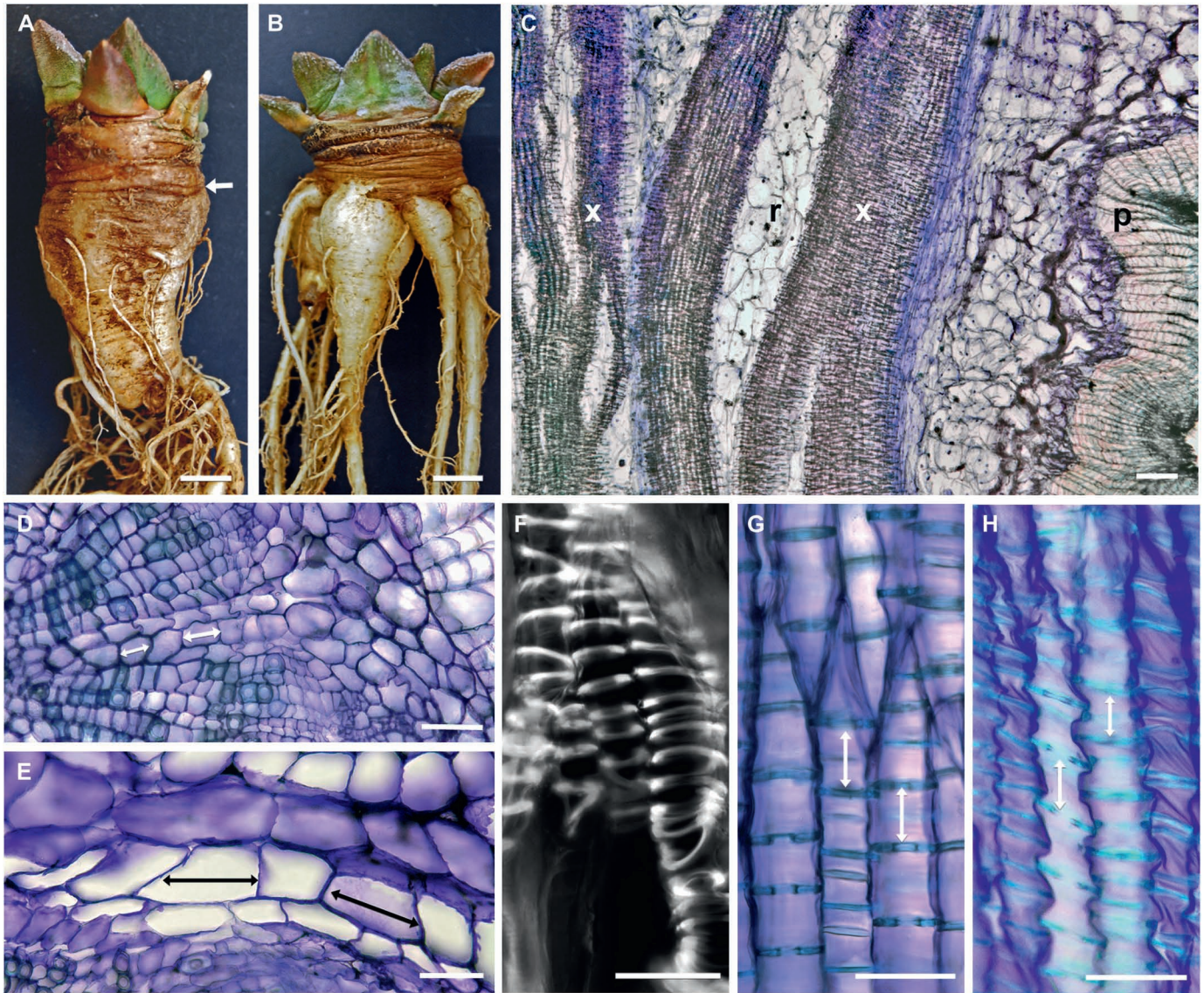
Fig. 2.   Morphology and anatomy of roots of *Ariocarpus fissuratus*. Transverse wrinkling of the periderm in whole plants (A) with a single taproot (arrow indicates root–shoot transition) and (B) with several swollen taproots (scale bars in A and B = 10 mm). (C) Longitudinal section through compressed basal region of taproot (p = periderm, r = ray, x = xylem; scale bar = 50 μm). Parenchyma cells in rays between xylem wedges in (D) noncontracted root regions (white arrows) and (E) contracted regions (black arrows; scale bars in D and E = 100 μm). Wide-band tracheids with lignified annuli, as seen in longitudinal sections of (F) contracted root region, (G) noncontracted root region, and (H) contracted root region (distance between lignified bands indicated by arrows in G and H; scale bars in F–H = 50 μm). C–E, G, and H were stained with toluidine blue O, viewed in transmitted light, and F was unstained, viewed with ultraviolet epifluorescence.

(Hemenway and Breazeale, 1935), species of *Pediocactus* (Benson, 1982), and *Lophophora williamsii* (Terry and Mauseth, 2006), though rates and mechanisms of contraction have not been reported previously. Presumably, the rate of contraction for *A. fissuratus* compensates for its shoot growth, with the result that the surface of the shoot in the field remains nearly flush with the soil surface (Benson, 1982). Shoots as well as roots contracted longitudinally, but shoot contraction accounted for less than 10% of the overall decrease in plant height above the soil. Thus, contractile roots of *A. fissuratus* were responsible for the downward movement of the shoot.

Root contraction was significantly greater during fall/winter than during the summer and was affected by soil moisture only after 90 d of reduced watering in late winter, when plants exhibited the maximum rate of descent. *Ariocarpus fissuratus* is the only member of its genus to occur in the continental United States, specifically, in the Chihuahuan Desert in southwest Texas. Typical annual minimum and maximum temperatures in its range, on the basis of the latitude and longitude of herbarium specimens collected near Brewster, Texas, USA (MOBOT; http://www.tropicos.org/NameSpecimens.aspx?nameid=5100291) are $1.2°C \pm 0.27°C$ and $35.8°C \pm 7.2°C$, respectively (years 1980–2005, data from PRISM, http://prism.oregonstate.edu), thus the low temperatures in the Occidental College greenhouse (about 5.0°C) were somewhat above those experienced by plants in the field. In any case, low temperatures combined with
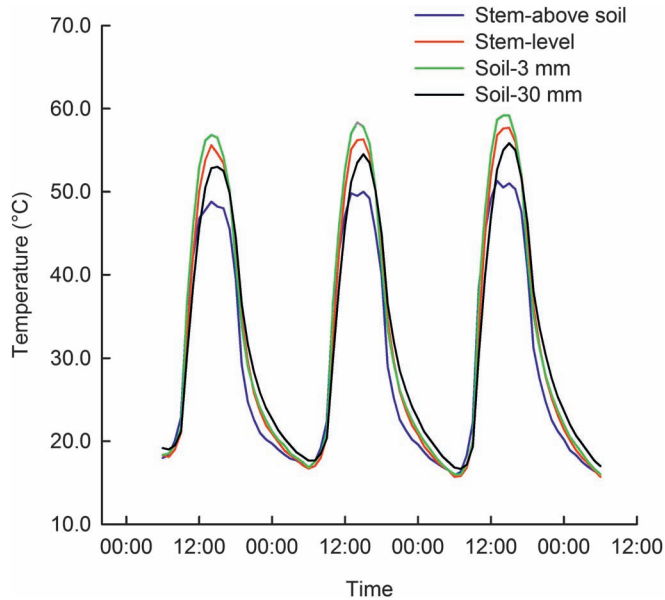
Fig. 3. Temperatures recorded in June 2007 for soil and plants in large containers on the roof of the biology building of Occidental College, Los Angeles, California, USA. Thermal sensors were embedded in plants with surface 30 mm above the soil (stem—above soil; blue line), in plants flush with the soil (stem—level; red line), in soil at 3 mm (green line), and in soil at 30 mm deep (black line). $N = 5$ plants and $N = 3$ thermal sensors in soil for each condition; SE (not shown for sake of legibility) was less than 2% of the mean in all cases.

relatively low soil moisture would be effective cues for contraction, resulting in more favorable shoot position during the subsequent spring and summer.

The morphology and anatomy of contractile roots in *A. fissuratus* indicated that contraction in the basal (older) region of the taproot involved radial enlargement, an increase in girth that was accompanied by longitudinal compression, as shown by transverse wrinkling of the periderm. In contracted root regions, parenchyma cells in the rays between wedges of xylem were much longer in the radial direction than were ray cells in noncontracted, distal regions of the taproot. Similar elongation of cells in the radial direction occurs in contractile roots of *Agave* and *Yucca* as well as in *Hyacinthus orientalis* (Jernstedt, 1984), *Trifolium repens* (Cresswell et al., 1999), and roots and hypocotyls of young plants of several species from fire-prone habitats (Fisher, 2008). Although ray cell height (axial dimension) was not measured in contractile roots of *A. fissuratus*, axial compression in adjacent xylem conduits was evident in the sinuous appearance of older xylem. In addition, individual conducting cells of the xylem, which are WBTs, indicated compression by the closer spacing of radial lignified bands in contracted than in noncontracted root regions. On the basis of the decrease in the distance between bands, WBTs in contracted root regions were about 17% shorter than WBTs in noncontracted regions, an amount of contraction that can be extrapolated to the root itself. The decrease in length that accompanied the increase in girth implicates a process of contraction similar to that of a Chinese finger trap or puzzle, in which an interconnected cylindrical lattice must decrease in length as it increases in diameter (Pütz, 2002). Such a mechanism has been thoroughly characterized in roots of *Freesia* (Ruzin, 1979), as well as in those of *T. repens*, in which the framework of the lattice is composed of fibers (Cresswell et al., 1999). For roots of *A. fissuratus*, the lattice framework consisted of WBTs because no lignified fibers were present.

Wide-band tracheids are well suited to longitudinal contraction because of their unlignified lateral walls, which allow compression without breakage. In addition, the width and positioning of the lignified bands (or annuli) are such that the lateral walls are prevented from total inward collapse, as discussed with respect to WBTs and water stress in cacti (Mauseth, 2004) and species of Portulacaceae (Landrum, 2006). In the most extreme case of compression, the lignified bands would resemble a stack of records, yet the pathway of water would remain unobstructed as long as tracheid lumens were aligned (see Fig. 2F). In contractile
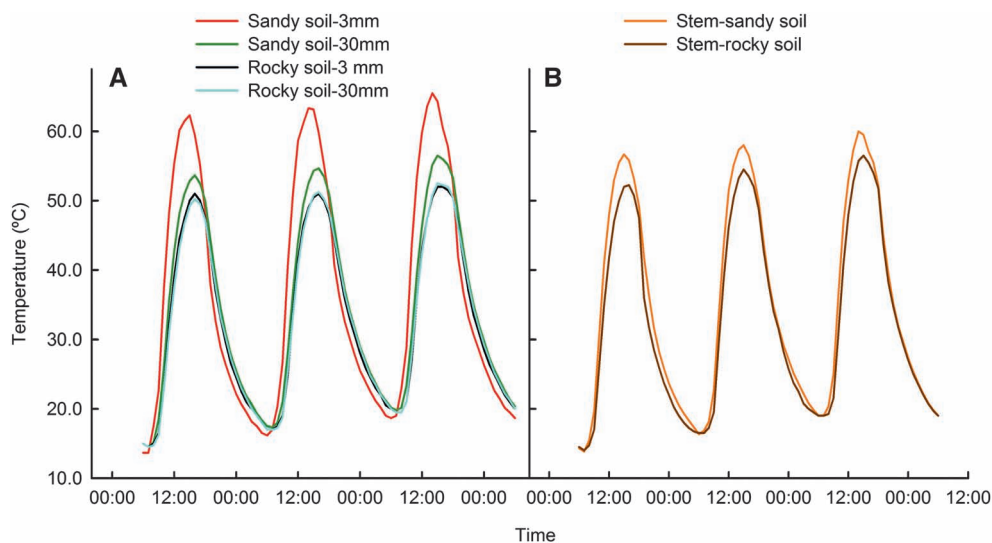

Fig. 4. Temperatures recorded in June 2008 for (A) soil and (B) plants in large containers on the roof of the biology building of Occidental College, Los Angeles, California, USA. Thermal sensors were embedded (A) in sandy soil at 3 mm (red line) and at 30 mm deep (dark green line) and in rocky soil at 3 mm (black line) and at 30 mm (light-blue line); and (B) in plants level with sandy soil (orange line) and in plants level with rocky soil (brown line). $N = 3$ thermal sensors in soil, and $N = 5$ plants for each condition; SE (not shown for sake of legibility) was less than 2% of the mean in all cases.
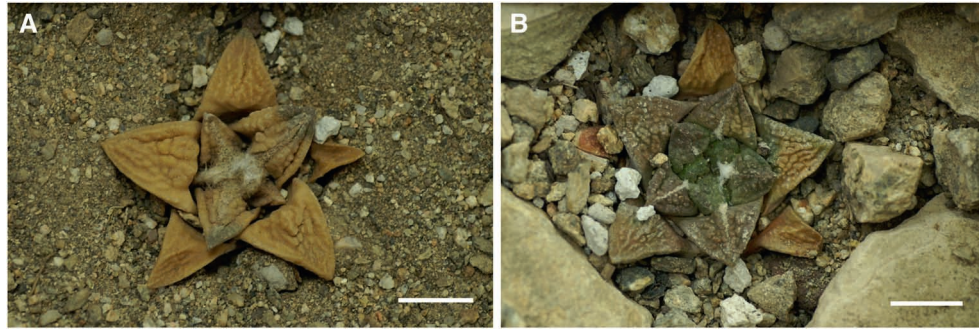
Fig. 5.   Plants of *Ariocarpus fissuratus* in large containers on the roof of the biology building of Occidental College, Los Angeles, California, USA, in June 2008 after 8 d of high temperatures. Plants were embedded in sandy soil without surface rocks (A) or in soil covered with rocks (B); scale bars = 10mm.

roots of *A. fissuratus*, the oldest (innermost) xylem showed the greatest compression, whereas the most recently formed (outermost) xylem showed little (Fig. 2C).

Consequences of root contraction—The effect of root contraction in field-grown plants of *A. fissuratus* is that their shoots are almost always level with the surrounding soil. Three adaptationist hypotheses can be invoked to explain this growth habit; namely, plants lower in the soil (1) have more favorable water relations, (2) experience more favorable temperatures, and/or (3) are subject to less herbivory because of plant concealment or camouflage. The first two hypotheses were tested in this study; only the second was supported, and only in the case of plants in rock-covered soil exposed to high temperatures.

With respect to water relations, plants of *A. fissuratus* lost similar amounts of water over a 24-h period whether their shoots were level with sandy or rocky soil or were 30 mm above rocky soil. These results are in contrast to measurements of water loss by two other living rocks, *Lithops karasmontana* and *L. lesliei*, which transpired less when the stems were embedded in soil than when exposed to air (Eller and Ruess, 1982). A possible explanation for the difference between the plants measured in this study and the *Lithops* species is the latter had their roots in water and were transpiring freely, whereas plants of *A. fissuratus* were in moderately dry soil on a hot summer day, when stomata appeared to be closed (little late night water loss was recorded). Differences in water loss due to plant position and soil type might have been greater had stomatal opening been greater. Also no difference existed in the amount of water lost from pots containing the two soil types without plants, suggesting the rocks did little to prevent evaporation of water from the soil. In the field, there may be more and larger rocks on the surface, leading to a more effective barrier against water loss. In any case, plants of *A. fissuratus* have a number of traits that confer drought tolerance, including an extremely thick cuticle and a central cavity in the stem, hypocotyl, and base of the taproot filled with mucilage, which has been shown to increase water storage capacitance in other species (Morse, 1990; Nobel et al., 1992). Thus, pulling shoots further into the soil may be less critical than are other traits for the water relations of *A. fissuratus*.

The second hypothesis, that root contraction positions plants of *A. fissuratus* in more favorable thermal environments, was tested for both low and high temperatures. The evidence in this study with regard to low temperatures was mixed in that the greatest contraction was measured on plants during the winter,

but in low temperatures in the growth chamber stem temperatures were lower than soil temperatures in almost all cases. Moreover, stem temperatures for plants in rocky soil were lower than for plants in sandy soil. The lowest temperature to which plants were exposed in the growth chamber, 3°C, is higher than the mean annual minimum temperature at field sites for *A. fissuratus* in Texas and considerably higher than the low LT_{50} of –10.2°C measured in this study. In addition, the mucilage in the stems of *A. fissuratus* may help plants tolerate low temperatures in the field as well as prolonged drought (Loik and Nobel, 1991). Thus, root contraction does not seem essential to protecting *A. fissuratus* from potentially lethal low temperatures.

With respect to high temperatures, in the summer of 2007, when plants were in large containers of sandy soil without rocks on the surface, stem temperatures were actually higher for shoots level with the soil than for shoots 30 mm above the soil,
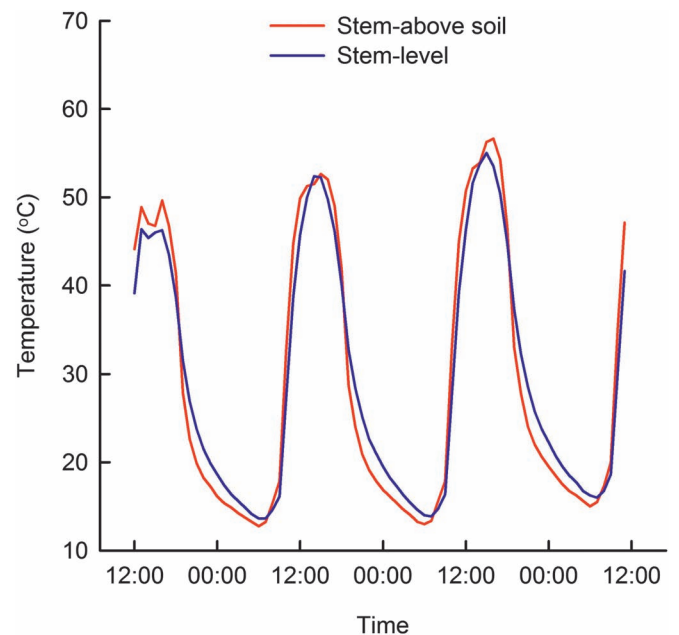

Fig. 6.   Temperatures recorded in July 2010 for plants in large containers of rocky soil on the roof of the biology building of Occidental College, Los Angeles, California, USA. Thermal sensors were embedded in plants level with rocky soil (blue line) and in plants with stem surfaces 30 mm above rocks (red line). $N = 4$ plants for each condition; SE (not shown for sake of legibility) was less than 5% of the mean in both cases.
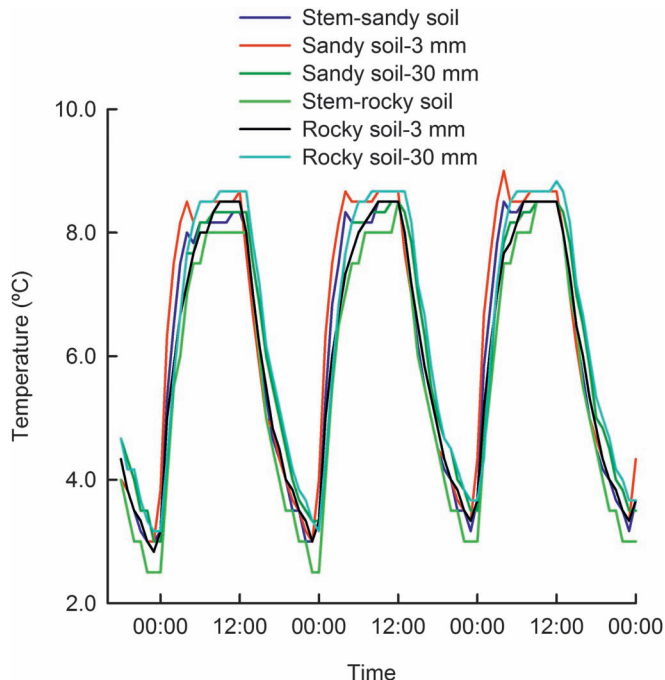
Fig. 7. Temperatures recorded for soil and plants in large containers in a growth chamber (8°C day/3°C night). Thermal sensors were embedded in plants in sandy soil (dark-blue line), in sandy soil at 3 mm (red line) and at 30 mm deep (dark-green line), in plants embedded in rocky soil (light-green line), and in rocky soil at 3 mm (black line) and at 30 mm (light-blue line). $N = 3$ for each condition; SE (not shown for sake of legibility) was less than 2% of the mean in all cases.

indicating that root contraction would confer little thermal advantage in the absence of surface rocks. The following summer, in containers of both rocky and sandy soil, not only were stem temperatures lower for plants in rocky soil than for plants in sandy soil, but after 8 d of intense summer heat, all plants in sandy soil were dead, whereas all plants in rocky soil survived. The highest temperature recorded for plants in rocky soil was 56.5°C, just under the LT_{50} of 56.8°C measured in this study, whereas the high temperature for plants in sandy soil was well

over that mark at 60°C. In a subsequent experiment to test whether plant position per se affected shoot temperatures in rocky soil, plants that were level with rocky soil had lower maximum temperatures than did plants elevated above the rocks. Thus, for *A. fissuratus* in the field, root contraction combined with rocks on the soil surface could help keep plant shoots from attaining lethal high temperatures.

The rocks used to cover the soil in this study were taken from a hillside near Palm Desert, California, USA, a site where succulents such as *Agave deserti* and small species of *Mammillaria* are most frequently established in rocky soil (Nobel et al., 1992; Martre et al., 2002). The rocks were chosen to be similar to the soil in color but tended to be a bit lighter (see Fig. 5). Thus, it is possible that the greater albedo of the rocks than of the sandy soil contributed to the lower temperatures of the rocky soil and the plants in it, as is the case for the small succulent *Dudleya saxosa* in the northwestern Sonoran Desert (Nobel and Zutta, 2007) and species of Aizoaceae in the South African Karoo (Schmiedel and Jürgens, 2004). In both these cases, the surrounding rock cover was quartz, which presumably reflects more light than did the granitic rocks used in this study and which has been shown to be critical for plant survival in the Karoo (Musil et al., 2009). In this study, at 3 mm below the surface, the mean maximum temperature of the rocky soil was approximately 10°C cooler than that of the sandy soil (53.3°C vs. 63.7°C), whereas at 30 mm below the surface, the difference was about 4°C (51.2°C vs. 55.0°C). The most probable explanation for this cooling is the rocks reduced the amount of sunlight reaching the underlying soil (Geiger et al., 2003). The plants themselves were not shaded by the small rocks used in this study, in contrast to the disadvantageous situation that can arise in the field when small succulents are shaded by nurse plants(G odínez-Álvareze ta l.,2003 ; Peterse ta l.,2 008).

It is worth noting that even when pulled down in rocky soil, plants of *A. fissuratus* reached internal temperatures only a few degrees lower than the high LT_{50} measured in this study. At a field site for *A. fissuratus* in Texas, maximum annual air temperatures exceeded 37°C in 7 y between 1980 and 2005, and they exceeded 38°C in 2 y, temperatures similar to air temperatures in this study when sandy soil temperatures exceeded 60°C, and the plants in sandy soil died. Thus, *A. fissuratus* occurs in regions where near-lethal temperatures are not uncommon and
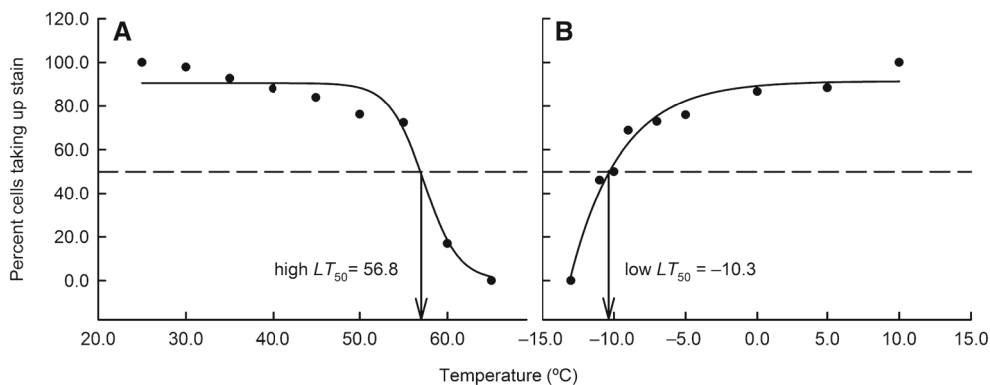

Fig. 8. Percentage of cells taking up the vital stain neutral red (= percent living cells) for plant tissue held at the target temperature for 60 min and then examined under a microscope at 200×. Plants in (A) were exposed to step increases in temperature and in (B) were exposed to step decreases. Curves were fitted using SigmaPlot (Systat Software, San Jose, California, USA). Equation for fitted curve in (A) is $f = a/(1+exp(-(x-x0)/b))$, $r^2 = 0.97$. Equation for fitted curve in (B) is $f = y0+a/(1+exp(-(x-x0)/b))$, $r^2 = 0.97$. The temperature at which 50% of the cells were dead (LT_{50}) was determined graphically (dashed line indicates 50%).
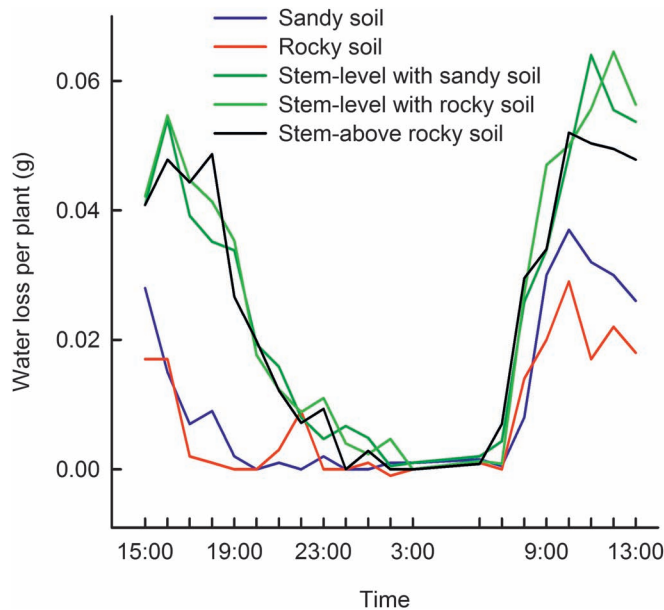
Fig. 9.   Water loss determined by hourly weighing of containers on the roof of the biology building at Occidental College, Los Angeles, California, USA, in July 2008: sandy soil (blue line), rocky soil (red line), plants level with sandy soil (dark green line), plants level with rocky soil (light green line), and plants above rocky soil (black line). Data are shown as means of $N = 6$, and SE (not shown for the sake of legibility) averaged less than 10% of the mean.

where rocks may be critical for plant survival. In an earlier investigation, the high LT_{50} for *A. fissuratus* was measured at 66.4°C (Nobel et al., 1986); however, this was determined by exposing tissue to high temperatures for 1 h, in contrast to the prolonged exposure experienced by plants in this study.

Although we did not test the third hypothesis, that maintaining the shoot level with the soil helps protect plants from herbivory, the camouflage benefits of being embedded in rocky soil for living rock cacti would seem apparent (Skelhorn et al., 2010). In addition to having abundant mucilage in the central cavity, however, *A. fissuratus* is well defended chemically by alkaloids, similar to those in *Lophophora williamsii* (peyote; Bruhn and Bruhn, 1973). Thus, selection for contractile roots as a means of protection against herbivory may be relatively weak.

In summary, plants of *A. fissuratus* moved down in the soil at a rate of 6–30 mm y^{-1} due to root contraction, which resulted from the longitudinal shortening that accompanied radial enlargement of basal root regions. Wide-band tracheids formed a compressible vascular framework that facilitated root contraction. Plants level with the surface of sandy soil attained higher temperatures than did plants with shoots above sandy soil, but temperatures for plants level with rocky soil were lower than for plants in sandy soil, as well as lower than for plants with shoots above rocky soil. Moreover, all plants in sandy soil died after 8 d of high summer temperatures, whereas all plants in rocky soil survived. Shoot positioning level with rocky soil did not lead to higher plant temperatures in a low-temperature experiment, nor did it decrease plant water loss. Thus, root contraction was clearly beneficial for *A. fissuratus* only when shoots were level with soil shaded by surface rocks. As global temperatures increase, rocky microhabitats will become even more critical ref-uges for plants such as the living rock cactus that otherwise will be likely to experience lethal temperatures more frequently.

LITERATURE CITED

Anderson, E. F. 1960.   A revision of *Ariocarpus* (Cactaceae). I. The status of the proposed genus *Roseocactus*. *American Journal of Botany* 47: 582–589.

Anderson, E. F. 1999.   *Ariocarpus*: Some reminiscences. *Cactus and Succulent Journal* 71: 180–190.

Benson, L. 1982.   The cacti of the United States and Canada. Stanford University Press, Stanford, California, USA.

Bobich, E. G., and G. B. North. 2008.   Structural implications of succulence: Architecture, anatomy, and mechanics of photosynthetic stem succulents, pachycauls, and leaf succulents. *In* E. De la Barrera E. and W. K. Smith [eds.], Perspectives in biophysical plant ecophysiology: A tribute to Park S. Nobel, 3–38. UNAM Press, Mexico City, Mexico.

Britton, N. L., and J. N. Rose. 1922.   The Cactaceae: Descriptions and illustrations of plants of the cactus family, vol. 3, 80–83. Carnegie Institute of Washington, Washington, D.C., USA.

Bruhn, J. G., and C. Bruhn. 1973.   Alkaloids and ethnobotany of Mexican peyote cacti and related species. *Economic Botany* 27: 241–251.

Cresswell, A., N. R. Sackville Hamilton, H. Thomas, R. B. Charnock, A. R. Cookson, and B. J. Thomas. 1999.   Evidence for root contraction in white clover (*Trifolium repens* L.). *Annals of Botany* 84: 359–369.

Eller, B. M., and B. Ruess. 1982.   Water relations of *Lithops* plants embedded into the soil and exposed to free air. *Physiologia Plantarum* 55: 329–334.

Fisher, J. B. 2008.   Anatomy of axis contraction in seedlings from a fire proneha bitat. *American Journal of Botany* 95: 1337–1348.

Geiger, R., R. H. Aron, and P. Todhunter. 2003.   The climate near the ground, 6th ed. Rowman and Littlefield Publishers, New York, New York, USA.

Godínez-Álvarez, H., T. Valverde, and P. Ortega-Baes. 2003.   Demographic trends in the Cactaceae. *Botanical Review* 69: 173–203.

Hemenway, A. F., and L. Breazeale. 1935.   A study of *Neomammillaria macdougali* (Rose). *American Journal of Botany* 22: 493–499.

Hoagland, D. R., and D. I. Arnon. 1950.   The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347: 1–32.

Jernstedt, J. A. 1984.   Root contraction in hyacinth. I. Effects of IAA on differential cell expansion. *American Journal of Botany* 71: 1080–1089.

Klooster, M. R., D. L. Clark, and T. M. Culley. 2009.   Cryptic bracts facilitate herbivore avoidance in the mycoheterotrophic plant *Monotropsis odorata* (Ericaceae). *American Journal of Botany* 96: 2197–2205.

Landrum, J. V. 2006.   Wide-band tracheids in genera of Portulacaceae: Novel, non-xylary tracheids possibly evolved as an adaptation to waters tress. *Journal of Plant Research* 119: 497–504.

Loik, M. E., and P. S. Nobel. 1991.   Water relations and mucopolysaccharide increases for a winter hardy cactus during acclimation to subzerot emperatures. *Oecologica* 88: 340–346.

Lopez, B. R., Y. Bashan, M. Bacilio, and G. De la Cruz-Agüero. 2009.   Rock-colonizing plants: Abundance of the endemic cactus *Mammillaria fraileana* related to rock type in the southern Sonoran Desert. *Plant Ecology* 201: 575–588.

Martre, P., G. B. North, E. G. Bobich, and P. S. Nobel. 2002.   Root deployment and shoot growth for two desert species in response to soilr ockiness. *American Journal of Botany* 89: 1933–1939.

Mauseth, J. D. 2004.   Wide-band tracheids are present in almost all species of Cactaceae. *Journal of Plant Research* 117: 69–76.

Morse, S. R. 1990.   Water balance in *Hemizonia luzulifolia*: The role of extracellularpol ysaccharides. *Plant, Cell & Environment* 13: 39–48.

Musil, C. F., P. D. R Van Heerden, C. D. Hilliers, and U. Schmiedel. 2009. Mild experimental climate warming induces mild metabolic impairment and massive mortalities in southern African quartz field succulents. *Environmental and Experimental Botany* 66: 79–87.

Nobel, P. S., M. Castañeda, G. North, E. Pimienta-Barrios, and A. Ruiz. 1998. Temperature influences on leaf CO_2 exchange, cell viability and cultivation range for *Agave tequilana*. *Journal of Arid Environments* 39: 1–9.

Nobel, P. S., G. N. Geller, S. C. Kee, and A. D. Zimmerman. 1986. Temperatures and thermal tolerances for cacti exposed to high temperatures near the soil surface. *Plant, Cell & Environment* 9: 279–287.

Nobel, P. S., P. M. Miller, and E. A. Graham. 1992. Influence of rocks on soil temperature, soil water potential, and rooting patterns for deserts ucculents. *Oecologia* 92: 90–96.

Nobel, P. S., and B. R. Zutta. 2007. Rock associations, root depth, and temperature tolerances for the "rock live-forever," *Dudleya saxosa*, at three elevations in the north-western Sonoran Desert. *Journal of Arid Environments* 69: 15–28.

North, G. B., E. K. Brinton, and T. Y. Garrett. 2008. Contractile roots in succulent monocots: Convergence, divergence and adaptation to limited rainfall. *Plant, Cell & Environment* 31: 1179–1189.

Onwueme, I. C. 1979. Rapid plant-conserving estimation of heat tolerance in plants. *Journal of Agricultural Science, Cambridge* 92: 527–536.

Peters, E. M., C. Martorell, and E. Ezcurra. 2008. Nurse rocks are more important than nurse plants in determining the distribution and establishment of globose cacti (*Mammillaria*) in the Tehuacán Valley, Mexico. *Journal of Arid Environments* 72: 593–601.

Pütz, N. 2002. Contractile roots. *In* Y. Waisel, A. Eshel, and U. Kafkafi [eds.], Plant roots: The hidden half, 3rd ed., 975–987. Marcel Dekker, New York, New York, USA.

Repetto, G., A. del Peso, and J. L. Zunita. 2008. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nature Protocols* 3: 1125–1131.

Ruzin, S. E. 1979. Root contraction in *Freesia* (Iridaceae). *American Journal of Botany* 66: 522–531.

Schmiedel, U., and N. Jürgens. 2004. Habitat ecology of southern African quartz fields: Studies on the thermal properties near the ground. *Plant Ecology* 170: 153–166.

Sivinski, R. C., and C. McDonald. 2007. Knowlton's cactus (*Pediocactus knowltonii*): Eighteen years of monitoring and recovery actions. *In* P. Barlow-Irick, J. Anderson, C. McDonald [eds], Southwestern rare and endangered plants: Proceedings of the 4th Conference, March 22–26, 2004, 98–107, Las Cruces, New Mexico. U.S. Deptartment of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado, USA.

Skelhorn, J., H. M. Rowland, M. P. Speed, and G. D. Ruxton. 2010. Masquerade: Camouflage without crypsis. *Science* 327: 51.

Terry, M., and J. D. Mauseth. 2006. Root-shoot anatomy and post-harvest vegetative clonal development in *Lophophora williamsii* (Cactaceae: Cacteae): Implications for conservation. *SIDA, Contributions to Botany* 22: 565–592.

Turner, J. S., and M. D. Picker. 1993. Thermal ecology of an embedded dwarf succulent from South Africa (*Lithops* spp: Mesembryanthemaceae). *Journal of Arid Environments* 24: 361–385.