Vegetative Reproduction as Related to Biomechanics, Morphology and Anatomy of Four Cholla Cactus Species in the Sonoran Desert

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Vegetative reproduction via the rooting of detached stem segments (joints) is well recognized for certain cylindropuntias (chollas). This mode of reproduction was characterized in the field for *Opuntia acanthocarpa*, *O. bigelovii*, *O. echinocarpa* and *O. ramosissima* in the northwestern Sonoran Desert and the southern Mojave Desert and related to the following: (1) morphology of terminal joints and their junctions; (2) the biomechanics and anatomy of terminal joint junctions; and (3) the rooting of detached terminal joints. Species that typically reproduce vegetatively were hypothesized to possess mechanically weak terminal joint junctions and terminal joints with high rooting abilities. In general, resistance to failure of terminal joint junctions depended on the diameter of the junction, with larger diameters providing greater resistance to mechanical failure. Junction strength also depended on the presence or absence of fibres and the amount of parenchyma cells per cross-sectional area. Rooting ability appeared to depend on joint diameter, which determines the amount of stored carbohydrates and water. Of the four species, only *O. bigelovii* showed evidence of vegetative reproduction in the field and was also the only species that had both relatively weak junctions and joints with a high rooting ability. Furthermore, joints of *O. bigelovii* had the most spines per tubercle area, which increases the chance that their spines will catch on a passing vertebrate, allowing for greater joint dispersal. © 2001 Annals of Botany Company

Key words: Biomechanics, Cactaceae, *Cholla*, libriform fibres, *Opuntia*, vascular tracheids, vessel elements.

INTRODUCTION

Vegetative reproduction is the process by which a plant part eventually detaches from an existing plant and roots independently (Bell, 1991). Vegetative reproduction can disperse new individuals as well as facilitate survival during unfavourable conditions as dormant structures, such as bulbs and corms, which can function as storage reservoirs for carbohydrates (Fitter, 1986). For certain cylindropuntias (chollas) of North American deserts, vegetative reproduction by rooting of detached stem segments (joints) or sterile fruits serves as a dispersal mechanism (Benson, 1982; Gibson and Nobel, 1986; Allen *et al.*, 1991). In fact, certain cholla species may depend almost entirely on vegetative reproduction (Benson, 1982). Cholla branches are sympodial axes comprised of joints that taper at their ends. The resulting smaller cross-sectional areas at their bases lead to relatively small connections between joints (Gibson and Nobel, 1986). Joint junctions are hypothesized to be regions of mechanical weakness because of their limited size and because the tensile and compressive forces resulting from bending moments increase toward the fixed end of stems (Niklas, 1992). The terminal joint junctions of chollas that reproduce vegetatively should be especially susceptible to mechanical failure.

For platyopuntias (Bobich and Nobel, 2001) and for other cacti (Niklas and Buchman, 1994; Molina-Freaner *et al.*, 1998; Niklas *et al.*, 1999), junction strength and overall stem strength are correlated with junction and stem cross-sectional area and the accumulation of secondary xylem (wood). Such a correlation is hypothesized to occur for the terminal joint junctions of chollas as well. However, vessel elements in the secondary xylem of chollas have smaller diameters and lengths (Gibson, 1977) and tend to occur in larger clusters than vessel elements of certain platyopuntias and species in subfamily Cactoideae (Gibson, 1978; Gibson and Nobel, 1986; Mauseth and Plemons-Rodriguez, 1998). These differences could affect the strength of the wood of chollas relative to other cacti. Furthermore, the positive correlation of vessel element and libriform fibre size with overall plant size and growth habit observed for platyopuntias and other cacti (Gibson, 1973, 1978; Mauseth and Plemons-Rodriguez, 1998; Bobich and Nobel, 2001) has not been observed for cylindropuntias (Gibson, 1977). Thus, the correlation between junction strength and cellular anatomy is hypothesized to be less strong for chollas than for platyopuntias (Bobich and Nobel, 2001).

In the deserts and certain grasslands of North America, cholla species that apparently depend on vegetative reproduction are sympatric with other cholla species that depend on sexual reproduction (Benson, 1982). A characteristic of certain vegetatively reproducing species is that the spines of their terminal joints tend to catch in the skin or pelts of passing vertebrates, causing the joints to dislodge. These joints either fall near the existing plant or are transported to other sites by vertebrates (Gibson and Nobel, 1986; Allen

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et al., 1991). Thus, the terminal joints of species that repro- duce vegetatively are hypothesized to have more spines per area than sexually reproducing species. The spine surfaces for these species are also hypothesized to have more strongly barbed epidermal cells than those of other species, which would allow the spines to catch and remain in the epidermis of a passing animal. Because vegetative reproduction also depends on the rooting of joints, it is further hypothesized that a higher percentage of experimentally detached joints will root for vegetatively- rather than sexually-reproducing species. These hypotheses were tested using biomechanical, anatomical and morphological analyses in conjunction with field and greenhouse observations of four cholla species that are sympatric in the northwestern Sonoran Desert. A primary objective is to identify the characteristics that facilitate vegetative reproduction via detached joints for the four cholla species considered.

MATERIALS AND METHODS

Characterization of vegetative reproduction

Vegetative reproduction was examined for four cylindro- puntia species (Cactaceae) differing in growth form at the University of California Philip L. Boyd Deep Canyon Desert Research Center in the northwestern Sonoran Desert (USA). When mature, *Opuntia acanthocarpa* var. *ganderi* (C. B. Wolf) L. Benson (gander cholla) is a shrub that has three or more main branches; *Opuntia bigelovii* Engelmann var. *bigelovii* (teddy-bear cholla) is an upright shrub with a main trunk and few lateral branches; *Opuntia echinocarpa* Engelmann and Bigelow var. *echinocarpa* (golden cholla) is shrubby with a short trunk and many branches; and *Opuntia ramosissima* Engelmann (diamond cholla, pencil cholla) is a shrub with two or more main lower branches and many secondary branches. Vegetative reproduction was also investigated for *O. bigelovii*, *O. echinocarpa* and *O. ramosissima* in the southern Mojave Desert in Joshua Tree National Park, where the maximum height of these species was approximately twice that found at the Deep Canyon Desert Research Center. Because progeny can develop from detached terminal joints, the presence or absence of detached terminal joints near conspecific plants and the condition of these joints (apparent water status and mortality) was noted for each species (detached joints could be distinguished from seedlings, when present, by having larger stem diameters and a greater number of spines). Vegetative reproduction for each species was estimated by the number of plants ⩽0·2 m tall that appeared to have developed from joints detached from nearby plants that were ⩾0·4 m tall at Deep Canyon Desert Research Center and plants ⩽0·4 m tall near those ⩾0·8 m tall at Joshua Tree National Park. For a smaller plant to be considered to be produced vegetatively, its base had to be closer to the taller plant than the height of the taller plant.

Biomechanical analyses and morphology

Deflections of terminal joints, resulting from incre- mentally increasing applied loads and hence increasing bending moments (Bobich and Nobel, 2001), were deter- mined in the field for ten branches from ten different plants for each species. Loads causing failure and the accompany- ing critical bending moments were also determined for each terminal joint junction. Upon removal of a branch from a plant, length and mean diameter of the terminal joint were measured to within 0·1 mm using calipers. The subterminal joint was then secured 10 mm below the joint junction with a wooden clamp that was bolted to an aluminium tripod and covered with 10 mm of rubber foam to prevent tissue damage; the subterminal joint was clamped so that the terminal joint was oriented vertically (Nobel and Meyer, 1991; Bobich and Nobel, 2001). Forces were applied perpendicularly to the axis of a terminal joint through its centre of mass with a force gauge (Shimpo Instruments, Itasca, IL, USA). Bending moments were calculated as the applied force × moment arm (half of the joint length). Horizontal deflections were read on a scale mounted directly above the terminal joint, using a fine needle inserted into its apex (Bobich and Nobel, 2001). Angular deflection was calculated as $\sin^{-1}$ (horizontal deflection/ joint length) (Nobel and Meyer, 1991).

The diameters of joint junctions were measured after failure of the terminal junctions. The section modulus (the geometric expression describing resistance to bending moments; Timoshenko and Young, 1962; Niklas, 1992) was calculated for each junction as $I/a = \pi a^3/4$, where I is the second moment of area (m^4) and a is the radius of the junction (m). The diameter of a cross-section including the xylem plus pith and the diameter of the pith alone were also recorded and used to calculate the cross-sectional area of xylem.

Morphological features of tubercles (enlarged succulent modified leaf bases) and spines were also characterized for terminal joints. Mean length and width of the tubercles were measured to calculate their surface area, which was approximated as a cylinder for all species except *O. ramosissima*, which has flat tubercles. The number of spines arising from the areole (axillary bud) on each tubercle and the mean length of the central spines were also determined. The surfaces of the spines were examined for all four species using a compound microscope.

Tissue preparation and anatomical analyses

After the removal of spines and glochids, samples of the entire junction from six terminal joints of each species were removed using a razor blade. These samples were then fixed in FAA (formalin-acetic acid-alcohol; 10 % v/v 37 % formaldehyde, 5 % acetic acid, 50 % ethyl alcohol, 35 % water), dehydrated using a tertiary butanol series, and embedded in Paraplast Plus (Oxford Labware, St. Louis, MO, USA; Jensen, 1962). After embedding, cross-sections approx. 15 µm thick were cut using a rotary microtome for the junctions of *O. bigelovii*, *O. echinocarpa* and *O. ramosissima* and a sliding microtome for the more heavily lignified junctions of *O. acanthocarpa*. The sections were stained using a combination of safranin (10 mg ml^{-1} 50 % ethyl alcohol) and fast green (10 mg ml^{-1} 50 % ethyl alcohol, 50 % clove oil; Jensen, 1962). Digital images of the

junction cross-sections were taken at $200\times$ using a digitizing camera (Pixera Corporation, Los Gatos, CA, USA) connected to an Olympus BH-2 light microscope (Lake Success, NY, USA). Cross-sectional areas were measured for 50 cells per junction of the following cell types: the axial parenchyma of secondary xylem, vessels, libriform fibres and vascular tracheids (wide-band tracheids; Mauseth *et al.*, 1995). Cell wall thicknesses, which were taken as the average of three measurements for each cell wall, were determined from images for vessels, libriform fibres, and vascular tracheids at $200\times$ using Image Pro Plus (Media Cybernetics, Silver Spring, MD, USA).

Cell lengths for vessels, libriform fibres, and vascular tracheids were determined from macerations of 3 mm thick samples of six terminal joint junctions for each species. Samples were placed in Jeffrey's solution (10 % chromic acid plus 10 % nitric acid by weight) at 40 °C for 48 h and then washed with distilled water (Berlyn and Miksche, 1976). After affixing to slides, the samples were stained with safranin (10 mg ml^{-1} 50 % ethyl alcohol). Measurements of cell length were taken from digital images at $100\times$ using Image Pro Plus.

Rooting of terminal joints

Rooting ability was determined for 40 terminal joints of each species that were removed from plants at the Deep Canyon Desert Research Center in March 2000 after rainfall totalling 33 mm in the previous 20 d. The segments were placed on top of soil from the Deep Canyon Desert Research Center (33° 38′ N, 116° 24′ W, 820 m elevation) in 0·33 m $\times$ 0·18 m $\times$ 0·14 m tubs and maintained in a glasshouse with maximum/minimum air temperatures averaging 30/14 °C. A depth of 10 mm of water was applied to the soil each week, which is representative of the average weekly winter/early spring rainfall at the Desert Research Center (Nobel, 1988). Joints were examined weekly for the production of adventitious roots that extended into the soil.

Statistical analyses, including comparisons among species, were performed using SigmaStat 2·03 (Jandel Corporation, San Rafael, CA, USA). Data are presented as means ± s.e.

RESULTS

Vegetative reproduction

Detached terminal joints, which could lead to the production of new plants, were found near the bases of three cholla species but not near *Opuntia acanthocarpa*. The detached terminal joints of *O. bigelovii* were relatively well hydrated compared to those of *O. echinocarpa* and *O. ramosissima*, which were extremely dehydrated or dead. In this regard, smaller plants within a radial distance equal to the height of taller conspecifics (thus assumed to be progeny produced from detached stems) were much more common for *O. bigelovii* than for the other three cholla species (Table 1). At Deep Canyon, the number of progeny (plants ⩽ 0·2 m tall) per established plant (⩾ 0·4 m tall) was 0·04 for *O. acanthocarpa*, 0·66 for *O. bigelovii*, and 0·01

for *O. echinocarpa* and *O. ramosissima* (Table 1). Observations at Joshua Tree National Park were similar with 0·62 progeny (plants ⩽ 0·4 m tall) per established plant (⩾ 0·8 m tall) for *O. bigelovii*, 0·01 for *O. echinocarpa*, and 0·02 for *O. ramosissima* (Table 1).

Terminal joint, joint junction and spine morphology

Terminal joints of *O. acanthocarpa* were the largest, followed by those of *O. bigelovii*, *O. echinocarpa* and then *O. ramosissima*. Specifically, terminal joints of *O. acanthocarpa* were twice as long and similar in diameter to those of *O. bigelovii*, 2·5-times longer and 1·9-times greater in diameter than those of *O. echinocarpa*, and 3·0-times longer and 4·6-times greater in diameter than those of *O. ramosissima* (Table 2). The greater joint dimensions of *O. acanthocarpa* were reflected in their mass, which was 1·3-times that of *O. bigelovii*, 9·2-times that of *O. echinocarpa*, and 29-times that of *O. ramosissima* (Table 2). Joint junctions had the greatest cross-sectional areas and the greatest wood cross-sectional areas for *O. acanthocarpa*, both averaging 4·4-times those of *O. bigelovii* and *O. echinocarpa* and 9·0-times those of *O. ramosissima* (Table 2).

Tubercles projected from the joints of *O. acanthocarpa*, *O. bigelovii* and *O. echinocarpa*, whereas joints of *O. ramosissima* were flat. Tubercle surface area was 2·3-times greater for *O. acanthocarpa* than for *O. echinocarpa* and 4·2-times greater than for *O. bigelovii* and *O. ramosissima* (Table 3). *Opuntia acanthocarpa* had 1·6-times more spines per areole than *O. bigelovii* and *O. echinocarpa*, and 32-times more than *O. ramosissima* (Table 3). However, *O. bigelovii* had 1·6-times more spines per tubercle surface area than *O. echinocarpa*, 2·2-times more than *O. acanthocarpa*, and 20-times more than *O. ramosissima*. Spines for *O. ramosissima*, which never exceeded one per tubercle, were 50 % longer than the central spines of the other three taxa (Table 3). The spine epidermal cells were longer for *O. bigelovii* than for

TABLE 1. *Shorter plants associated with taller plants that were presumed to have been produced vegetatively at the University of California Philip L. Boyd Deep Canyon Desert Research Center and Joshua Tree National Park*

Site and Species	Number of taller plants examined	Number of shorter plants found within a distance equal to the height of the taller plants
Deep Canyon		
O. acanthocarpa	144	5
O. bigelovii	210	139
O. echinocarpa	202	3
O. ramosissima	174	2
Joshua Tree		
O. bigelovii	431	269
O. echinocarpa	374	4
O. ramosissima	326	5

Taller plants were ⩾ 0·4 m and ⩾ 0·8 m tall at Deep Canyon and Joshua Tree, respectively, and shorter plants were ⩽ 0·2 m and ⩽ 0·4 m tall, respectively.

TABLE 2. *Morphological characteristics of terminal joints and their junctions*

	Species				P
Character	*O. acanthocarpa*	*O. bigelovii*	*O. echinocarpa*	*O. ramosissima*	
Joint length (mm)	97.1 ± 4.4^a	47.9 ± 1.2^b	39.1 ± 3.3^{bc}	32.6 ± 1.7^c	<0.001
Average joint diameter (mm)	29.2 ± 0.9^a	30.3 ± 0.9^a	15.1 ± 0.6^b	6.3 ± 0.2^c	<0.001
Joint mass (g)	32.2 ± 2.0^a	24.2 ± 2.5^b	3.5 ± 0.2^c	1.1 ± 0.1^d	<0.001
Junction cross-sectional area (mm^2)	63.7 ± 4.4^a	14.8 ± 2.2^b	13.4 ± 1.3^b	6.9 ± 0.9^c	<0.001
Junction xylem cross-sectional area (mm^2)	36.1 ± 2.8^a	8.7 ± 0.9^b	8.4 ± 1.1^b	4.1 ± 0.7^c	<0.001

Data for joint lengths, diameters, and masses ($n = 25$) and junction wood areas ($n = 10$) are means $\pm$ s.e. The P value for joint length comparisons is for a Kruskall-Wallis one-way ANOVA; all others correspond to a one-way ANOVA performed on log-transformed data. Values with different superscripts within a row are significantly different ($P < 0.05$) for Dunn (for joint length) or Tukey pairwise comparisons.

TABLE 3. *Morphological characteristics of tubercles and spines*

	Species				P
Character	*O. acanthocarpa*	*O. bigelovii*	*O. echinocarpa*	*O. ramosissima*	
Tubercle area (mm^2)	188 ± 11^a	49.2 ± 3.1^b	81.3 ± 9.9^c	40.0 ± 3.5^b	<0.001
Number of spines per areole	16.0 ± 1.2^a	9.9 ± 0.5^b	9.7 ± 0.6^b	0.5 ± 0.1^c	<0.001
Spine length (mm)	17.1 ± 0.9^a	18.3 ± 0.2^a	16.6 ± 1.3^a	26.5 ± 2.0^b	<0.001
Epidermal cell length (μm)	108 ± 7^{ab}	125 ± 8^a	101 ± 6^{ab}	91 ± 4^b	<0.001
Epidermal cell projection (μm)	8.8 ± 0.5^a	9.8 ± 0.7^a	8.2 ± 0.7^a	5.5 ± 0.5^b	0.003

Data for tubercle area, number of spines per areole, and length of central spines ($n = 10$ terminal joints from 10 different plants) as well as spine epidermal cell length and epidermal cell extension from the spine surface ($n = 10$ central spines from 10 different plants) are means $\pm$ s.e. P values correspond to one-way ANOVA. Values with different superscripts within a row are significantly different ($P < 0.05$) after Tukey pairwise comparisons.

O. ramosissima, and those of *O. acanthocarpa* and *O. echinocarpa* were intermediate (Table 3). Projections of the epidermal cells from the spine surface were 38 % less for *O. ramosissima* than for the other three taxa, which had similar projections (Table 3).

Biomechanics of terminal joints and joint junctions

The deflection of terminal joints increased with increasing applied loads for all four species (Fig. 1). Terminal joints of *O. acanthocarpa* and *O. bigelovii* deflected less than those of *O. echinocarpa* and *O. ramosissima* for a given applied load ($P < 0.001$, one-way ANOVA of log-transformed data; Fig. 1). The initial slope for the deflection curves averaged $11.7 \pm 1.9°$ kg^{-1} for *O. acanthocarpa*, $18.5 \pm 1.9°$ kg^{-1} for *O. bigelovii*, $77.3 \pm 7.3°$ kg^{-1} for *O. echinocarpa*, and $84.9 \pm 14.9°$ kg^{-1} for *O. ramosissima* (Fig. 1). The maximum deflection of terminal joints prior to terminal junction failure was least for *O. bigelovii*, intermediate for *O. acanthocarpa*, and greatest for *O. echinocarpa* and *O. ramosissima* ($P < 0.001$, one-way ANOVA). Specifically, the maximum deflection of terminal joints averaged $31.3 \pm 3.5°$ for *O. acanthocarpa*, $10.8 \pm 1.3°$ for *O. bigelovii*, $65.0 \pm 1.9°$ for *O. echinocarpa* and $49.9 \pm 5.9°$ for *O. ramosissima*. The critical applied loads causing mechanical failure of terminal joint junctions were least for *O. bigelovii* and *O. ramosissima*, intermediate for *O. echinocarpa*, and greatest for *O. acanthocarpa* ($P < 0.001$, one-way ANOVA). The magnitude of the critical loads averaged 1.9 ± 0.2 kg for *O. acanthocarpa*, 0.49 ± 0.2 kg for *O. bigelovii*, 0.77 ± 0.1 kg for *O. echinocarpa*, and 0.42 ± 0.1 kg for *O. ramosissima*.

The critical bending moments of joint junctions increased logarithmically with the logarithm of increases in the junction section modulus among the four species ($P < 0.001$; Fig. 2). Critical bending moments for terminal junctions were greatest for *O. acanthocarpa*, followed by *O. echinocarpa*, *O. bigelovii* and *O. ramosissima* in that order ($P < 0.001$, one-way ANOVA; Fig. 2). The critical bending moments averaged 0.96 ± 0.07 N m for junctions of *O. acanthocarpa*, 0.11 ± 0.02 N m for those of *O. bigelovii*, 0.18 ± 0.02 N m for those of *O. echinocarpa*, and 0.07 ± 0.01 N m for those of *O. ramosissima*. The section modulus for terminal junctions was also greatest for *O. acanthocarpa*, followed by *O. bigelovii* and *O. echinocarpa*, which did not differ ($P = 0.97$, Tukey pairwise comparisons), and *O. ramosissima* ($P < 0.001$, one-way ANOVA for log-transformed data; Fig. 2). The section modulus averaged 71.6 ± 7.2 mm^3 for *O. acanthocarpa* junctions, 8.1 ± 1.7 mm^3 for *O. bigelovii* junctions, 6.9 ± 1.0 mm^3 for *O. echinocarpa* junctions, and 2.5 ± 0.4 mm^3 for *O. ramosissima* junctions.

Anatomy of terminal joint junctions

The primary tissue type in the terminal junctions of the four species was parenchyma, which occurred in the pith, the rays, and the axial regions of the secondary xylem
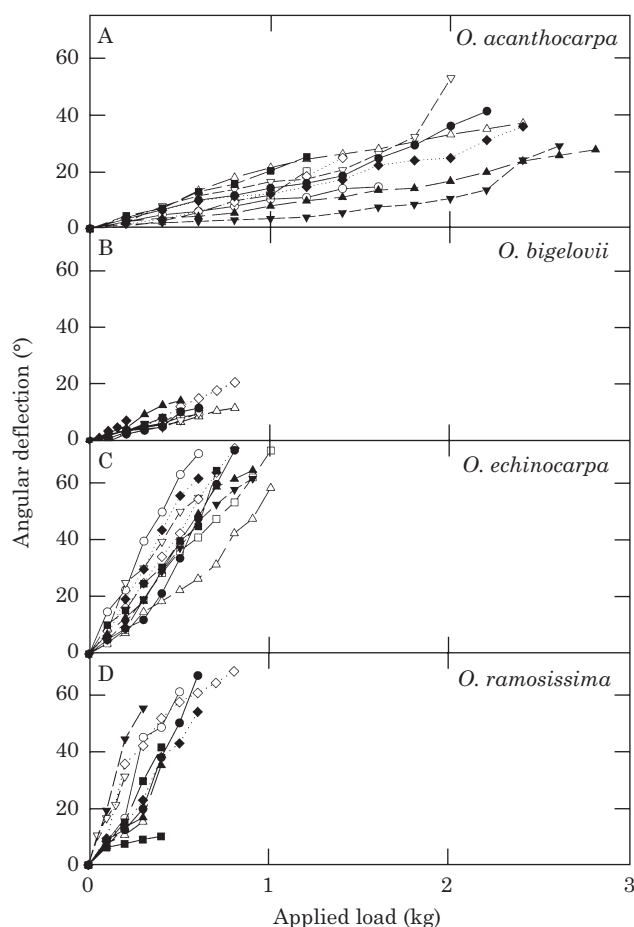
FIG. 1. Relationship between an applied load and the resulting angular deflection for terminal joints of *O. acanthocarpa* (A), *O. bigelovii* (B), *O. echinocarpa* (C) and *O. ramosissima* (D) (*n* = 10 branches for each taxon). Different symbols correspond to different terminal joints.
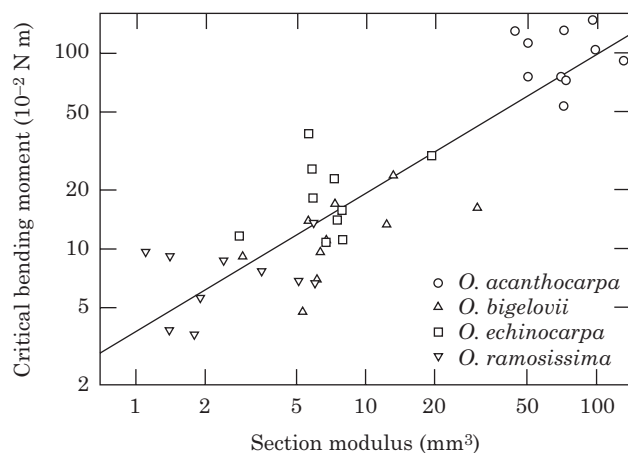

FIG. 2. Relationship between the critical bending moment and the section modulus for terminal joint junctions of *O. acanthocarpa* (○), *O. bigelovii* (△), *O. echinocarpa* (□), and *O. ramosissima* (▽) (*n* = 10 joint pairs for each species). The equation for the regression line is log (critical bending moment) = 3·89 + 0·70 log (section modulus), $r^2 = 0.77$.

(Fig. 3). Parenchyma cells tended to be largest for *O. bigelovii* and smallest for *O. ramosissima*. For instance, in the axial region (Fig. 3), parenchyma cell diameters averaged 12·2 ± 0·6 μm for *O. acanthocarpa*, 14·9 ± 1·3 μm for *O. bigelovii*, 11·0 ± 0·6 μm for *O. echinocarpa*, and 8·4 ± 0·1 μm for *O. ramosissima* (*P* < 0·001, one-way ANOVA).

The lateral cell walls of vessel elements had either helical or helical-reticulate secondary thickenings. The vessel distribution in the metaxylem was similar for the four species, with vessels either solitary or in clusters of usually no more than four. The secondary xylem of *O. acanthocarpa*, *O. echinocarpa* and *O. ramosissima* was ring-porous, usually with only one cycle of larger early-wood vessels adjacent to smaller latewood vessels (Fig. 3A, C, D), whereas the entire secondary xylem of *O. bigelovii* appeared to be diffuse-porous (Fig. 3B). Earlywood vessels of *O. acanthocarpa*, *O. echinocarpa* and *O. ramosissima* and the vessels of *O. bigelovii* were usually solitary or paired, whereas latewood vessels occurred in radial files of three or more. Terminal junctions of *O. acanthocarpa* had the greatest number of vessels per junction: 38 % more than those of *O. bigelovii*, 95 % more than those of *O. echinocarpa*, and 179 % more than those of *O. ramosissima* (Table 4). Vessel diameter for *O. bigelovii* was twice that for *O. ramosissima*, with vessels of *O. acanthocarpa* and *O. echinocarpa* having intermediate diameters. Vessels of *O. bigelovii* also had cell walls that were, on average, 27 % thicker than for the other three species. Vessel elements of *O. acanthocarpa* and *O. bigelovii* were 31 % longer than those of *O. echinocarpa*, on average, and 100 % longer than those of *O. ramosissima* (Table 4).

Libriform fibres, which occurred only in the terminal junctions of *O. acanthocarpa* (Fig. 3A), were tapered and had uniformly thickened lateral walls. The presence of lignin in libriform fibre cell walls varied with location in the junction cross-section; fibres in some regions had lignin throughout their walls, whereas in other regions lignin was present only in the middle lamella, primary wall, and rarely in the outer layers of the secondary wall, thus appearing to be gelatinous. Regions with heavily lignified fibres usually had lignified ray parenchyma. The inner layers of the secondary cell wall of fibres in these regions often appeared to separate from the rest of the wall (Fig. 3A). Fibres occurred in clusters of at least ten, and often completely replaced the axial parenchyma in latewood. Considering the cells in terminal junctions with thickened secondary cell walls, libriform fibres of *O. acanthocarpa* were the longest and most abundant (Table 4).

Vascular tracheids were fusiform with two to six annular secondary thickenings that extended far into the cell lumen. Although absent from the junctions of *O. acanthocarpa*, they were present in the metaxylem and secondary xylem of *O. bigelovii*, *O. echinocarpa* and *O. ramosissima*. For *O. bigelovii*, these cells usually occurred in clusters of two to six in the metaxylem and were either solitary or paired in the rays and in the axial regions of the secondary xylem (Fig. 3B). Vascular tracheids in *O. echinocarpa* and *O. ramosissima* were concentrated in the metaxylem, frequently occurring in clusters of ten or more, being less
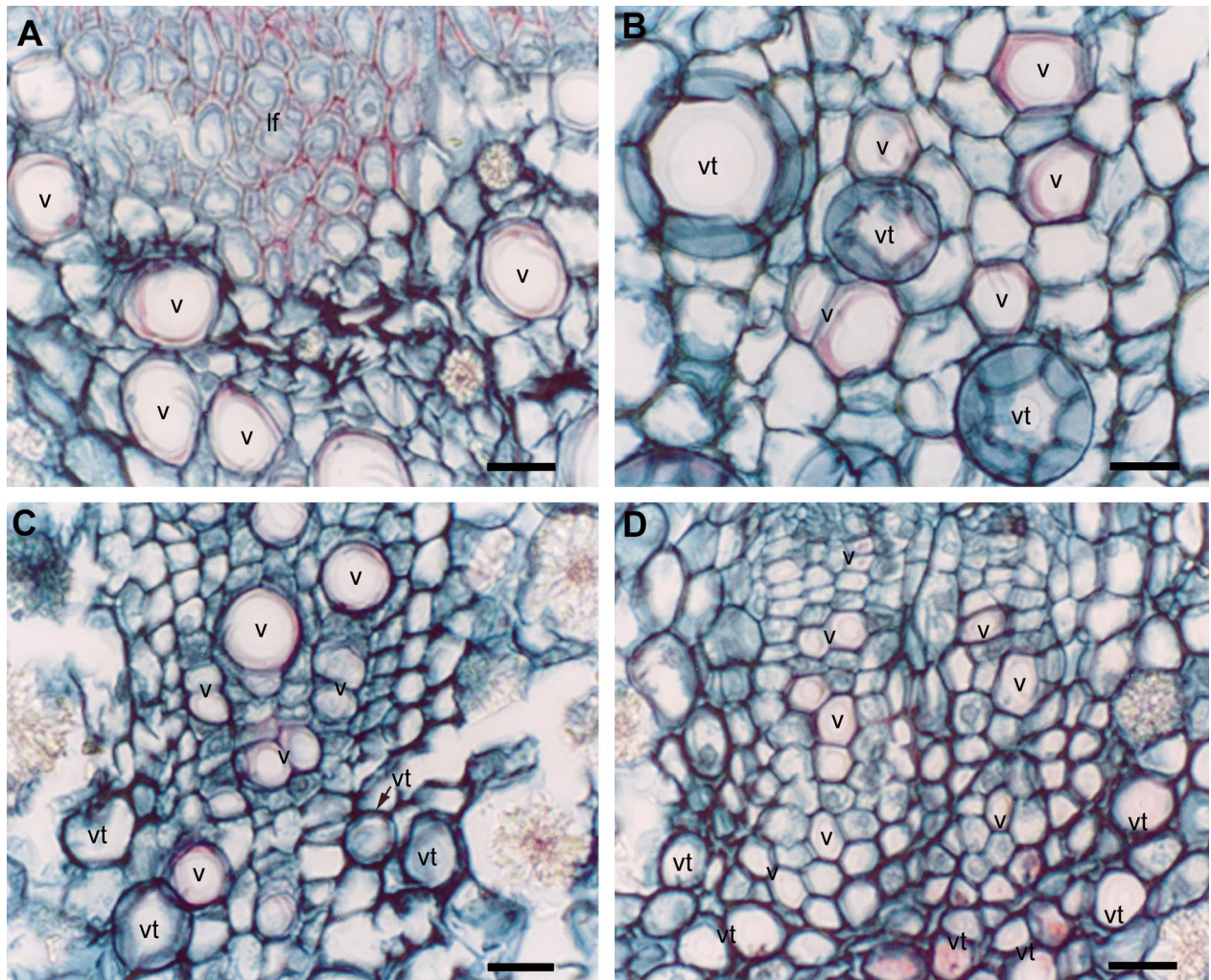
FIG. 3. Photomicrographs of cross-sections of joint junction xylem: A, non-fibrous and fibrous secondary xylem of *O. acanthocarpa* showing earlywood vessels (v) and libriform fibres (lf); B, metaxylem and secondary xylem of *O. bigelovii* showing vessels and vascular tracheids (vt); C, metaxylem and secondary xylem of *O. echinocarpa* showing earlywood vessels and vascular tracheids; D, metaxylem and secondary xylem of *O. ramosissima* showing both earlywood and latewood vessels and vascular tracheids (bars = 20 μm).

common in the ray and axial regions (Fig. 3C and D). Vascular tracheids were most abundant for junctions of *O. ramosissima* and least abundant for those of *O. bigelovii* (Table 4). The diameter, secondary thickenings, and cell length were greatest for vascular tracheids of *O. bigelovii*, averaging 58 % more than for the vascular tracheids of *O. echinocarpa* and 115 % more than for the vascular tracheids of *O. ramosissima* (Table 4).

Rooting of terminal joints

During the first 2 weeks, detached joints of all four species failed to root in the greenhouse (Fig. 4). Half of detached joints rooted within 8 weeks for *O. bigelovii* and at 9 weeks for *O. acanthocarpa*. Rooting was maximal at 14 weeks with *O. acanthocarpa* having 63 % rooted joints, *O. bigelovii* 80 %, *O. echinocarpa* 17 %, and *O. ramosissima* 0 % (Fig. 4). In fact, 68 % of *O. ramosissima* joints died during the observation period.

DISCUSSION

Resistance to mechanical failure for terminal joint junctions of the four cholla species examined generally depended on the section modulus of the joint junctions, which depends on the joint junction diameter cubed. When all four species were considered together, the bending moment causing junction failure increased logarithmically with the section modulus of the junctions. This critical bending moment also increases logarithmically with section modulus for cladode junctions of platyopuntias (Bobich and Nobel, 2001). Similar relationships exist between stem length and stem diameter for columnar cacti (Niklas and Buchmann, 1994; Cornejo and Simpson, 1997; Molina-Freaner *et al.*, 1998; Niklas *et al.*, 1999). Resistance to mechanical failure for cholla joint junctions clearly depends on the section modulus of the junctions for *Opuntia acanthocarpa*, which resisted failure the most, and *O. ramosissima*, which resisted failure the least. However, the section moduli, junction

TABLE 4. *Characteristics of vessel elements, libriform fibres, and vascular tracheids in terminal joint junctions*

Cell type and Characteristic	O. acanthocarpa	O. bigelovii	O. echinocarpa	O. ramosissima	P
Vessel element					
Number per junction	1760 ± 151[a]	1285 ± 131[ab]	908 ± 70[bc]	632 ± 93[c]	<0·001
Diameter (μm)	16·3 ± 0·8[a]	20·8 ± 0·6[b]	13·9 ± 0·8[a]	9·9 ± 0·6[c]	<0·001
Cell wall thickness (μm)	2·23 ± 0·04[a]	2·61 ± 0·09[b]	2·02 ± 0·05[ac]	1·93 ± 0·04[c]	<0·001
Length (μm)	126 ± 3[a]	118 ± 6[a]	93 ± 3[b]	61 ± 5[c]	<0·001
Libriform fibre					
Number per junction	21084 ± 6250	0	0	0	—
Diameter (μm)	11·6 ± 0·7	—	—	—	—
Cell wall thickness (μm)	2·49 ± 0·17	—	—	—	—
Length (μm)	222 ± 16	—	—	—	—
Vascular tracheid					
Number per junction	0	450 ± 144[a]	511 ± 160[ab]	724 ± 85[b]	<0·001
Diameter (μm)	—	37·3 ± 2·5[a]	26·4 ± 2·0[b]	17·1 ± 0·8[c]	<0·001
Cell wall thickness (μm)	—	12·5 ± 0·5[a]	6·39 ± 0·38[b]	5·27 ± 0·09[b]	<0·001
Length (μm)	—	116 ± 4[a]	85 ± 2[b]	61 ± 6[c]	<0·001

Diameters and cell wall thicknesses for vascular tracheids are for the annularly thickened secondary walls. Data are means ± s.e. ($n = 6$ terminal joint junctions for each species). *P* values correspond to one-way ANOVA across each row. Values followed by different superscripts are significantly different ($P < 0·05$) for Tukey pairwise comparisons.

areas, and wood areas of joint junctions did not differ between *O. echinocarpa* and *O. bigelovii*, yet the load causing junction failure and the mean critical bending moment were greater for *O. echinocarpa*, suggesting that factors other than the section modulus affect joint junction strength.

Terminal joint junctions were more flexible for *O. echinocarpa* and *O. ramosissima* than for *O. acanthocarpa* and *O. bigelovii*. By readily deflecting in the direction of the force, the bending moments and stresses in joint junctions of *O. echinocarpa* and *O. ramosissima* would be lessened, which is similar to the effects of wind on leaves with flexible petioles (Vogel, 1994; Nobel, 1999). By minimizing deflection per unit force, the stiffer joint junctions of *O. acanthocarpa* and *O. bigelovii* maximize the applied bending moment for any external force. Stem stiffness is related to xylem accumulation and lignification of stem tissues for woody plants in general (Carlquist, 1975; Niklas,

1992), and for cacti in particular (Niklas and Buchman, 1994; Molina-Freaner *et al.*, 1998; Niklas *et al.*, 2000; Bobich and Nobel, 2001). Even though joints of *O. acanthocarpa* and *O. bigelovii* experienced similar deflections per applied force, they differed greatly in their junction wood cross-sectional areas and their junction anatomies.

Parenchyma cells in the pith, primary rays, vascular rays, and axial parenchyma were generally greater in diameter for *O. bigelovii* than for the other three species. Cell wall deformation for parenchymatous tissue of a given cross-sectional area decreases as the number of parenchyma cells increases (Niklas, 1988, 1992). Thus, the parenchyma of *O. acanthocarpa*, *O. echinocarpa* and *O. ramosissima* should be stronger on a per area basis and contribute more to junction strength than the parenchyma of *O. bigelovii*. The resulting lower junction strength leading to breakage at lower forces for *O. bigelovii* than for the other three species partially explains why its joint junctions have lower critical bending moments than those of *O. echinocarpa* for similar section moduli.

Vessels in the xylem of joint junctions of *O. bigelovii* had thicker cell walls than those of the other three species. Such vessels should therefore aid in resisting deflection more than the vessels of *O. acanthocarpa*, *O. echinocarpa* and *O. ramosissima*, and are probably why *O. bigelovii* joints deflected less under a particular applied force (were less flexible) than those of *O. echinocarpa* and *O. ramosissima*. Vessel elements resist deformation better than parenchyma cells because of their lignified secondary cell wall thickenings (Niklas, 1992). However, vessels were not as abundant in junction cross-sections of the four cholla species as were parenchyma cells. Also, in the terminal cladode junctions of platyopuntias, vessel elements have little influence on junction strength because of their low frequency compared to parenchyma cells (Bobich and Nobel, 2001). Furthermore, the vessel elements of all four species also had helical to helical-reticulate secondary thickenings on their lateral
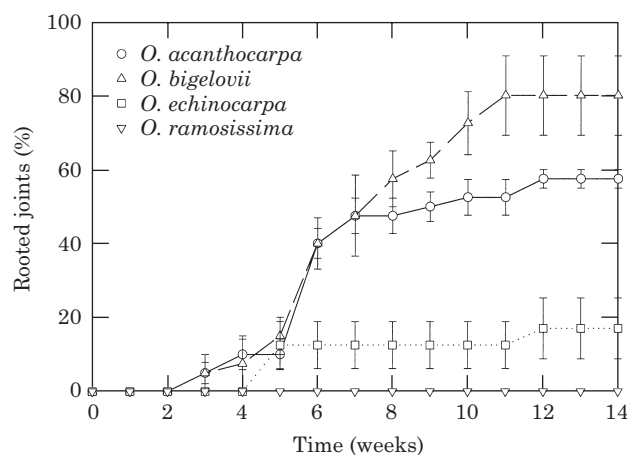

FIG. 4. Time course for rooting of detached terminal joints. Four groups consisting of ten joints each were analysed for each species; data are means ± s.e.

walls, which makes them less rigid than pitted vessel elements and more able to deform axially (Carlquist, 1975; Fahn, 1990).

Vascular tracheids in cacti are believed to function in water storage and cavitation prevention (Mauseth and Plemons, 1995; Mauseth *et al.*, 1995) and probably do not provide much resistance to tensile and compressive forces because their secondary cell wall thickenings are annular (Bobich and Nobel, 2001). However, vascular tracheids probably reduce shearing between cells because the secondary thickenings of adjacent vascular tracheids usually alternate, creating an interlocking of their lateral walls (Bobich and Nobel, 2001). Vascular tracheids, which were absent in terminal junctions of *O. acanthocarpa*, occurred in smaller clusters and were more evenly distributed throughout the metaxylem and secondary xylem for *O. bigelovii* than for the junction xylem of *O. echinocarpa* and *O. ramosissima*, where they occurred primarily in large clusters in the metaxylem. The vascular tracheids in terminal joint junctions should therefore experience less shearing for *O. echinocarpa* and *O. ramosissima* than for *O. bigelovii*.

The greater strength of *O. acanthocarpa* junctions than those of the other three cholla species was due not only to their comparatively larger junction cross-sectional area and section modulus, but also to the presence of libriform fibres, which were absent from the terminal junctions of the other three species. Libriform fibres, which function primarily in support (Fahn, 1990), were long and tapered, occurred in large clusters, and had lignified middle lamellae; about half had lignin throughout the secondary cell wall and the other half were gelatinous. Fibres with lignin present throughout their cell walls are more rigid and would be primarily responsible for resisting stresses resulting from bending moments (Niklas, 1992). Gelatinous fibres with lignin absent from the inner layers of the secondary wall are a characteristic of reaction wood (Fahn, 1990) and apparently function in re-orienting stems vertically (Gartner, 1995). Cladode junctions of shrubby and arborescent platyopuntias also have gelatinous fibres, but unlike the case for *O. acanthocarpa*, terminal cladode junctions are usually fibreless, with gelatinous fibres occurring only in older junctions (Bobich and Nobel, 2001). The large numbers of libriform fibres and the relatively large junction section modulus not only make terminal joint junctions of *O. acanthocarpa* stronger than those of the other three cholla species, but also make it unlikely that these junctions will fail in nature.

Opuntia spines have epidermal cells with pointed tips that project toward the base of the spine (Robinson, 1974; Gibson and Nobel, 1986). Such projecting barbs help the spines of all four species to lodge in the skin or coat of a passing vertebrate, although this ability is lessened for *O. ramosissima* because its barbs do not project away from the spine as far as for the other three species. By having more spines per tubercle area than the other three species, terminal joints of *O. bigelovii* increase the chance that their spines will lodge in the skin or coat of a vertebrate and also allow larger bending moments. Specifically, catching on a spine near the apex allows the length of the joint to act as a moment arm, which increases the bending moment for a given applied force. Spines can also reduce overheating for detached joints lying on the ground. For instance, temperatures of detached joints of *O. bigelovii* in the northwestern Sonoran Desert decrease by 1 °C when joints are 1 cm above the soil surface and by 5 °C when 2 cm above, compared with being on the soil surface (Nobel *et al.*, 1986). The lower spine frequencies of the other three species, especially *O. ramosissima* (which averaged less than one spine per areole), may allow the joint to contact the soil surface, leading to possible overheating. The greater spine frequency of terminal joints of *O. bigelovii* may also allow them to be cooler than joints of the other three species by shading the joint surface (Nobel *et al.*, 1986).

The greatest percentage of detached terminal joints rooted for *O. bigelovii*, followed by *O. acanthocarpa* and *O. echinocarpa*; no detached joints rooted for *O. ramosissima*. The low rooting percentages for joints of *O. echinocarpa* and the absence of rooting for joints of *O. ramosissima* may be due to their thin stems, which probably have low amounts of stored carbohydrates and water. In fact, 67 % of the detached joints of *O. ramosissima* died under relatively mild conditions in the greenhouse. The time required for joints of *O. acanthocarpa*, *O. bigelovii* and *O. echinocarpa* to root was less than the time required for joints of *O. bigelovii* and two varieties of *O. fulgida* to root on the soil surface in the Sonoran Desert in Arizona (Holthe and Szarek, l985). The differences could be due to lower air and soil temperatures and wetter soil conditions in the greenhouse, along with the fact that the joints were collected in the late winter after the plants had been growing under relatively favourable conditions.

Opuntia bigelovii has been widely recognized for its ability to reproduce vegetatively (Benson, 1982; Holthe and Szarek, 1985; Gibson and Nobel, 1986; Nobel *et al.*, 1986) and was the only one of the four species with evidence of extensive vegetative reproduction in the field. The traits possessed by terminal joints of *O. bigelovii* favouring vegetative reproduction are a high spine frequency, biomechanically stiff but weak junctions, and a high rooting ability. None of the other species in this study—*O. acanthocarpa*, *O. echinocarpa*, or *O. ramosissima*—possessed more than two of these traits. Joints of *Opuntia fulgida*, another vegetatively reproducing species, also have weak junctions with little xylem production (Benson, 1982), diffuse-porous secondary xylem (Gibson, 1977), and a high rooting ability (Holthe and Szarek, 1985). Chollas that proliferate mainly using vegetative reproduction must have biomechanical and morphological properties favouring ready detachment as well as the ability to root on the soil surface under harsh desert conditions.

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