

## BIOMECHANICS AND ANATOMY OF CLADODE JUNCTIONS FOR TWO *OPUNTIA* (CACTACEAE) SPECIES AND THEIR HYBRID<sup>1</sup>

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Hybridization between the introduced arborescent *Opuntia ficus-indica* and the native shrubby *O. littoralis* has led to populations, referred to as *O. "occidentalis,"* which form thickets that can dominate hillsides of chaparral and that can survive fires. Because the thickets apparently develop via vegetative reproduction, *O. "occidentalis"* was hypothesized to have a greater ability than its parent species to reproduce vegetatively due to weaker cladode junctions. Of the three taxa, the junctions for *O. "occidentalis"* had the least amount of wood, despite having cladode masses and junction cross-sectional areas similar to those of *O. littoralis*. The cladodes of *O. "occidentalis"* resisted deflection about their junctions the least and their junctions required the least amount of applied mass and the smallest bending moment to fail mechanically. The junction wood for all three taxa consisted mostly of parenchyma, with lesser amounts of cells with thickened secondary cell walls, indicating that some junction strength depended on hydrostatic pressure, especially for terminal junctions. Libriform fibers, which contribute to support and resist bending moments, were about 80% less frequent in the sub-subterminal junctions of *O. "occidentalis"* than in *O. ficus-indica* and *O. littoralis*. Vascular tracheids, which probably reduced shear among cells in the wood, were 90% less frequent in the terminal and sub-subterminal junction wood of *O. "occidentalis"* compared to *O. littoralis*. Thus wood characteristics can account for the weaker junctions of *O. "occidentalis"* compared to those of *O. ficus-indica* and *O. littoralis*, which apparently increases the ability of the hybrid to reproduce vegetatively.

**Key words:** biomechanics; Cactaceae; cladode; cladode junction; hybrid; libriform fibers; *Opuntia*; vascular tracheids; vessel elements.

Branches occur either as monopodial axes formed from the extension of one apical meristem or sympodial axes comprised of shoot units that develop from an axillary bud of a previous unit (Bell, 1991). In the Cactaceae, prominent examples of sympodial axes are found in the genus *Opuntia*, for which photosynthetic branch units are either cylindrical (cylindropuntias) or flattened (platyopuntias; Gibson and Nobel, 1986). A common characteristic of both cylindrical and flattened stem segments, referred to as joints and cladodes, respectively, is that they are smaller in cross section at their bases (Gibson and Nobel, 1986; Nobel and Meyer, 1991). For instance, junctions between cladodes along a branch of *Opuntia ficus-indica* have a cross-sectional area equal to 22% of the area at mid-cladode (Nobel and Meyer, 1991).

A woody dicotyledon branch can resemble a tapered cantilever, with a greater cross-sectional area at the fixed end of the branch compensating for the local increase of tensile and compressive forces when the branch is subjected to applied forces (Mosbrugger, 1990; Niklas, 1992). In platyopuntias, forces due to applied loads are concentrated in relatively small cross-sectional areas of the cladode junctions, which could contribute to a local mechanical weakness and possibly lead to stem failure (Gibson and Nobel, 1986; Nobel and Meyer, 1991). Therefore, the ability of cladode junctions to resist mechanical failure was hypothesized to depend on the cross-sectional area of the junctions. Because detached *Opuntia* stems have the ability to root (Gibson and Nobel, 1986), such mechanical failure of the cladode junctions could facilitate vegetative reproduction (Grant and Grant, 1980; Mandujano, Montaña, and Eguiarte, 1996; Mandujano et al., 1998).

Platyopuntia wood is generally composed of fibrous axial regions produced by fascicular vascular cambium and poorly lignified, or unlignified, vascular rays produced by interfascicular cambium (Gibson, 1976, 1978; Gibson and Nobel, 1986). However, variation occurs in wood characteristics among platyopuntias, especially for the amount of fibers in the axial regions (Gibson, 1978). Furthermore, wood composition in the Cactaceae can change during development (Gibson, 1973; Mauseth and Plemons, 1995), e.g., trunks of *Browningia candelaris* have fibrous wood at maturity while its branches have fibrous wood early in development and nonfibrous wood at maturity (Mauseth, 1993). Preliminary observations have revealed that only wood, pith, and phloem of cladode junctions directly connect the older cladodes to the younger cladodes for *Opuntia ficus-indica*. The preponderance of wood in the cladode junction led to the hypothesis that the constituents of such wood affect branch deflection and the ability of the cladode junctions to resist mechanical failure.

Hybridization has long been recognized among platyopuntias in southern California (Philbrick, 1963; Benson and Walkington, 1965; Goeden, Fleschner, and Ricker, 1967). An interesting case involves the introduced, arborescent, agricultural species *Opuntia ficus-indica* and the native, low, shrubby species *Opuntia littoralis*. Progeny of this hybridization can vary morphologically between putative F<sub>1</sub> hybrids and *O. littoralis* backcrosses and are referred to as *Opuntia "occidentalis"* (Benson and Walkington, 1965; Benson, 1982). In the southern California chaparral where platyopuntias occur, the fire frequency has changed due to human disturbances and the arrival of Mediterranean grasses (Mensing, 1998; Keeley, Fotheringham, and Morais, 1999). Although fire is detrimental to most cacti (Nobel, 1988), populations of *O. "occidentalis"* are often able to survive fires because it forms large thickets due to vegetative reproduction, unlike its putative parents, and only the outer plants are damaged (Gibson and Nobel, 1986). Clad-

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ode detachment caused by failure at cladode junctions and leading to vegetative reproduction may be caused by passing animals, extreme environmental conditions, or extensive self-loading due to biomass accumulation. The greater ability of *O. "occidentalis"* to reproduce vegetatively compared with *O. ficus-indica* and *O. littoralis* was hypothesized to reflect the biomechanical and anatomical properties of the cladode junctions of the three taxa. Also, because different *Opuntia* growth forms have different types of wood and wood development (Gibson, 1978), it is further hypothesized that the hybridization of *O. ficus-indica* and *O. littoralis* may have led to different wood characteristics in *O. "occidentalis."* The present study therefore investigated biomechanical properties and cellular anatomy of the cladode junctions for *O. ficus-indica*, *O. littoralis*, and *O. "occidentalis."* Cellular wood composition, wood development, and measurements of wood elements with secondary cell walls were interpreted with respect to biomechanics and vegetative reproduction for each taxon.

## MATERIALS AND METHODS

**Plant materials and field observations**—Branches of *Opuntia ficus-indica* (L.) Miller (2.4-m-tall plants, accession number 1279 of Texas A & M University, Kingsville, Texas, USA) were studied at the Agricultural Experiment Station, University of California, Riverside, California, USA. Branches of *O. littoralis* var. *vaseyi* (Coulter) Benson and Walkington (0.6-m-tall plants, clone number 3355) and *O. "occidentalis"* (0.7-m-tall plants, clone number 13230) were studied at Rancho Santa Ana Botanic Garden, Claremont, California. The branches used for the biomechanical and morphological measurements consisted of a linear series of four cladodes with three intervening junctions; the youngest to oldest cladode was referred to as first, second, third, and fourth cladode, and the youngest to oldest junction as terminal, subterminal, and sub-subterminal junction. To characterize the vegetative reproduction of *O. "occidentalis"* under natural conditions in the field, observations of the branches of *O. "occidentalis"* were also made on a south-facing slope in Frank G. Bonelli County Park, San Dimas, California, USA. The plants of *O. "occidentalis"* both at Rancho Santa Ana and at Bonelli County Park were similar, having a sprawling habit with prostrate branches and upright branches, the latter rarely having more than three cladodes above the ground. Cladodes were glaucous, had very few spines when young although spination increased with age, and had lengths and widths of  $0.185 \pm 0.006$  and  $0.102 \pm 0.002$  m, respectively, at Rancho Santa Ana and  $0.161 \pm 0.005$  and  $0.096 \pm 0.002$  m, respectively, at Bonelli County Park.

**Biomechanical and morphological measurements**—Deflections caused by incrementally increased masses applied perpendicularly to the face of the younger cladode of each cladode junction were determined for seven branches of each taxon analyzed in the order of terminal, subterminal, and then sub-subterminal junction. The masses that caused failure of each of the junctions were determined. Branches were held in place using two wooden clamps bolted to a rigid scaffold and covered with 10 mm of rubber foam to prevent damaging the cladodes (Fig. 1). The upper clamp was placed 10 mm below the cladode junction being analyzed, with the younger cladode oriented vertically, and the lower clamp was used to help immobilize the branch (Nobel and Meyer, 1991). Forces were applied to the upper cladode using a steel wire attached to a 7-mm-diameter j-bolt that was placed through the cladode center of mass and secured with nuts, washers, and rubber foam padding (Nobel and Meyer, 1991; Fig. 1). Preliminary measurements indicated that the center of mass of the upper cladodes for all three taxa was 40% of the distance from the base to the tip of a cladode. The steel wire passed over a low-friction pulley adjusted so that the applied forces were always perpendicular to the face of the upper cladode (Fig. 1). Linear deflections were read on a scale with millimetre gradations mounted directly above the upper cladode, which had a fine steel needle inserted into its apex (Fig. 1). The deflection angle was calculated as  $\sin^{-1}$  (linear deflection/cladode length; Nobel and Meyer, 1991).

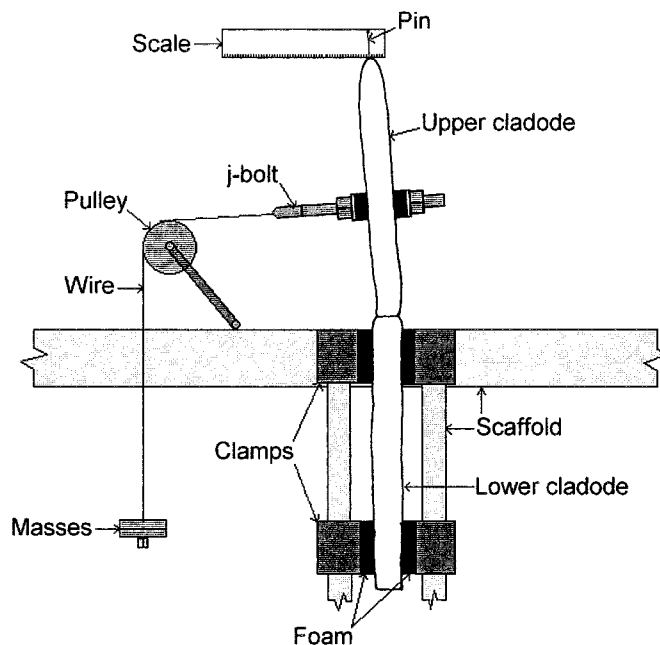


Fig. 1. Schematic of the experimental arrangement used for the biomechanics measurements. Branches had four sequential cladodes with three intervening cladode junctions.

After each junction failure, the length, width, and thickness of each of the upper cladodes as well as of the major and minor axes of the approximately elliptical cladode junctions (which included the wood, pith, phloem, and the areas where the chlorenchyma of the lower and upper cladodes came into contact) were measured. The major and minor axes were also measured for the regions including the wood, pith, and phloem, the wood and pith, and the pith. The bending moments (in N m) were calculated by multiplying the applied force (applied mass  $\times$  gravitational constant)  $\times$  the moment arm ( $0.4 \times$  cladode length). Cladode lengths averaged 0.377 m for *O. ficus-indica* and 0.193 m for the other two taxa. The section modulus ( $I/a$ ), which is the geometric component that describes resistance to the bending moment (Timoshenko and Young, 1962; Niklas, 1992), was calculated from the semi-minor axis ( $a$ , in direction of applied bending moment) and the semi-major axis ( $b$ ) of the junction area that included the wood, pith, and phloem, using the following equation:  $I/a = \pi a^2 b/4$ , where  $I$  is the second moment of area.

**Tissue preparation and cell measurements**—Samples for sectioning were taken from the terminal and the sub-subterminal cladode junctions of four branches for each taxon. After the epidermis, hypodermis, and areoles had been removed with a razor blade, terminal and sub-subterminal junctions were cut into four 90° sectors and eight 45° sectors, respectively. The sectors were then fixed in formalin, acetic acid, and ethyl alcohol (FAA) and dehydrated through a tertiary butanol series (Jensen, 1962). Once embedded in Paraplast Plus (Oxford Labware, St. Louis, Missouri, USA), sections  $\sim 15 \mu\text{m}$  thick were prepared using a rotary microtome with a 12-cm blade (Leica Instruments, Heidelberg, Germany). The sections were stained using safranin as the primary stain and fast green as the counter stain (Jensen, 1962).

Digital images of the cross-sections at  $200\times$  were obtained using an Olympus BH-2 light microscope (Lake Success, New York, USA) and a digital image analysis system (Pixera Corporation, Los Gatos, California, USA). Measurements of cell wall thickness and the cross-sectional area of cells with secondary cell walls were recorded from the images using Image Pro Plus (Media Cybernetics, Silver Spring, Maryland, USA). Mean external cell diameter was calculated for vessels, libriform fibers, and vascular (wide-band) tracheids (Gibson, 1973, 1978; Mauseth, 1995) from the cell cross-sectional area of 150 cells for each junction; mean cell wall thicknesses of these cells were determined from the average of three measurements per cell. The num-

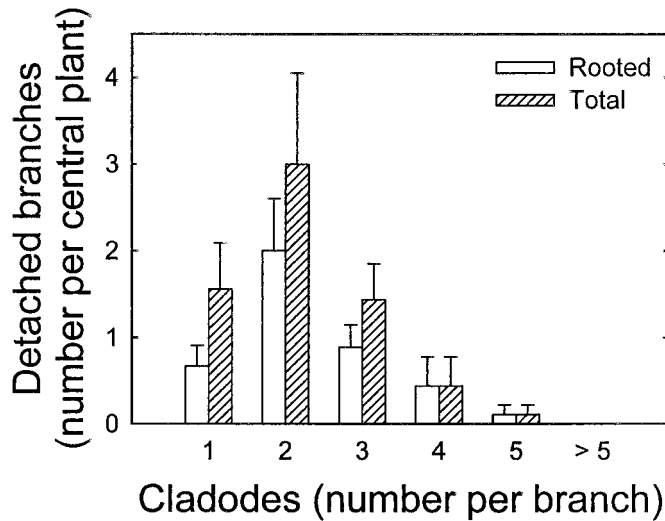


Fig. 2. Frequency of detached branches for *O. "occidentalis"* at Frank G. Bonelli County Park, San Dimas, CA. Data are means  $\pm$  1 SE for the rooted number and the total number of detached branches around the periphery of a central plant ( $N = 9$  central plants).

bers of vessels, libriform fibers, and vascular tracheids per unit area were determined for two entire sectors of terminal junctions and for four entire sectors of sub-subterminal junctions.

The lengths of vessel elements, libriform fibers, and vascular tracheids were determined from macerations of 5-mm-thick tissue samples from four sub-subterminal cladode junctions of each taxon. Tissue samples were immersed in Jeffrey's solution (10% chromic acid and 10% nitric acid) at 40°C (Berlyn and Miksche, 1976). After 24 h, a sample was removed, washed with distilled water, affixed to slides, and stained with safranin. Cell lengths were measured from digital images taken at 30 $\times$  for libriform fibers or at 100 $\times$  for vessel elements and vascular tracheids and recorded using Image Pro Plus.

Statistical analyses were performed using SigmaStat (Jandel Corporation, San Rafael, California, USA). All means are presented with standard errors.

RESULTS

**Field observations**—Under natural conditions in the field, *O. "occidentalis"* increased its ground cover either by spreading, due to prostrate stems, or by the rooting of detached branches around the periphery of a central plant. Each central plant covered  $5.5 \pm 0.8$  m<sup>2</sup> and had an average of  $6.5 \pm 1.4$  detached branches (Fig. 2). Of the detached branches, 46% consisted of two cladodes, while <9% had four or more cladodes (Fig. 2). Detached branches consisting of two or more cladodes were 1.8 times more likely to be rooted than detached single cladodes ( $P = 0.03$ ; Fig. 2); detached branches with four or five cladodes were always rooted (Fig. 2).

**Cladode morphology**—The first (youngest), second, and third cladodes of a branch were 5–6 times greater in mass for the arborescent *O. ficus-indica* than for the frutescent *O. littoralis* and *O. "occidentalis"* (Table 1). The cladode masses for the three cladode positions did not differ between *O. littoralis* and *O. "occidentalis."* The cladode junction cross-sectional area of *O. ficus-indica* was 2.5–4 times larger than for either *O. littoralis* or *O. "occidentalis"* (Table 1). As for cladode masses, cladode junction cross-sectional areas of *O. littoralis* and *O. "occidentalis"* did not differ for any of the three positions. On the other hand, wood cross-sectional area for the junctions differed among the three taxa for all three junction positions, with *O. ficus-indica* having 2.7–3.4 times more wood area per junction than did *O. littoralis* and 4.8–5.7 times more than did *O. "occidentalis"* (Table 1). The ratio of wood area to cladode junction area did not differ between *O. ficus-indica* and *O. littoralis* for any junction position ( $P > 0.05$ ), averaging 0.39 for terminal, 0.48 for subterminal, and 0.56 for sub-subterminal junctions. However, both species had 46% more wood area per junction area in terminal and subterminal junctions and 30% more in sub-subterminal junctions than did *O. "occidentalis"* ( $P < 0.05$ ; Table 1). The section modulus ( $I/a$ ), computed from the region containing the wood, pith, and

TABLE 1. Characteristics of cladodes and cladode junctions. Cladode masses ( $N = 12$ ), junction areas ( $N = 7$ ), the wood areas ( $N = 7$ ), and the section moduli ( $N = 7$ ) for each junction are means  $\pm$  1 SE.  $P$  values correspond to a one-way ANOVA performed for log-transformed data for each row; values followed with different letters across taxa are significantly different ( $P < 0.05$ ) after Tukey pairwise multiple comparisons.

Parameter Position	<i>O. ficus-indica</i>	<i>O. littoralis</i>	<i>O. "occidentalis"</i>	$P$
Mass (kg)				
Cladode				
First (youngest)	1.32 $\pm$ 0.10a	0.251 $\pm$ 0.026b	0.236 $\pm$ 0.031b	<0.001
Second	1.41 $\pm$ 0.12a	0.256 $\pm$ 0.018b	0.259 $\pm$ 0.017b	<0.001
Third	1.83 $\pm$ 0.21a	0.324 $\pm$ 0.025b	0.309 $\pm$ 0.040b	<0.001
Cross-sectional area (10 <sup>-4</sup> m <sup>2</sup> )				
Cladode junction				
Terminal	14.0 $\pm$ 1.1a	4.7 $\pm$ 1.1b	3.8 $\pm$ 0.4b	<0.001
Subterminal	19.6 $\pm$ 1.9a	7.6 $\pm$ 0.6b	6.4 $\pm$ 0.4b	<0.001
Sub-subterminal	37.7 $\pm$ 5.0a	12.2 $\pm$ 1.0b	9.1 $\pm$ 0.8b	<0.001
Wood cross-sectional area (10 <sup>-4</sup> m <sup>2</sup> )				
Cladode junction				
Terminal	5.3 $\pm$ 0.5a	2.0 $\pm$ 0.3b	1.1 $\pm$ 0.2c	<0.001
Subterminal	9.7 $\pm$ 1.1a	3.5 $\pm$ 0.3b	2.2 $\pm$ 0.3c	<0.001
Sub-subterminal	22.7 $\pm$ 3.8a	6.7 $\pm$ 0.8b	4.0 $\pm$ 0.6c	<0.001
Section modulus (10 <sup>-6</sup> m <sup>3</sup> )				
Cladode junction				
Terminal	2.40 $\pm$ 0.40a	0.63 $\pm$ 0.12b	0.23 $\pm$ 0.05c	<0.001
Subterminal	5.75 $\pm$ 0.61a	1.38 $\pm$ 0.22b	0.74 $\pm$ 0.13c	<0.001
Sub-subterminal	19.8 $\pm$ 4.1a	3.31 $\pm$ 0.52b	0.97 $\pm$ 0.14c	<0.001

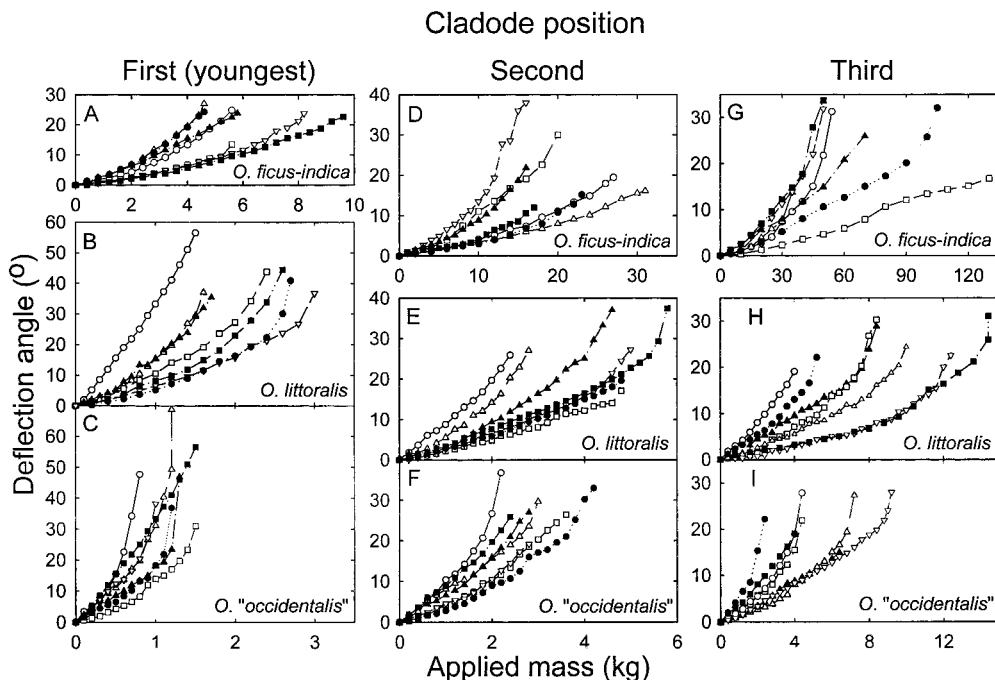


Fig. 3. Effects of applied forces on the angular deflection for upper cladodes adjoining terminal (A–C), subterminal (D–F), and sub-subterminal (G–I) cladode junctions for *O. ficus-indica* (A, D, G), *O. littoralis* (B, E, H), and *O. "occidentalis"* (C, F, I). Seven branches were used for each taxon, designated by different symbols for each branch. Scale intervals on each ordinate are the same for each cladode position, whereas intervals on the abscissa are 3–10 times greater for *O. ficus-indica* than for *O. littoralis* and *O. "occidentalis"*.

phloem, was also greatest for terminal, subterminal, and sub-subterminal junctions of *O. ficus-indica*, being 3.8–6.0 times greater than that of *O. littoralis* and 10–20 times greater than that of *O. "occidentalis"* (Table 1).

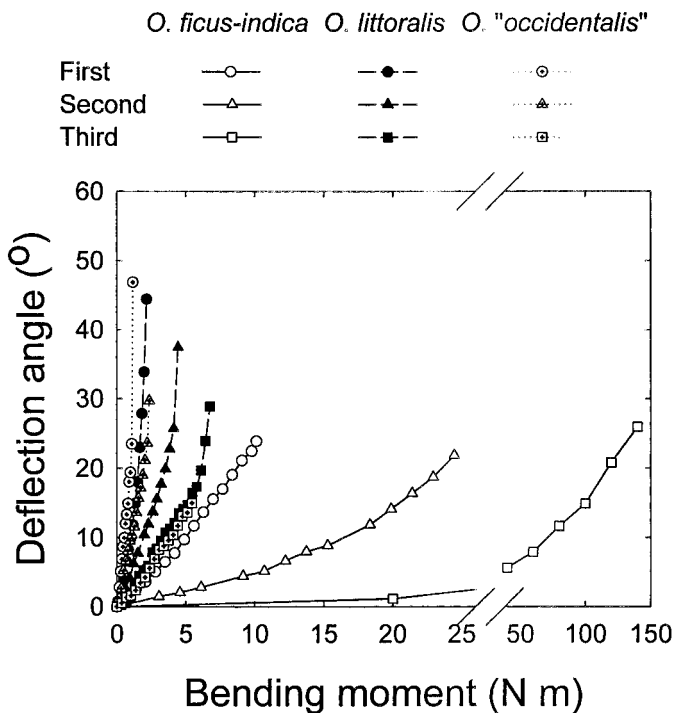


Fig. 4. Effects of bending moments on the angular deflections for first, second, and third cladodes of *O. ficus-indica*, *O. littoralis*, and "*O. occidentalis*." Data for bending moments are for curves with solid triangles (▲) in Fig. 3.

**Biomechanics of cladode junctions**—Cladodes initially tended to have a linear increase in deflection with increasing applied mass followed by a larger deflection per unit applied mass (Fig. 3). Cladodes of *O. ficus-indica* resisted deflection the most, *O. littoralis* was intermediate, and *O. "occidentalis"* resisted deflection the least ( $P < 0.05$ ; Fig. 3). The initial slope of the curve for deflection angle vs. applied mass for first (youngest) cladodes averaged  $2.9^\circ \pm 0.5^\circ/\text{kg}$  for *O. ficus-indica*,  $10.3^\circ \pm 1.7^\circ/\text{kg}$  for *O. littoralis*, and  $21.2^\circ \pm 3.2^\circ/\text{kg}$  for *O. "occidentalis"* (Fig. 3A–C); for second cladodes the slopes were  $0.90^\circ \pm 0.18^\circ/\text{kg}$ ,  $4.8^\circ \pm 0.7^\circ/\text{kg}$ , and  $7.0^\circ \pm 1.0^\circ/\text{kg}$ , respectively (Fig. 3D–F), and for third cladodes they were  $0.30^\circ \pm 0.04^\circ/\text{kg}$ ,  $2.0^\circ \pm 0.4^\circ/\text{kg}$ , and  $3.4^\circ \pm 0.44^\circ/\text{kg}$ , respectively (Fig. 3G–I). First cladodes experienced a greater amount of deflection prior to cladode junction failure for *O. littoralis* and *O. "occidentalis"* (average of  $42^\circ \pm 3^\circ$ ) than for *O. ficus-indica* ( $23^\circ \pm 2^\circ$ ;  $P < 0.05$ ; Fig. 3A–C), but no difference occurred among taxa in deflection prior to junction failure for second or third cladodes (averages of  $26^\circ \pm 2^\circ$  and  $25^\circ \pm 1^\circ$ , respectively; Fig. 3D–I). The amount of angular deflection for a given bending moment decreased from first to third cladodes and was least for *O. ficus-indica*, intermediate for *O. littoralis*, and greatest for *O. "occidentalis"* (Fig. 4). The initial slope of the curve for bending moment vs. angular deflection for first cladodes averaged  $2.3^\circ \pm 0.6^\circ \text{ N}^{-1} \text{ m}^{-1}$  for *O. ficus-indica*,  $15.7^\circ \pm 3.4^\circ \text{ N}^{-1} \text{ m}^{-1}$  for *O. littoralis*, and  $28.1^\circ \pm 5.4^\circ \text{ N}^{-1} \text{ m}^{-1}$  for *O. "occidentalis"*"; for second cladodes the slopes averaged  $0.42^\circ \pm 0.06^\circ$ ,  $6.3^\circ \pm 1.1^\circ$ , and  $9.1^\circ \pm 1.5^\circ \text{ N}^{-1} \text{ m}^{-1}$ , respectively; and

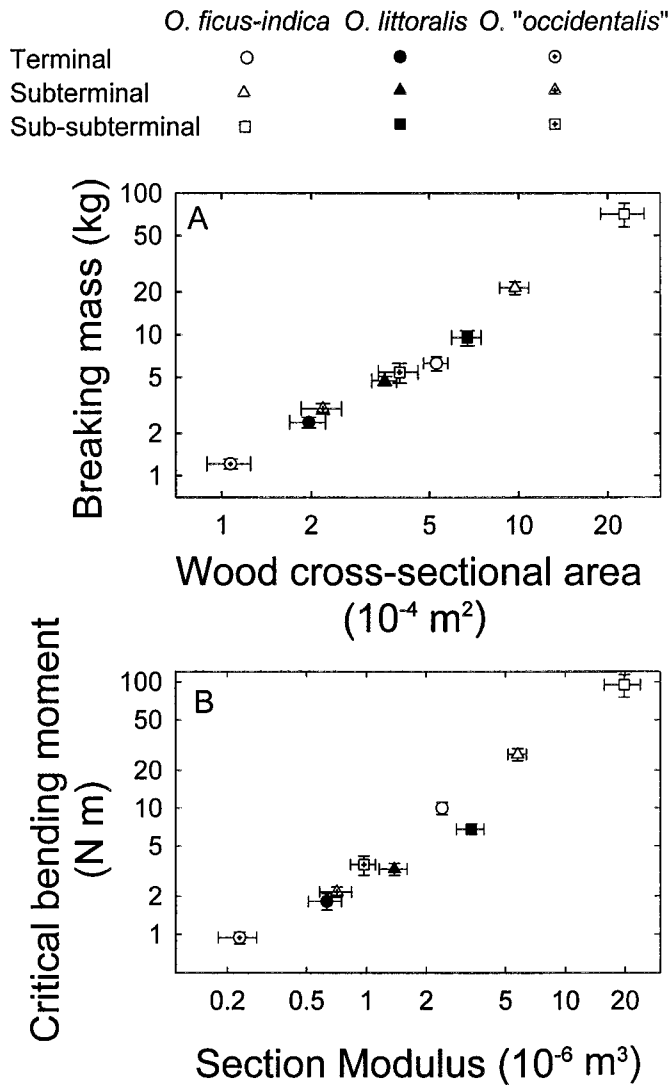


Fig. 5. Log-log relationship between the (A) breaking masses and the mean wood cross-sectional area and (B) the critical bending moment and the section modulus for terminal, subterminal, and sub-subterminal cladode junctions of *O. ficus-indica*, *O. littoralis*, and *O. "occidentalis"*. Data are means  $\pm$  1 SE for both independent and dependent variables ( $N = 7$  branches for each taxon).

for third cladodes the slopes averaged  $0.18^\circ \pm 0.02^\circ$ ,  $2.4^\circ \pm 0.4^\circ$ , and  $4.8^\circ \pm 1.1^\circ \text{ N}^{-1} \text{ m}^{-1}$ , respectively.

The mass causing failure of cladode junctions differed among the three taxa for each junction position (Fig. 5A). The breaking masses for terminal, subterminal, and sub-subterminal junctions of *O. ficus-indica* were 2.6, 4.6, and 7.5 times greater, respectively, than those of *O. littoralis* and 5.3, 7.1, and 13.2 times greater, respectively, than those of *O. "occidentalis"*. Cladode junction breaking masses increased from terminal to subterminal to sub-subterminal junctions within each taxon (Fig. 5A). Such increases in the junction breaking mass reflected increases in the junction wood area, with log (breaking mass in kg) equaling  $-1.98 + 1.46 \log$  (wood area in square metres  $\times 10^{-4}$ ) for *O. ficus-indica* ( $r^2 = 0.89$ ),  $0.28 + 0.97 \log$  (wood area) for *O. littoralis* ( $r^2 = 0.91$ ), and  $0.42 + 0.93 \log$  (wood area) for *O. "occidentalis"* ( $r^2 = 0.83$ ). The critical bending moments (the moment that caused junction

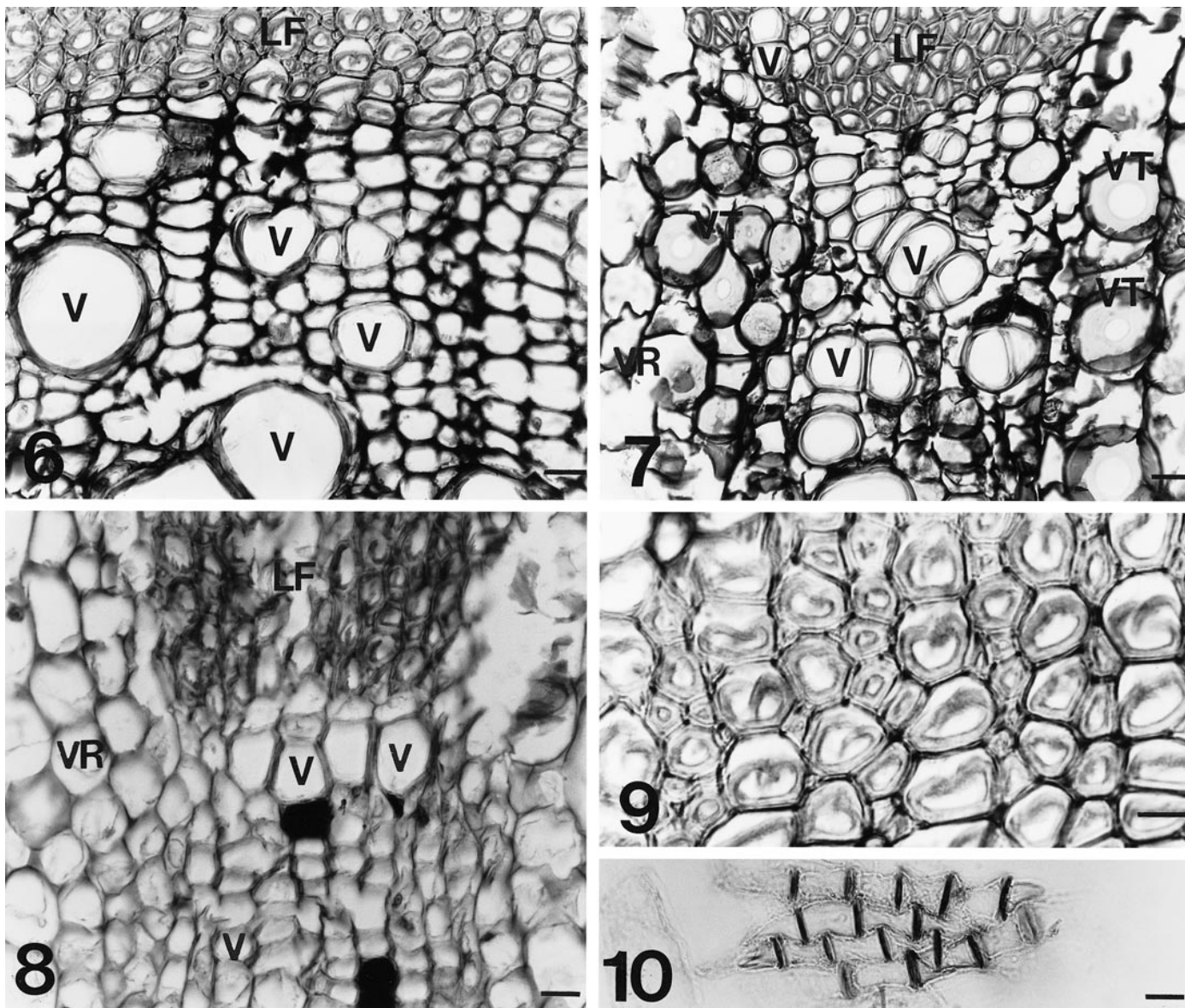
failure) for terminal, subterminal, and sub-subterminal junctions of *O. ficus-indica* were 5.5, 8.2, and 13.8 times greater than for *O. littoralis* and 10.5, 12.4, and 26.6 times greater than for *O. "occidentalis"* (Fig. 5B). There was also a log-log relationship between the critical bending moment and the section modulus, with log (critical bending moment) equaling  $0.75 + 0.96 \log$  (section modulus in  $\text{m}^3 \times 10^{-6}$ ) for *O. ficus-indica* ( $r^2 = 0.88$ ),  $0.69 + 0.74 \log$  (section modulus) for *O. littoralis* ( $r^2 = 0.92$ ), and  $0.89 + 0.54 \log$  (section modulus) for *O. "occidentalis"* ( $r^2 = 0.56$ ; Fig. 5B).

**Cell types and frequencies in cladode junction wood**—The wood of terminal and sub-subterminal cladode junctions for all three taxa consisted primarily of thin-walled parenchyma cells in the rays and the axial xylem; lesser amounts of cells with thickened secondary cell walls occurred in the axial xylem (Figs. 6–8). Cells with secondary cell walls consisted of vessel elements, libriform fibers, and vascular tracheids. Vessel elements differed in distribution and in the type of secondary cell wall thickening according to growth form. For *O. ficus-indica*, the cladode junction wood was diffuse-porous with primarily solitary or paired vessels (Fig. 6). Secondary cell wall thickenings for vessel elements of its metaxylem and early secondary xylem were helical, whereas in later formed wood its vessel elements had reticulate wall thickenings. The cladode junction wood for *O. littoralis* and *O. "occidentalis"* was ring-porous and the vessel elements were arranged in radial files (Figs. 7 and 8). The secondary cell wall thickenings of the earlywood vessel elements were primarily reticulate and always stained positively for lignin, whereas their wall thickenings for latewood vessel elements were primarily helical and approximately half stained positively for lignin.

For the three taxa, libriform fibers occurred as clusters of ten or more cells (Figs. 6–8). Unlike the secondary wall thickening in vessel element walls, libriform fibers appeared to have uniformly thickened secondary walls. The middle lamellae of fibers stained positively for lignin and the primary cell wall stained somewhat, but the inner layers of the secondary wall did not stain for lignin and were often separated from the rest of the cell wall (Fig. 9).

Vascular tracheids occurred only in the wood of *O. littoralis* and *O. "occidentalis"*. These cells were fusiform, having two to six annular secondary thickenings along their length, and were arranged in radial files bordering the metaxylem, in the axial xylem, and adjacent to the axial xylem in the rays (Fig. 7). The vascular tracheids in the rays had a greater diameter than did those of the axial xylem. Adjacent vascular tracheids often had an interlocking appearance due to the positions of the annular bands, which usually alternated (Fig. 10).

The average number of cell types per square millimetre differed between terminal and sub-subterminal cladode junction wood. Vessel frequencies in junction wood decreased 30–45% from terminal to sub-subterminal junctions (Table 2). Libriform fibers were absent in terminal junction wood for *O. ficus-indica* and nearly absent for *O. littoralis* and *O. "occidentalis"* (Table 2). The libriform fiber frequency in sub-subterminal junction wood was 5 times greater than the vessel frequency for *O. ficus-indica*, about the same for *O. littoralis*, but only one-third as much for *O. "occidentalis"* (Table 2). Vascular tracheids were absent from terminal and sub-subterminal junctions of *O. ficus-indica* (Table 2). Vascular tracheid frequency increased 114% from terminal to sub-subterminal junction wood for *O. littoralis* but decreased 62% for *O. "occidental-*



Figs. 6–10. Cladode junction wood. Figs. 6–8. Representative cross sections of the peripheral wood of sub-subterminal cladode junctions (bars = 40  $\mu\text{m}$ ). 6. Diffuse-porous wood of *O. ficus-indica* showing vessels (v), libriform fibers (lf), and unlignified axial parenchyma. 7. Ring-porous wood of *O. littoralis* showing latewood vessels, libriform fibers, vascular tracheids (vt), and vascular rays (vr). 8. Ring-porous wood of *O. "occidentalis."* 9. Cross section of libriform fibers of *O. ficus-indica* showing lignified middle lamellae (bar = 10  $\mu\text{m}$ ). 10. Vascular tracheids from a tissue maceration of *O. littoralis* displaying an interlocking pattern due to annular secondary thickenings (bar = 10  $\mu\text{m}$ ).

is," reflecting the lack of vascular tracheids in the outer half of sub-subterminal junctions of *O. "occidentalis."* Comparisons among the three taxa showed that *O. ficus-indica* averaged only 42% as many vessels per square millimetre in terminal and sub-subterminal junctions as the other two taxa (Table 2). However, libriform fibers in sub-subterminal junctions of *O. ficus-indica* were 2.0 times more frequent than for *O. littoralis* and 7.0 times more frequent than for *O. "occidentalis"* (Table 2). Vascular tracheids in terminal junctions and sub-subterminal junctions were common only for *O. littoralis* (Table 2).

**Cell dimensions in cladode junction wood**—The most frequent vessel diameter class for terminal and sub-subterminal

cladode junctions was 30–35  $\mu\text{m}$  for *O. ficus-indica*, whereas diameters were most frequent under 20  $\mu\text{m}$  for *O. littoralis* and *O. occidentalis* (Fig. 11). However, the average vessel diameter did vary between terminal and sub-subterminal junctions for all three taxa. For terminal junctions of *O. ficus-indica*, the average vessel diameter was 33  $\mu\text{m}$ , increasing 29% for sub-subterminal junctions (Fig. 11). Vessels of *O. littoralis* had an average diameter of 25  $\mu\text{m}$  for terminal junctions, increasing 15% for sub-subterminal junctions (Fig. 11). For *O. "occidentalis,"* vessel diameters averaged 22  $\mu\text{m}$  in terminal junctions, increasing 15% for sub-subterminal junctions (Fig. 11). The other measured dimensions for vessel elements, libriform fibers, and vascular tracheids did not vary between terminal and sub-subterminal junctions.

TABLE 2. Frequency of cells with thickened secondary cell walls in cladode junctions. Two entire sectors were examined for each terminal junction, and four sectors were examined for each sub-subterminal junction. Data are means  $\pm$  1 SE for terminal and sub-subterminal junctions ( $N = 4$  branches per taxon).  $P$  values correspond to a one-way ANOVA performed for each row; values followed with different letters across taxa are significantly different ( $P < 0.05$ ) after Tukey pairwise multiple comparisons. Vascular tracheid frequencies for sub-subterminal junctions were square root-transformed prior to ANOVA.

Cladode junction Cell type	Cell frequency (no./mm <sup>2</sup> )			$P$
	<i>O. ficus-indica</i>	<i>O. littoralis</i>	<i>O. "occidentalis"</i>	
Terminal junction				
Vessels	13.3 $\pm$ 1.4a	27.5 $\pm$ 4.3ab	34.5 $\pm$ 5.5b	0.015
Libriform fibers	0.0 $\pm$ 0.0a	1.1 $\pm$ 0.6a	3.9 $\pm$ 2.9a	0.291
Vascular tracheids	0.0 $\pm$ 0.0a	18.5 $\pm$ 1.6b	2.9 $\pm$ 1.5a	<0.001
Sub-subterminal junction				
Vessels	8.4 $\pm$ 1.3a	19.3 $\pm$ 2.9b	19.1 $\pm$ 1.2b	0.005
Libriform fibers	40.8 $\pm$ 3.1a	22.1 $\pm$ 3.7b	6.2 $\pm$ 1.2c	<0.001
Vascular tracheids	0.0 $\pm$ 0.0a	39.5 $\pm$ 6.3b	1.1 $\pm$ 0.4a	<0.001

Dimensions of vessel elements and of libriform fibers were largest for *O. ficus-indica*. In particular, length, diameter, and cell wall thickness for vessel elements of *O. ficus-indica* were 35–50% greater than for *O. littoralis* and *O. "occidentalis"* (Table 3). Length of libriform fibers was 37% greater for *O. ficus-indica* than for *O. littoralis* and *O. "occidentalis,"* while their diameter was 15% greater (Table 3). Cell wall thickness

of libriform fibers did not differ among the three taxa (Table 3). The length and diameter of vascular tracheids and the radial extent of the annular thickenings did not differ between *O. littoralis* and *O. "occidentalis"* (Table 3). Of the three cell types, libriform fibers had the greatest length, averaging 3.0 times longer than vessel elements and 3.5 times longer than vascular tracheids.

DISCUSSION

Because wood is the stiffest of any axial plant material and the volume fraction of wood positively correlates with stem stiffness in other cacti (Niklas, 1993; Molina-Freaner, Tinoco-Ojanguren, and Niklas, 1998), any increases in cladode junction wood production should increase the section modulus, and, consequently, the junction strength for *O. ficus-indica*, *O. littoralis*, and their hybrid *O. "occidentalis."* Cladode mass, junction cross-sectional area, the wood cross-sectional area, and the section modulus of such junctions were greater for the arborescent *O. ficus-indica* than for the frutescent *O. littoralis* and *O. "occidentalis."* This is consistent with the trend for platyopuntias that plant height is proportional to wood accumulation (Gibson, 1978). Although cladode mass and junction area did not differ between *O. littoralis* and *O. "occidentalis,"* the junction wood area averaged 70% greater and the section modulus averaged 210% greater for *O. littoralis* than those for *O. "occidentalis."* Also, the ratios of wood area to junction area for *O. ficus-indica* and *O. littoralis*, while similar to each other, were greater than the ratios for *O. "occidentalis,"* again indicating that the hybrid produced less junction wood and thus had a smaller region resisting bending moments.

Many allometric relationships describing plant structure are logarithmic (Niklas and Buchman, 1994; Niklas, 1997), as are the relationships between the applied breaking mass of cladode junctions and the junction wood area, and the critical bending moment and the section modulus for the three platyopuntias considered. The increase for log (breaking mass) with log (wood area) averaged 54% greater for *O. ficus-indica* than for *O. littoralis* and *O. "occidentalis";* the increase for log (critical bending moment) with log (section modulus) for *O. ficus-indica* averaged 29% greater than for *O. littoralis* and 78% greater than for *O. "occidentalis."* Not only was the resistance to junction failure correlated with plant height but also the junction of the hybrid did not increase in strength as much with age as did the junctions of its parents. The resistance to angular deflection of cladodes depended on junction wood area, with *O. ficus-indica* deflecting the least per applied mass

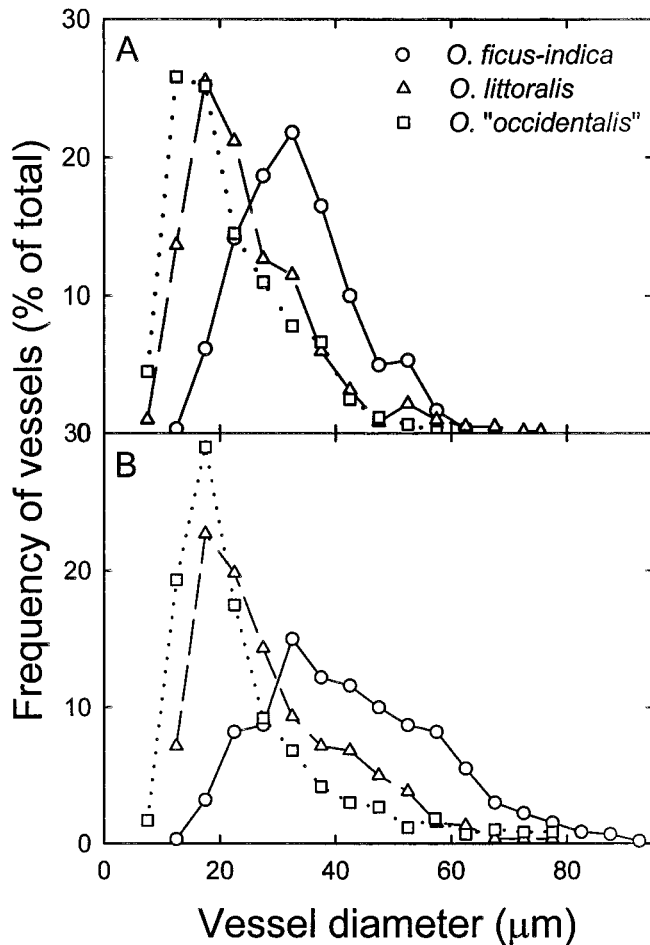


Fig. 11. Vessel diameters for (A) terminal and (B) sub-subterminal cladode junctions of *O. ficus-indica*, *O. littoralis*, and *O. "occidentalis."* For each junction, 150 vessels were measured and then placed into 5- $\mu$ m-wide size classes ( $N = 4$  branches for each taxon).

TABLE 3. Characteristics of wood in sub-subterminal cladode junctions. Diameters and cell wall thicknesses for vascular tracheids, which were absent for *O. ficus-indica*, are for the annular secondary thickenings. Data are means  $\pm$  1 SE ( $N = 4$  branches per taxon; 150 cells were examined for each branch).  $P$  values either correspond to a one-way ANOVA (for vessel elements and libriform fibers) or a  $t$  test (for vascular tracheids) performed for each row; values followed with different letters across taxa are significantly different ( $P < 0.05$ ) after pairwise comparisons.

Xylem cell Feature	Cell dimensions ( $\mu\text{m}$ )			$P$
	<i>O. ficus-indica</i>	<i>O. littoralis</i>	<i>O. "occidentalis"</i>	
Vessel elements				
Length	194 $\pm$ 6a	142 $\pm$ 5b	152 $\pm$ 6b	0.001
Diameter	42.9 $\pm$ 1.8a	28.6 $\pm$ 1.4b	24.7 $\pm$ 1.6b	0.001
Wall thickness	3.5 $\pm$ 0.1a	2.8 $\pm$ 0.1b	2.5 $\pm$ 0.1b	0.002
Libriform fibers				
Length	592 $\pm$ 17a	424 $\pm$ 17b	439 $\pm$ 11b	<0.001
Diameter	20.5 $\pm$ 0.4a	17.0 $\pm$ 0.6b	17.0 $\pm$ 0.8b	0.004
Wall thickness	3.9 $\pm$ 0.1a	3.3 $\pm$ 0.1a	3.5 $\pm$ 0.2a	0.059
Vascular tracheids				
Length	—	112 $\pm$ 6a	135 $\pm$ 8a	0.063
Diameter	—	33.7 $\pm$ 1.1a	43.0 $\pm$ 4.8a	0.093
Annular wall thickness	—	11.8 $\pm$ 0.3a	12.9 $\pm$ 0.8a	0.234

and per bending moment, and *O. "occidentalis"* deflecting the most. Increases in junction wood area and, consequently, section modulus from terminal to sub-subterminal junctions influenced the angle of cladode deflection prior to junction failure for *O. littoralis* and *O. "occidentalis"* but not for *O. ficus-indica*.

Cellular contributions to stem strength can change with the age of the wood, especially for polymorphic woods in the Cactaceae (Gibson, 1973, 1978; Mauseth and Plemons, 1995). The wood of terminal, subterminal, and sub-subterminal cladode junctions for the three taxa consisted mostly of parenchyma, suggesting that the strength of the junctions depended on the hydrostatic pressure of the parenchyma cells (Niklas, 1992). The contribution of the parenchyma apparently decreased from terminal junctions to sub-subterminal junctions because the proportion of cells with secondary cell wall thickenings increased with junction age, especially for *O. ficus-indica* and *O. littoralis*. A similar cellular phenomenon may explain the stiffening of wood in *Carnegiea gigantea* as plants increase in height (Niklas and Buchman, 1994). The ability to resist junction failure appeared to correlate with wood type, with the diffuse-porous wood of *O. ficus-indica* being more resistant than the ring-porous woods of *O. littoralis* and *O. "occidentalis."* However, these differences are more likely dependent on the properties of the cell types present rather than on the size and distribution of the vessels in the wood.

Libriform fibers contribute to support and resist bending moments (Fahn, 1990). Although libriform fibers were virtually absent from terminal junctions of *O. ficus-indica*, *O. littoralis*, and *O. "occidentalis,"* additional biomass due to the production of daughter cladodes probably was a signal for fiber production (Gibson, 1978), explaining the occurrence of the fibers in their sub-subterminal junctions. Sclerenchyma, which often includes libriform fibers, is more resistant to elastic deformation than is parenchyma (Niklas, 1993). Thus libriform fibers in sub-subterminal junctions can support the mass of the cladodes distal to the junction with a smaller amount of area than would parenchyma. As in other platyopuntias, libriform fibers for *O. ficus-indica*, *O. littoralis*, and *O. "occidentalis"* were longer than the two other cell types investigated (vessel elements and vascular tracheids). The greater length of libriform fibers is due to intrusive growth (Gibson, 1978), which creates extra contact between cell walls

in the clusters of fibers. This cell wall contact, along with the lignification of the middle lamellae, prevents the fibers from shearing past each other, making the clusters of fibers act as a unit when resisting bending moments. Moreover, secondary cell walls of libriform fibers were thickened along the length of a cell, indicating that fiber cell walls were more resistant to deformation due to tensile or compressive forces than if the walls were reticulately, helically, or annularly thickened (Carlquist, 1975). Therefore, the properties of libriform fibers indicate that the greater their frequency in the junction wood, the stronger the wood, as is shown by the greater resistance of *O. ficus-indica* and *O. littoralis* compared with *O. "occidentalis."*

Reaction wood in stems forms in response to a gravitational stimulus and acts to orient them vertically (Fahn, 1990; Gartner, 1995). A distinguishing feature of reaction wood in dicotyledons, which is referred to as tension wood, is the presence of gelatinous fibers, which have an inner gelatinous layer in the secondary cell wall that has little or no lignin and that often separates from the rest of the wall during sectioning (Satiat-Jeunemaitre, 1986; Fahn, 1990). Because the libriform fibers in the junction wood of the three taxa investigated had these characteristics and cladodes are rarely perfectly vertical, the libriform fibers most likely aid in orienting cladodes vertically and the junction wood of these three taxa is apparently tension wood. However, further research is necessary to describe the development of reaction wood in cladode junctions of platyopuntias.

The contributions of vessel elements and vascular tracheids to junction strength are less apparent than the contributions of libriform fibers. The strength of vessel elements usually increases with increasing secondary cell wall thickening (Carlquist, 1975). Thus the vessel elements of *O. ficus-indica*, which were larger and had greater thickening of the secondary cell walls, should be stronger than those of *O. littoralis* and *O. "occidentalis."* However, vessel elements are probably weaker than libriform fibers because the amount of cell wall thickening per lumen area for vessel elements is less than that of libriform fibers.

Vascular tracheids are common in shrubby and caespitose forms of the Cactaceae, but are absent in taller growth forms (Gibson, 1973, 1978). In polymorphic woods they are seldom produced after the appearance of fibers (Gibson, 1978; Mau-

seth and Plemons, 1995), suggesting that they do not provide much support. Because vascular tracheids interlock due to their annular secondary thickenings, they may provide resistance against shearing stresses resulting from bending forces in the junction, preventing vascular tracheids and adjacent parenchyma from sliding past each other. Vascular tracheids are also located close to the center of the junction where shearing stresses are maximal (Niklas, 1992). The greater frequency of vascular tracheids in the junction wood of *O. littoralis* than in that of *O. "occidentalis"* may then explain why, along with greater wood area, *O. littoralis* cladodes showed greater resistance to angular deflection about their respective junctions than did those of *O. "occidentalis."*

Vegetative reproduction by the rooting of detached branches can increase the ground area covered by platyopuntias (Grant and Grant, 1980; Gibson and Nobel, 1986; Mandujano et al., 1998). For *O. "occidentalis,"* detached branches with two to five cladodes rooted more often than single cladodes; the larger branches probably had more water and stored carbohydrates, which could enable them to survive drought better. Only 9% of detached branches had four or more cladodes, indicating that failure for junctions older than sub-subterminal junctions is unlikely. The ability to form large thickets by vegetative reproduction gives *O. "occidentalis"* a selective advantage over its putative parents, because these thickets are able to survive the fires in the chaparral of southern California (Benson and Walkington, 1965; Gibson and Nobel, 1986). Although there are many morphological variants as a result of the repeated hybridization between the introduced arborescent *O. ficus-indica* and the native shrubby *O. littoralis*, the hybrid types believed to dominate the chaparral are backcrosses with *O. littoralis* (Benson and Walkington, 1965). Because *O. littoralis* and the *O. "occidentalis"* of this study were similar in cladode mass and junction area and both taxa had ring-porous wood, this *O. "occidentalis"* probably represents much of the platyopuntia hybrid population in the local chaparral.

Although morphological and anatomical evidence describes how *O. "occidentalis"* junctions can fail mechanically, what causes the junction to fail in nature is not fully known. Perturbations by coyotes and small mammals of the region may cause junction failure. Wind has been previously shown to have little effect on the deflection of *O. ficus-indica* (Nobel and Meyer, 1991), but wind effects may be different for lower growth forms of platyopuntias that have weaker junction wood. The most likely junction failure may occur when a branch or individual cladode is at a large angle from the vertical, providing a bending moment due to gravity that is large enough to cause stress fractures in the wood, thus gradually weakening the wood until it either fails under its own mass or by other forces.

In summary, the traits of the cladode junction wood of *O. "occidentalis"* caused their junctions to be weaker than those of *O. ficus-indica* and *O. littoralis*. These traits included a decreased amount of wood compared to junctions of *O. ficus-indica* and *O. littoralis*. The junction wood of *O. "occidentalis"* also had fewer libriform fibers, cells that offer the most support and resistance to bending moments, than both parents and fewer vascular tracheids than *O. littoralis*, which probably decreased the amount of shear between cells in the wood. These traits, which could be deficiencies for the hybrid in another environment, probably have contributed to the enhanced ability of *O. "occidentalis"* to form dense thickets in the local chaparral compared to its parents, *O. ficus-indica* and *O. littoralis*.

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