BOT. GAZ. **144**(3):371–375. 1983.

0006-8071/83/4403-0009$02.00

THE ANATOMICAL BASIS FOR HYGROSCOPIC MOVEMENT IN PRIMARY RAYS OF DAUCUS CAROTA SSP. CAROTA (APIACEAE)

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Polarization microscopy was used to examine the cause of hygroscopic movement in umbellet peduncles, or primary rays, of *Daucus carota* (Apiaceae). Net microfibril orientation of thick-walled parenchyma cells is perpendicular to the long axis of the cell. When the relative humidity rises, these parenchyma cells elongate. In contrast, net microfibril orientation of sclerified fibers is parallel to the long axis of the cell. These fibers do not elongate with increasing relative humidity. Because thick-walled parenchyma cells predominate in the abaxial half of a ray and fibers in the adaxial half, and because the long axis of both cell types is parallel to that of the whole ray, the ray bends adaxially with increasing relative humidity and abaxially with decreasing relative humidity.

Introduction

Hygroscopic movement, which is caused by the absorption and loss of water with changes in relative humidity, influences propagule dispersal in a number of plant species (KERNER VON MARILAUN 1895; HABERLANDT 1914; FITTING et al. 1921). LACEY (1980) showed that such movement in the primary rays of *Daucus carota* ssp. *carota* L. influences both the spatial and temporal patterns of seed dispersal. These primary rays are peduncles of the umbellets that constitute the umbel, or fruit cluster. When the relative humidity falls, the rays open and expose the fruits to dispersal agents; when the humidity rises, these rays close around the mature fruits, preventing dispersal. RICOME (1898) and FUNK (1913) described the anatomy of these rays and the asymmetrical distribution of cell types in the rays of living plants; however, neither mentioned hygroscopic movement, which begins only after a plant dies. THELLUNG (1926) first reported that hygroscopic movement occurs, but he did not examine the anatomy. Because the anatomical basis for hygroscopic movement will augment our understanding of dispersal patterns in *D. carota,* we examined the ray anatomy in this subspecies.

ZIMMERMAN (mentioned by HABERLANDT [1914]) first proposed that hygroscopic movement in a cell could be explained by the orientation of cellulose microfibrils in the cell wall; the absorption of water causes the cell to enlarge in a direction perpendicular to the predominant axis of the microfibrils. Specifically, we explored the application of ZIMMERMAN's hypothesis to *D. carota.* We asked (1) whether ray cells expand and contract perpendicular to the predominant orientation of the microfibrils, (2) whether the net microfibril orientation in cells on the abaxial side of the ray differs from the net orientation in adaxial cells, (3) what the differences in orientation are, and (4) when these proposed differences arise during plant development.

Manuscript received October 1982; revised manuscript received March 1983.

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Material and methods

We examined both cross and longitudinal sections of more than 30 dead rays and fresh rays at different developmental stages. Specimens either were sectioned by hand or were embedded in paraffin and then sectioned with a microtome (SASS 1958). Because preliminary studies showed that most, if not all, of the bending occurs in the distal half of those primary rays on the periphery of the umbel (fig. 1), data from only distal halves of these rays are presented. We examined all sections through an Olympus research microscope (VANOX) equipped with bright-field, polarizing, and first-order red plate optics. The polarizing and red plate optics were used to determine the net microfibril orientation (PRESTON 1974).

In addition, we isolated individual cells from the adaxial and abaxial parts of the ray by macerating rays in an equal mixture of 10% chromic and 10% nitric acids for 2–4 h. The cells were then placed on a microscope slide covered with a thin layer of Tween-20, which reduced the adhesion of the cells to the slide. Special attention was given to the parenchyma cells that predominate in the abaxial half of the ray and the sclerified fibers that predominate in the adaxial half. After placing the cells under the microscope, we determined their net microfibril orientation and their direction of hygroscopic movement by blowing alternately moist (mouth open wide) and dry (mouth narrow) air on them.

Results

Sclerified fibers, whose long axis paralleled that of the ray, were always found in the vascular bundles of dead rays (fig. 2) and rays with partially

developed fruits (fig. 3). On the adaxial side, they were also abundant in interfascicular areas (figs. 2, 3). Under polarizing light, the walls of these cells appeared somewhat birefringent in cross section. In longitudinal section, they appeared very birefringent when oriented at a 45° angle to the polarizing filters (fig. 4). When these sections were additionally oriented at 90° to the red plate, the fibers appeared bright yellow (fig. 4), which indicated that the net direction of microfibrils in the fiber walls closely paralleled the long axis of the cell and, thus, the whole ray.

The abaxial side of these same ray sections comprised mostly shorter but broader thick-walled parenchyma cells whose long axis also paralleled that of the ray (figs. 2, 3). The walls of these cells were highly birefringent in cross section but only in areas where the wall was 45° to both polarizing filters. This would be expected if the predominant microfibril orientation was perpendicular or almost perpendicular to the long axis of the cell. In longitudinal section, the parenchyma cells appeared uniformly bright when oriented at 45° (fig. 4). The red plate indicated that the predominant direction of the microfibrils in these cells was perpendicular to the long axis of the cell and to the whole ray. Thus, when viewed with yellow fibers, the parenchyma cells appeared blue (fig. 4).

The differences in microfibril arrangement that we observed in both dead rays and living rays subtending developing fruits were not apparent in rays taken from umbels at anthesis. At this developmental stage the walls of both fibers and parenchyma cells were thinner (fig. 5) and showed little birefringence in longitudinal section (fig. 6). Most of the birefringence was found in the vascular tissue, e.g., the xylem. Our observations indicate, therefore, that additional cell wall is deposited throughout the ray after anthesis and that this additional material is associated with the appearance of the asymmetrical birefringence pattern of the ray as a whole.

Not all parenchyma cells undergo additional wall thickening after anthesis. During fruit development, the walls of parenchyma cells on the adaxial side remained thin and also appeared very irregular (fig. 3). These same cells were absent from cross sections of dead rays (fig. 2). Thus, while many of the parenchyma cells on the abaxial side thicken during fruit development and provide structural support for dead rays, those on the adaxial side collapse.

Isolated parenchyma cells from the abaxial half of dead rays always expanded in a direction parallel to the long axis of the cell (fig. 7) and perpendicular to the net microfibril orientation when we blew moist air on them. Isolated fibers, on the other hand, showed no detectable longitudinal or lateral expansion (fig. 8).

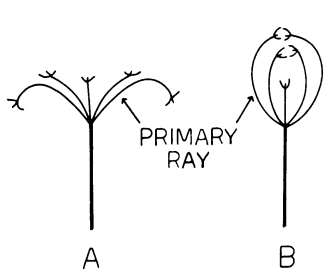


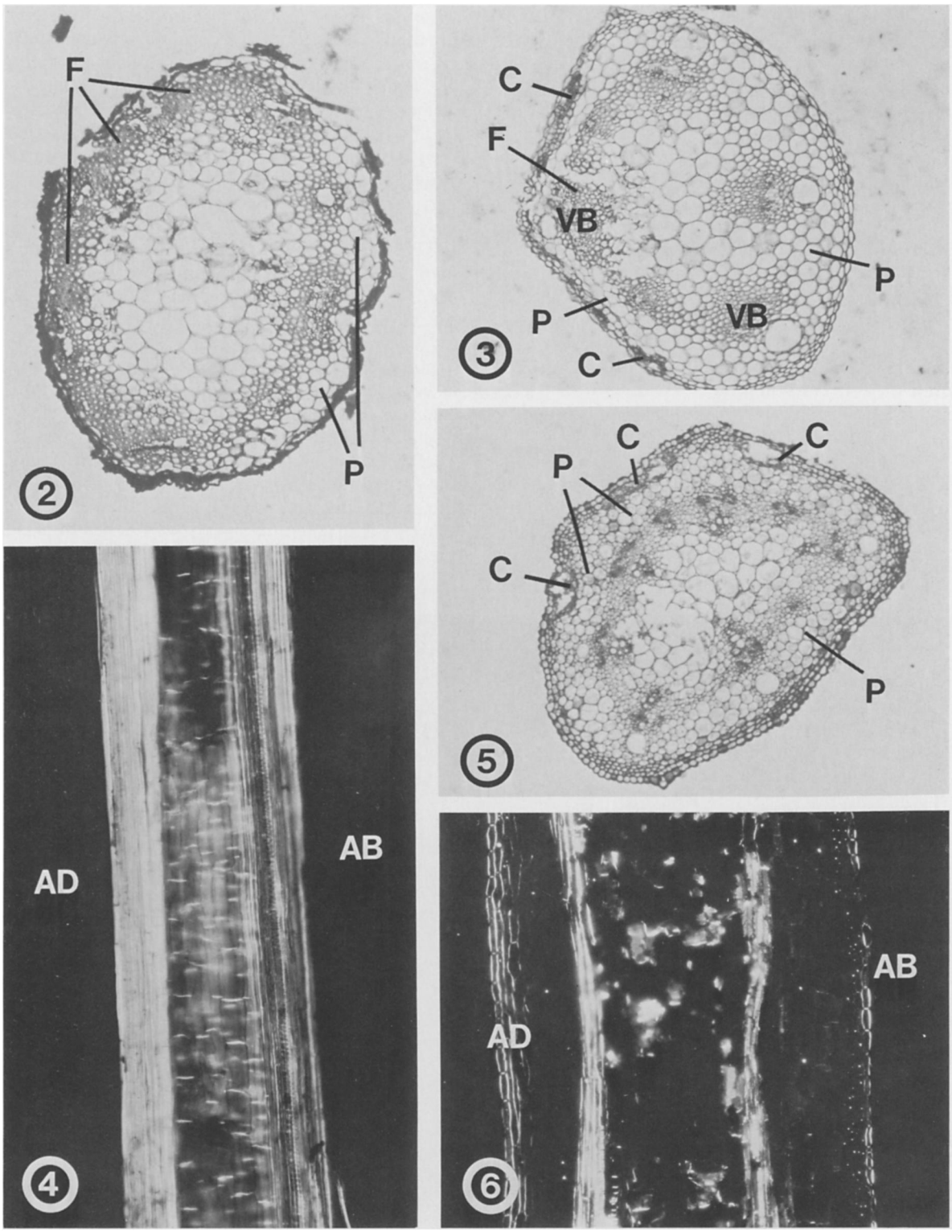
FIG. 1.—Schematic drawing of a dead compound umbel of *Daucus carota (A)* when the relative humidity is low and *(B)* when the relative humidity is high.

Discussion

Our observations indicate that expansion and contraction in thick-walled parenchyma cells occur in the direction predicted by ZIMMERMAN, i.e., perpendicular to the net microfibril orientation. Similar relationships have been observed in living cells of other plants (GREEN 1973; PALEVITZ and HEPLER 1976), and evidence that this orientation actually controls the direction of cell expansion comes from PROBINE and PRESTON's (1962) studies of *Nitella*.

The microfibrils associated with hygroscopic movement are deposited after initial cell growth. Typically, such microfibrils are helically oriented in the cell wall with the predominant pitch of the helices varying from steeply oblique, almost parallel to the long axis of a cell, to slightly oblique, almost perpendicular to the long axis (ESAU 1977). If the microfibrils are deposited in layers (e.g., secondary wall layers), the pitch can vary among layers within a single cell. Regardless of the number of layers, however, the net pitch, over all layers, can vary among cells. Our examinations have identified such variation between thick-walled parenchyma cells and sclerified fibers; the net pitch of the parenchyma cells is essentially perpendicular to the long axis of the cell, and that of the fibers essentially parallels the long axis.

The differential orientation of microfibrils in these two cell types and the asymmetrical distribution of these cells throughout a dead ray could produce the hygroscopic movement observed in *Daucus carota*. When the relative humidity rises, the parenchyma cells on the abaxial side of the ray elongate. The fibers on the adaxial side do not expand because of both their microfibril orientation and their wall lignification. Therefore, since the adaxial half of the ray undergoes no counterbalancing elongation, the ray bends inward toward the center of the umbel. Given the amount of expansion that we observed in single parenchyma cells, it is easy to see how many cells acting in concert could produce great changes in the angle of opening of a whole umbel. Because this process is purely mechanical



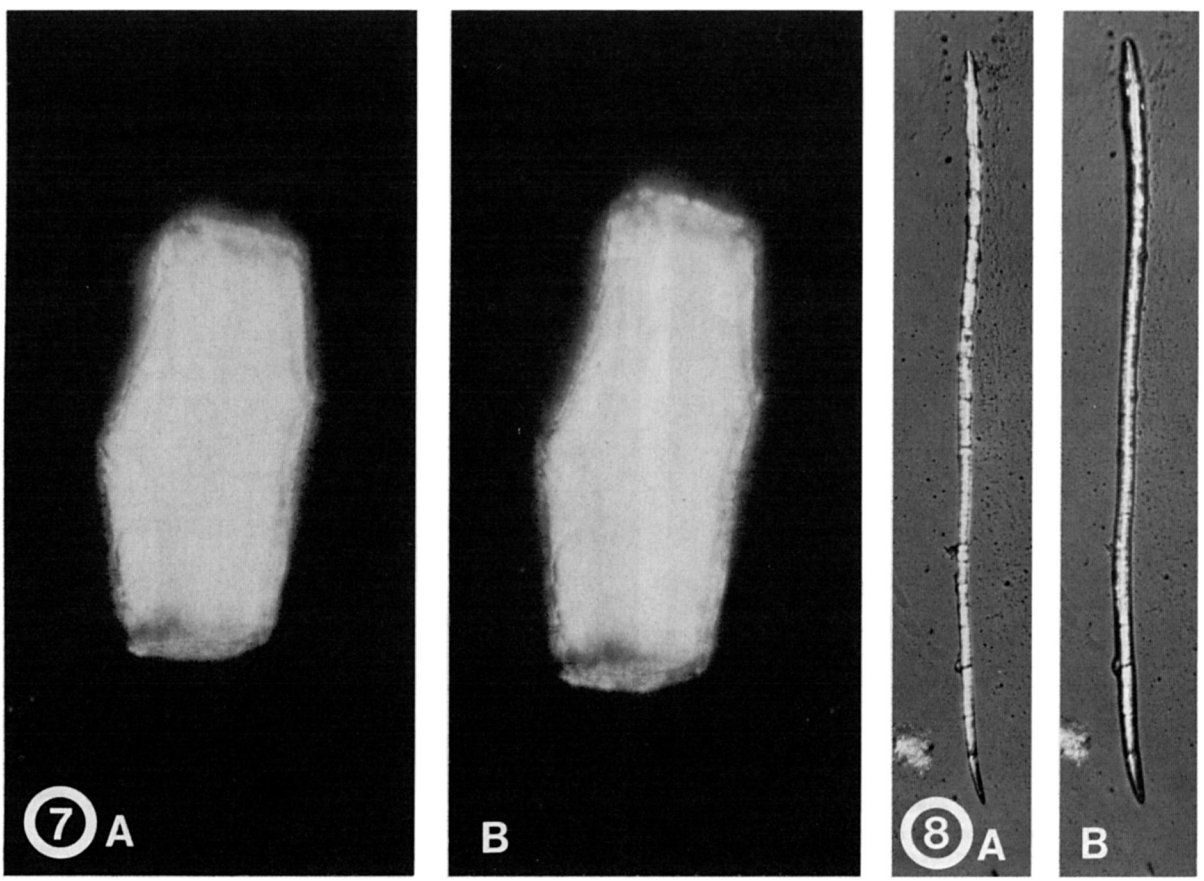
FIGS. 2–6.—All ×64. Figs. 2, 3, Cross sections of the distal half of a peripheral primary ray of *Daucus carota* stained with safranin and fast green. Fig. 2, Ray from a dead umbel. Fig. 3, Ray from an umbel beginning to close after anthesis. Fig. 4, Longitudinal section of the distal half of a primary ray as seen through polarizing filters and a red plate. For all the pictures taken through the polarizer, the polarizer was oriented in an N-S direction, the analyzer in an E-W direction, and when used, the red plate in a SW to NE direction. Fig. 5, Cross section of a ray taken from an umbel at anthesis. Fig. 6, Polarizing microscopic view of a longitudinal section of a ray taken from an umbel at anthesis. *AB* = abaxial side, *AD* = adaxial side, *C* = chlorenchyma, *F* = sclerified fibers, *P* = parenchyma, *VB* = vascular bundle.

and begins only after death of the tissue, it differs from other types of plant movement like tropisms that occur in living tissue (DAYANANDAN, FRANKLIN, and KAUFMAN 1981). It is similar, however, in that it reflects an asymmetry at the time of the response.

Asymmetrical cell differentiation in rays begins before anthesis (RICOME 1898; FUNK 1913). For example, chlorenchyma develops most, and many times only, on the adaxial side (figs. 3, 5). After anthesis, further differential deposition of wall material occurs in the rays. This delay in cell wall thickening may be a consequence of natural selection. If this deposition occurred before anthesis, the microfibrillar arrangement would prevent the expansion or opening of the umbel during anthesis while the cells are still alive, i.e., full of water. This expansion presumably facilitates pollination and is, therefore, necessary to ensure seed set.

The differential microfibrillar arrangement in the primary rays of *D. carota* ssp. *carota* and resultant hygroscopic movement influence the overall dispersal pattern in this subspecies (LACEY 1980). They may also influence the dispersal pattern in other members of the *D. carota* complex, which comprises two aggregates, the *gingidium* and *carota* groups (SMALL 1978). Although these groups are not clearly delineated, they seem to reflect an evolutionary divergence, the former to a maritime and the latter to an inland climate. SMALL (1978) reported that umbels in the *carota* group typically close during fruit development (i.e., form a bird's nest, as in ssp. *carota*), while those in *gingidium* group do not.

This observation suggests that hygroscopic movement and its associated anatomical asymmetry may occur only in the *carota* group. Further, we can postulate that the maritime climate, to which *gingidium* members are exposed, may actually select against such movement; the continuously high relative humidity along coastlines should keep umbels that are sensitive to relative humidity perpetually closed, preventing seed dispersal. Seed dispersal is particularly important in maintaining and expanding extant populations of *D. carota* ssp. *carota* and in establishing new ones (LACEY 1982). Its importance to other members of the *carota* group and to members of the *gingidium* group is currently ob-



FIGS. 7, 8.—Polarizing microscopic views. Fig. 7, Thick-walled parenchyma cell (×288) *(A)* when dry air was blown on it and *(B)* when moist air was blown on it. Fig. 8, Sclerified fiber (×72) *(A)* when dry air was blown on it and *(B)* when moist air was blown on it.

scure. All available information suggests, though, that further comparative studies of seed dispersal, hygroscopic movement, and the latter's associated anatomical features would be useful in elucidating the evolutionary trends in dispersal patterns within this taxonomically difficult complex.

Acknowledgments

We thank Dr. E. SMALL for helpful comments during this work and the University of Michigan for financial support to E. P. LACEY. We also thank T. BARKLEY for help in preparing this manuscript.

LITERATURE CITED

DAYANANDAN, P., C. I. FRANKLIN, and P. B. KAUFMAN. 1981. Gravity perception and asymmetric growth in plants: a model derived from the grass pulvinus. Physiologist **24:**113–114.

ESAU, K. 1977. Anatomy of seed plants. Wiley, New York.

FITTING, H., H. SCHENCK, L. JOST, and G. KARSTEN. 1921. Strasburger's textbook of botany. Macmillan, London.

FUNK, G. 1913. Beiträge zur Kenntniss der mechanischen Gewebesysteme in Stengel und Blatt der Umbelliferen. Bot. Centralb. Beih. 29:219–295.

GREEN, P. B. 1973. Morphogenesis of the cell and organ axis–biophysical models. Pages 166–190 *in* Brookhaven Symposia in Biology No. 25. Brookhaven National Laboratory, Upton, N.Y.

HABERLANDT, G. 1914. Physiological plant anatomy. Macmillan, London.

KERNER VON MARILAUN. 1895. The natural history of plants. Vol. **4.** F. W. OLIVER, ed. and transl. Blackie, London.

LACEY, E. P. 1980. The influence of hygroscopic movement on seed dispersal in *Daucus carota* (Apiaceae). Oecologia **47:**110–114.

———. 1982. The timing of seed dispersal in *Daucus carota* L. (Apiaceae). Oikos **39:**83–91.

PALEVITZ, B. A., and P. K. HEPLER. 1976. Cellulose microfibril orientation and cell shaping in developing guard cells of *Allium:* the role of microtubules and ion accumulation. Planta **132:** 71–93.

PRESTON, R. D. 1974. The physical biology of plant cell walls. Chapman Hall, London.

PROBINE, M. C., and R. D. PRESTON. 1962. Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca.* J. Exp. Biol. **13:**111–127.

RICOME, H. 1898. Symmetrie des rameaux floraux. Bot. Biol. Vég. Ser. 8, **7:**293–396.

SASS, J. E. 1958. Botanical microtechnique. 3d ed. Iowa State College Press, Ames.

SMALL, E. 1978. A numerical taxonomic analysis of the *Daucus carota* complex. Can. J. Bot. **56:**248–276.

THELLUNG, A. 1926. *Daucus* [in German]. Pages 1501–1526 *in* G. HEGI, ed. Illustrierte Flora von Mittel-Europa. Vol. **5.** Lehmans, Munich.