THE GENUS *ECHINACEA* (ASTERACEAE): FLORAL, STEM, AND PETIOLE MORPHOLOGY

Harold W. Keller

Botanical Research Institute of Texas
1700 University Drive
Fort Worth, Texas 76107, U.S.A.
haroldkeller@hotmail.com

ABSTRACT

The genus *Echinacea* (Asteraceae) has importance economically, medicinally, and ornamentally. Endemic to North America, distribution is centered in the states of Arkansas, Kansas, Missouri, and Oklahoma. Native Americans of the central Great Plains used *Echinacea* as a highly prized medicinal plant panacea. This anatomical study is based on R.L. McGregor's taxonomic treatment of the genus *Echinacea* that included 11 taxa: *E. angustifolia* var. *angustifolia*, *E. angustifolia* var. *strigosa*, *E. atrorubens*, *E. laevigata*, *E. pallida*, *E. paradoxa* var. *neglecta*, *E. paradoxa* var. *paradoxa*, *E. purpurea*, *E. sanguinea*, *E simulata*, and *E. tennesseensis*. Anatomy of *Echinacea tennesseensis* was not included because live plants were not available. Plants were collected at the height of anthesis from the experimental gardens at the University of Kansas. Samples were prepared for microtome and free-hand sectioning and staining. Macromorphology and microanatomy are described here, and photomicrographs illustrate the adaxial epidermal cells of ray ligules. Tissue map line drawings illustrate the pattern and distribution of stem trichomes, epidermal cells, cortex, vascular bundles, and pith. Measurements were included for stem diameters, epidermis, collenchyma, parenchyma, xylem vessels, sclerenchyma fibers, xylem and phloem vascularization, protoxylem points, and location and number of secretory canals for each *Echinacea* taxon. Sclerenchyma fibers (sclerotic cells with a black phytomelanin substance) are located in the pith tissue of all the varieties of *E. angustifolia*. Tissue maps and photomicrographs illustrate petiole transections and the presence of brachysclerids (stone cells) in *E. paradoxa* var. *neglecta* which were found nowhere else in this study. Plants resulting from crossings and introgression between *E. atrorubens* and *E. angustifolia* had many intermediate characteristics and were called "race *intermedia*." This name has no nomenclatural standing but the plants were found to have unique ray ligule adaxial epidermal cells. These multicelluar structures consist of an enlarged basal cell with a neck and a catenuliform series of one, two, or three discrete pyramidal cells that have not been described for any member of the Asteraceae or other flowering plant. A key to *Echinacea* taxa that includes the distinctive micromorphology of ray ligule adaxial epidermal cells is presented. A discussion of the structure and function of ray ligule microanatomy is included as this relates to insect pollinators. Questions still remain concerning the constancy of anatomical characters over a broad range of habitats based on statistically sampled populations.

Key Words: dichotomous key, *Echinacea* (coneflower), endangered species, ligule adaxial epidermal cells, macro-and microanatomy, sclerotic and stone cells

RESUMEN

El género *Echinacea* (Asteraceae) tiene importancia económica, medicinal y ornamentalmente. Endémica a Norteamérica, se centra en los estados de Arkansas, Kansas, Missouri y Oklahoma. Los americanos nativos de las Grandes Planicies (Llanuras Centrales), usaron *Echinacea* como una planta muy altamente apreciada como panacea medicinal. Este estudio anatómico se basa en el tratado taxonómico del género de R.L. McGregor que incluyó 11 taxa: *E. angustifolia, E. angustifolia* var. *strigosa, E. atrorubens, E. laevigata, E. pallida, E. paradoxa* var. *neglecta, E. paradoxa* var. *paradoxa, E. purpurea, E. sanguinea, E. simulata* y *E. tenneesseensis*. Las plantas se colectaron durante el pico de la floración en los Jardines Experimentales de la Universidad de Kansas para prepararlas para el micrótomo, seccionado a mano y tinción. En este estudio describimos la macromorfología y la microanatomía, y las fotomicrografías ilustran las células epidérmicas adaxiales de las flores radiales. Los dibujos de las células epidérmicas ilustran los patrones y distribución de los tricomas del tallo, células epidérmicas, córtex, conjuntos vasculares y médula, con medidas de los tipos de células que incluyen diámetro de los tallos, epidermis, colénquima, parénquima, vasos del xilema, fibras de esclerénquima, vascularización del xilema y líber, puntos del protoxilema y la ubicación y número de los canales secretores para cada taxón de *Echinacea*. Las fibras del esclerénquima (con una sustancia fitomelánica negra) se localizan en el tejido medular de *E. angustifolia* var. *angustifolia, E. angustifolia* "raza intermedia" y *E. angustifolia* var. *strigosa*. Los mapas de tejido y fotomicrografías ilustran los cortes transversales de los peciolos y la presencia de braquiscléridas (células pétreas) en *E. paradoxa* var. *neglecta* las cuales no se encontraron en ninguna otra parte en este estudio. Otro taxón llamado *E. angustifolia* var. *angustifolia* "raza intermedia" no tiene posición nomenclatural pero también se describió y se encontró que tiene células epidérmicas adaxiales únicas en las flores radiales. Estas células multicelulares consisten de una célula basal agrandada con un cuello y una serie cateluniforme de 1, 2 o 3 células piramidales discretas que no se han descrito para ningún miembro de las Asteraceae o alguna otra planta con flores. Se presenta una clave para los taxa de *Echinacea* que incluye la micromorfología distintiva de las células epidérmicas adaxiales de las hemilígulas. La estructura y función de las células epi-

dérmicas adaxiales de las hemilígulas tiene implicaciones ecológicas importantes que se relacionan con los insectos polinizadores. *Echinacea tennesseensis* no se incluyó en este estudio anatómico pero se discutió como un taxón de especial interés como una especie en peligro de extinción.

INTRODUCTION

Historical Overview

This study was conducted during a two year period (1960–1962) as part of the partial fulfillment of the requirements for the degree of Master of Arts at the University of Kansas (KU) under the direction of R.L. McGregor (Keller 1962). The author shortly thereafter served as an officer in the United States Army Medical Service Corps and was not able to publish his thesis at that time. Several years later McGregor (1968) published his monograph of *Echinacea* which represented 15 years of field studies throughout the range of the various taxa. This included growing transplants representing most taxa in the KU experimental garden and greenhouse for a period of eight years. Several thousand plants were started from seed both in the garden and greenhouse representing all possible crosses and backcrosses. Chromosome counts were made from over 2100 field collections of buds, root tips from transplants, and root tips from germinating seeds (McGregor 1968).

The Comparative Anatomy section of the paper by McGregor (1968) included a summation of taxonomically significant characters for certain taxa associated with stem, petiole, and adaxial ray ligule epidermal cell anatomy. A key to identify *Echinacea* taxa was constructed based on the combination of these characters. Detailed descriptions and illustrations of micromorphology for stems, petioles, and floral anatomy for each *Echinacea* taxon were not included.

Keller (1962) included a general key based on a combination of anatomical characters that included the number, size, and location of stem secretory canals, overall stem size and tissue patterns of xylem, presence or absence of sclerotic cells in pith tissue, presence or absence of trichomes, and differences in ray ligule adaxial epidermal cells. Additional keys were also included based solely on the marginal ray floret anatomy and stem secretory system. The 95 pages of text, 56 line drawings, and 34 photomicrographs of microanatomy in the thesis are included here in part to document a more complete descriptive anatomy of *Echinacea* taxa (http://hdl.handle.net/1808/11669).

In a study of root anatomy of *Echinacea angustifolia* var. *angustifolia*, *E. pallida*, and *E. purpurea*, Mistríková and Vaverková (2007) noted that "a description of the microscopic characteristics of a cross-section of the aerial parts of the *Echinacea* plants is not available." The question posed here was to determine differences in stem, petiole, and ray floret microanatomy that could separate and identify taxa as well as contribute to a better understanding of aerial plant microanatomy and morphology of the genus *Echinacea*. The purpose of this study was to emphasize both aspects and, where feasible, devise a key using a combination of macro- and microanatomical key characters.

Review of Past Literature

Echinacea Moench, Methodus, 591. 1794, is a member of the Asteraceae, a family that exhibits a wide range of distribution and habit. It is probably the largest family of flowering plants with an estimated 1535 genera and 23,000 species (Bremer et al. 1994) and numbers given as 1353 genera and 23,000 species in Judd et al. (2008). This family is comprised mostly of herbs, although sometimes shrubby and more rarely woody habits occur. *Echinacea* herein is treated as a herbaceous, perennial prairie forb, however, toward the end of the growing season a small amount of secondary growth may develop, not unlike many herbaceous plants. *Echinacea* may also serve as forage for grazing animals and is often an indicator of undisturbed prairies. It is one of many showy wild flowers that are such a familiar part of the landscape of Kansas. An ever-increasing demand for horticultural varieties led to the 'King', a pink variety, and 'White Lustre' among others, both developed from *E. purpurea* (McGregor 1968).

The genus *Echinacea* is native and endemic to central United States of America, extending into Canada, but is unknown from Mexico. Most taxa are more or less restricted to the states of Arkansas, Kansas, Nebraska, Oklahoma, Minnesota, Missouri, North Dakota, South Dakota, and Texas with scattered collections from

Colorado, Georgia, Illinois, Iowa, Kentucky, Louisiana, Massachusetts, Montana, New Mexico, North Carolina, South Carolina, Tennessee, and Virginia. Highest population density and species diversity occurs in the states of Arkansas, Kansas, Missouri and Oklahoma with Missouri heading the list (Richter 2013).

The Economic Importance section of McGregor (1968) discusses the use of *E. angustifolia* and *E. pallida* roots as a source of drug extracts for various medical ailments but was limited to early anglo settlers of the Great Plains region. Uses by Native Americans were not included in his discussion of Economic Importance; however, an excellent review is available in Kindscher (1989). Native Americans used the prairie forb genus *Echinacea* as a general panacea, but some of its reputed medicinal properties were: the root relieved the pain of toothache; the juice soothed burns and aided healing; and plants placed in steam baths acted as vaporizers cooling the body from heat discomfort. It even was thought to be beneficial in the treatment of mumps and distemper in horses. Even today an alcoholic tincture of *Echinacea* root is used for the healing of wounds and cure of sore throat (Stevens 1961).

Probably the best source of indigenous names and anglo folk use is detailed for *E. angustifolia* by Kindscher (1991). The indigenous tribes that had names and uses included the Apache, Cheyenne, Comanche, Crow, Dakota, Hidatsa, Kickapoo, Kiowa, Lakota, Mesquakie, Omaha, Pawnee, Ponca, Potawatomi, and Winnebago. As the most widely used medicinal plant of the plains, as noted by Kindscher (1991), the collective uses based on the aforementioned tribes included: the dried seed head was used to comb or brush hair; as a mushroom medicine because its seed head was similar in shape to a mushroom; root tissues for an eyewash, coughs, sore throat, or to stimulate saliva flow; applications for snake bites (rattlesnakes) and other venomous bites, stings and poisonings; a remedy for hydrophobia, wounds, tonsillitis, stomach ache, pain in bowels; root teas brewed for sore mouths or gums, rheumatism, arthritis, and measles. Coneflower roots were mixed with fungal puffballs (Gasteromycetes, *Lycoperdon* spp.) spores and skunk oil to treat boils. This list of broad medicinal uses of *Echinacea* gives a better idea of the past history of possible potential medical efficacy and future herbal uses in modern human cultures. More recent papers document the enhanced immune-stimulatory and anti-inflammatory activity of *Echinacea* and the impact of overharvesting, especially in areas in the state of Kansas (Kindscher 1989; Price and Kindscher 2007; Kindscher et al. 2008; Axentiev et al. 2010; Upton 2010).

Metcalf and Chalk (1950) in their classic two-volume work, listed numerous anatomical features that characterize the Asteraceae, including secretory canals, lacticiferous canals, glandular and nonglandular hairs, anomalous secondary thickening, and medullary and cortical bundles. Herbaceous stems among members of the family usually exhibit a ring of collateral vascular bundles, each accompanied in the pericyclic region by large strands of fibers, often forming distinct "bundle caps." Even though *Echinacea* is cited by Solereder (1908) and Metcalf and Chalk (1950), no detailed investigation of anatomical characteristics, either on a comparative or taxonomic basis, has been published. Since that time additional data were published that extends our knowledge of ray floret anatomy in the Asteraceae.

Papers published that relate to *Echinacea* ray ligule anatomical microcharacters (Table 1, Baagøe 1977a; 1977b) evaluate three epidermal types, including the helianthoid type, consisting of papillose and nearly isodiametric cells that come closest morphologically to *Echinacea*. The illustration of these papillose adaxial epidermal cells (see Baagøe 1977a, plate 1b) shows a three-dimensional SEM view of *Rudbeckia* sp. and plate 2 c of an *Aster* sp. ligule in optical plane cross section showing a light photomicrograph with median wall thickening, and plate 2e of a *Rudbeckia* ligule in cross section showing septa. A survey of the 111 genera and 275 species in the Asteraceae did not mention *Echinacea* by name or illustrate any of the taxa (Baagøe 1977a). Another paper by Baagøe (1977b) evaluates ray ligule microcharacters in the Asteraceae as they relate to taxonomic differences that separate taxa and applies them as characters in a key to tribes, sub-tribes, and genera. *Echinacea* is not mentioned in that paper nor was the *Echinacea* monograph of McGregor (1968) cited as a source of anatomical data.

Description of the adaxial epidermal cells of the Sub-tribe Helianthinae that have papillose cells are designated as the helianthoid type. These cells represented by *Rudbeckia hirta* and *R. speciosa* (see Baagøe 1977b, fig. 5a SEM) have the largest cells (length-width ratio) and thicker outer cell walls. In other words these cells

are more elongate vertically with a narrow neck and much wider at the base. None of these cells illustrated or described are multicellular.

Dome shaped adaxial epidermal cells with papillae were shown by Baagøe (1980) using SEM for certain members of the Lactuceae. Examples (Table 1) were represented by *Calycoseris wrightii* (fig. 1B) with hooked papillae pointing toward the distal end of the ligule, *Hieracium saxifragum* (fig. 2A) with more upright papillae, and *Rafinesquia neomexicana* (fig. 1I) with papillose ligule surfaces distally as well as several other examples (Baagøe 1980). Unfortunately some of the SEMs show distorted and collapsed adaxial epidermal cells caused by shrinkage after preservation as herbarium specimens. Critical point drying techniques were not used to preserve cell shapes.

The anatomy of ray florets in the Asteraceae is surprisingly understudied and examples must come from other unrelated floral taxa. References and examples are summarized in Table 1. These aforementioned combined images provide a historical context for the different sizes and shapes of ray floret adaxial epidermal cells observed here in *Echinacea* taxa.

An exhaustive review of taxonomic treatments and phylogenetic papers including *Echinacea* is beyond the scope of this paper; therefore, the *Echinacea* taxa discussed by McGregor (1968), the taxonomic treatment by Urbatsch et al. (2006) and by Flagel et al. (2008) will be followed because the anatomical data in Keller (1962) is associated with those names.

MATERIALS AND METHODS

Collections of *Echinacea* used in this study were made at the University of Kansas Experimental Gardens, Lawrence, Kansas, with some additional field collections. A complete list of the taxa with field notes is included under Collections in the thesis of Keller (1962). Voucher specimens were deposited in the R.L. McGregor Herbarium (KANU). Collections were cited by Keller (1962) as the source of live specimens used for this anatomical study of *Echinacea* taxa. All plants studied were collected during anthesis from 19 Jun to 22 Jun1961. Only specific portions of the plants were selected: flower head, stem, and node with attached leaf.

Voucher specimens are listed below for the source populations from which the KU garden collections were grown or from which collections were made.

E. angustifolia var. **angustifolia:** Kansas, Comanche Co., 17 mi E of Coldwater, prairie hillside, 18 Jun1957, *E. Lathrop 3827*.

E. angustifolia var. **angustifolia** race **intermedia:** Kansas, Mitchell Co., 7 mi N of Hunter, rocky prairie hillside, 22 Jun 1961, *B. Menhusen s.n.*

E. angustifolia var. **strigosa:** Oklahoma, Murray Co., 1 mi N of Sulphur, 29 May 1960, *R.L. McGregor 15607*.

E. atrorubens: Kansas, Douglas Co., 1 mi W and ½ mi S of KU Experimental Gardens, 22 Jun 1961, *H.W. Keller s.n.*

E. laevigata: North Carolina, Durham Co., near Durham, grown from seed sent by Bloomquist 5 (*leg. ign. s.n.*)

E. pallida: Kansas, Chautauqua Co., 3 mi E and 3 mi N of Sedan, 19 Aug 1959, *R.L. McGregor 15042*.

E. paradoxa var. **neglecta:** Oklahoma, Murray Co., rocky prairie hillside common in area at Platt National Park, 7 Jun 1959, *R.L. McGregor 14323*.

E. paradoxa var. **paradoxa:** Missouri, Barry Co., rocky hillside, 3 mi SE jct. Hwy 112 and F, Roaring River State Park, 12 Jun 1959, *R.L. McGregor 14367*.

E. purpurea: Arkansas, Baxter Co., wooded hillside, 2 ½ mi SE of Mountain Home, 6 Aug1959, *R.L. McGregor 14961*.

E. sanguinea: Texas, Angelina Co., sandy open bank at edge of pine forest, 2.6 mi S of Lufkin on Hwy 89, 12 May 1960, *R.L. McGregor 15557*.

E. simulata: Missouri, Oregon Co., roadside opening in oak-hickory woods, 2.2 mi N of Greer, Clark National Forest, 3 Jun 1960, *V. Harms 321*.

Live plant samples were based on not more than five plants and at least five transections made of five stems, petioles, and ray florets. Due to a small sample size taken at one point in time, a statistical comparison of population samples was not attempted. Consequently, the anatomical keys constructed herein are practical only within certain limitations with the caveat that recognizing qualitative differences in description of cell types previously unknown merits special consideration.

Table 1. References describing flower petal and ray ligule adaxial epidermal cells. SEM = scanning electron micrograph; LP = light photomicrograph; LD = line drawing.

Family: Tribe	Taxon	Cell shape	Source & illustration type
Asteraceae: Cichorieae	*Calycoseris wrightii*	Hooked papillae	Baagøe (1980, Fig. 1B); SEM
Asteraceae: Cichorieae	*Hieracium saxifragum*	Upright papillae	Baagøe (1980, Fig, 2A); SEM
Asteraceae: Cichorieae	*Rafinesquia neomexicana*	Hooked papillae	Baagøe (1980, Fig, 2I); SEM
Asteraceae: Heliantheae	*Aster* sp.	Papillose, dome-shaped	Baagøe (1977a, Plates 2c, 2e); LP
Asteraceae: Heliantheae	*Helianthus annuus*	Conical	Whitney et al. (2011); SEM
Asteraceae: Heliantheae	*Rudbeckia* sp.	Papillose, conical	Baagøe (1977a, Plate 1b); SEM
Gesneriaceae	*Saintpaulia ionantha*	Rounded papillae	Endress (1994, Fig. 5.10-2); SEM
Lentibulariaceae	*Pinguicula vulgaris*	Nipple-like	Eames & MacDaniels (1947, Fig. 169C); LD
Rosaceae	*Amelanchier laevis*	Hemispherical	Eames & MacDaniels (1947, Fig. 169A); LD
Rosaceae	*Rosa* sp.	Dome-shaped, columnar	Esau (1960, Fig. 20.1-A); LP
Verbenaceae	*Lantana camara*	Long, conical papillae	Endress (1994, Fig. 5.10-3); SEM
Violaceae	*Viola* × *wittrockiana* cultivar	Sharply pointed conical, some with cuticular striation	Weryszko-Chmielewska & Sulborska (2012, Figs. 1C, 5); LP, SEM

Organs such as stem and leaf were collected in the same morphological position on specimens representing each taxon to ensure validity of comparison. This was accomplished by determining a point midway between ground level and flower attachment which served as the source of material for the stem anatomy presented here. The first recognizable leaf (not to be confused with the reduced upper leaf) borne on the stem below the capitulum was selected for study purposes. This corresponds in some cases to a position just above leaves borne in somewhat of a rosette-like fashion, especially in the shorter species.

The leaves have either sheathing bases or, in the narrow-leafed species, are distinctly petiolate. Petiole anatomy was based on samples taken 0.6 cm from the point of departure on the stem axis for sheathing leaf bases and halfway between the stem axis and the leaf base for distinctly petiolate leaves. Floral parts such as ray ligules were sectioned at the approximate midpoint gauged by the overall length of the particular structure. After selection, the designated materials were placed in vials containing formalin-propiono-alcohol (Johansen 1940, p. 42).

Two methods of preparation were employed, each possessing certain merits. Tissue prepared by the free-hand sectioning method gave excellent preservation of detail, especially in the secretory canal system. However, producing sections uniform in thickness, less than 10 µm and truly parallel to the plane of cutting requires practice, skill, and patience. Furthermore, sections should be standardized to a certain thickness to study structural properties on a comparative basis. The microtome method has drawbacks caused by the previous dehydrative treatment before sectioning that tends to collapse and distort the thin-walled epithelial cells that surround the canal cavity. The graded alcohol series and staining in Coplin jars and eventual embedding in paraffin was a long, time-consuming process prior to sectioning with the microtome.

Free-hand transections were made by orienting live plant material in elderberry pith and then thinly slicing sections with a single edge razor blade. Transections of ray ligules were mounted directly into glycerol and photographed with a compound microscope. Stem transections were stained either with safranin and fast green or phloroglucinol in 18 percent HCL. After passing the stained sections through a graded alcohol series, they were permanently mounted in picolyte. Temporary phloroglucinol mounts were made by placing sections in glycerol. Moreover, the true nature of the cells (size, shape, and wall thickness) was greatly enhanced when fresh material could be cut, stained, and passed directly into glycerol without overuse of a harsh dehydrating agent such as alcohol. This holds especially for the collenchyma which is rich in water and tends to undergo a noticeable shrinkage when subjected to dehydration. Shorter preparation time for free-hand sections facilitated more rapid analysis.

Sections prepared by the paraffin method had cortical cells more compact, thinner walled, and intercellular spaces completely occluded. This method more readily demonstrated the vascularization of the stem axis because whole, intact sections could be obtained. Tissue prepared for the microtome was handled according to

Johansen (1940, p.130) using tertiary butyl alcohol as the dehydrating agent. Following impregnation and embedding in paraffin, the material was sectioned with a Spencer Rotary Microtome at blade settings of 10 µm and 15 µm. Difficulty in sectioning was encountered where extensive sclerification occurred throughout the pith region, exhibiting a tough, woody consistency.

Preliminary staining followed the schedule outlined by Johansen (1940, p. 80–82) with slight modifications to fit each taxon. Staining time was altered when safranin stain was used with the counterstain fast green to get brilliant color differentiation. The presence of a carbohydrate compound, presumably starch, was detected by applying Lugol's solution (I_2KI-potassium iodide) to freshly cut sections. Inulin reported in the literature as commonly found in roots, and sometimes in stems, gave a negative test. Phloroglucinol indicated the extent and relative degree of lignification among the taxa.

Macerations of stem material were prepared by slicing the stem longitudinally into small slivers to increase the disintegrating power and lessen the time required to free the cemented cells. Usually thirty minutes was sufficient time to freely suspend the parenchymatous and collenchymatous cells, but vascular elements often were teased apart with a pin probe. The macerating fluid was made according to the formula prescribed by Jeffrey in Johansen (1940, p. 104). The range and average measurements of pericyclic fibers, vessels, collenchyma, and parenchyma cells were tabulated from a minimum of 30 individual cells of each.

Thin strips of epidermal cells were peeled from the stem. These were then projected and drawn in surface view noting the frequency of stomata and trichomes, if any, the cuticle characteristics, epidermal patterns, and cell dimensions. Length in surface view refers to cell elongation and orientation in a vertical plane and width to that measurement in a horizontal plane.

Measurements made of the ray ligule adaxial epidermal cells have a vertical orientation so that length refers to the upward, vertical extension exaggerated by their papillose condition. The basal width is in a horizontal plane parallel with the surface of the ray florets. Trichomes and hairs are terms that also have been used for stem and leaf pubescence and also for ray florets. However, usage of ray florets and ray ligule adaxial epidermal cells will be followed here to be consistent with McGregor (1968).

The Bausch & Lomb TRI-SIMPLEX Micro-projector was used to project images of tissue sections upon white paper conveniently placed on a tabletop and then outlining the image to make tissue maps. This apparatus was equipped with a tri-objective revolving nosepiece that gave magnifications of 2.7×, 5×, and 12×. A special attachment, the 5× Huygenian eyepiece made possible even greater magnifications. Drawings of stem and petiole transections were made at a magnification of 40×. The different tissue systems are represented thusly: the xylem with associated fibers is vertically lined; the phloem is blank or white; the bundle caps are blackened; and stippling between vascular bundles indicates lignification.

Light photomicrographs were taken with a compound microscope with either a low power (10×) or high power (43×) objective lens with a 10× eyepiece at a total magnification of 100× or 430×. Scale bars were not used; magnifications were calculated using cell measurements. Photographic images were recorded using Kodak 35mm Panatomic X black and white negative film. The 35mm negatives were scanned at 6300 dpi using an Imacon Precision II film scanner. The 16-bit gray-scale images were inverted to positive tif images using Photoshop CS6 software.

GENERAL ANATOMICAL BODY PLAN OF *ECHINACEA*

Echinacea is a genus of herbaceous perennials usually occurring in undisturbed prairie or glade ecosystems. Most taxa usually arise from a taproot (only *E. purpurea* has a rhizome and fibrous root system) as a single erect stem, are most often unbranched with hairy to smooth leaves, basal and cauline, alternate, petiolate, and terminating in a single flower head (capitulum). Ray florets (8 to 21) represented by strap-shaped ray ligules are sterile, forming a showy head of dark purple to rose or pale pink, yellow, or white, either spreading, drooping, or reflexed ray florets with either two or three notched tips. The central cone is made up of perfect, fertile, disc flowers that are inconspicuous because of the surrounding colorful paleae (chaffy bracts) with orange to reddish purple ends that create the showy spiny cone.

The generic name is derived from the Greek word *echinos* meaning hedgehog that refers to the prominent spiny cone of disc florets that eventually matures into a bristly seed head. The morphological characters used to describe the general habit in monographic treatments (McGregor 1968; Urbatsch et al. 2006; Yatskievych 2006) do not mention anatomical characters and that includes stem diameters.

Stem anatomy of plants includes the spatial arrangement of cell types represented by parenchyma, collenchyma, sclerenchyma, and primary and secondary xylem and phloem (vascular tissue). The presence or absence, location, size, and number of external cells (trichomes) or internal secretory cells that include cavities, canals, resiniferous or mucilaginous cells, crystals (cystoliths, druse, raphides), and laticifers serve as diagnostic characters useful in taxonomic work (Eames & MacDaniels 1947; Esau 1958, 1960). Specific regions of the stem for example, epidermis, cortex, pericycle, endodermis, vascular bundles, and pith, may show modification due to thickness of cell walls, size, maturation, and taxon-specific differences. The stem tissue is typical of herbaceous perennial dicots with a pith region that occupies about 75 percent of the central core of the plant and collateral vascular bundles, forming a ring nearer the periphery and usually separated by interfascicular parenchyma or sclerenchyma. A descriptive anatomical evaluation of these morphological features has not been published for the stem, petiole, and ray ligule adaxial epidermal cells for *Echinacea* taxa.

Petiole anatomy offers additional sources of comparisons between *Echinacea* taxa as this relates to the number of major or minor collateral vascular bundles, petiole shape in transectional view, presence or absence of lacunae, and presence or absence of secretory cells. In *Echinacea* the petiole is supplied by three major collateral vascular bundles, a manifestation of departing foliar traces from the stem. Due to different methods of fusion, division, or twisting of the leaf traces, the number of vascular bundles traversing the cortex may or may not be the same as the number that enter the leaf. Minor vascular bundles are associated in different numbers with the major vascular bundles (Eames & MacDaniels 1947).

The arrangement of vascular bundles in the petiole is usually constant for a given species and often for families (three for the Asteraceae). In addition petiole shape can be used as a taxonomic character. Transversely cut petioles can be recognized by shapes, for example, horseshoe-shaped, V-shaped, and cylindrical-shaped. Moreover, secretory canals, universally present in the genus, differ in size, number and position in the petiole. Anatomically the petiole contains the same tissues as the stem: epidermis, collenchyma in varying amounts, and vascular bundles with associated fibrous sheaths.

The main emphasis of this study was the anatomy of the ray florets that are the showy parts of the composite flower. Petals are leaf-like in form, but they differ histologically in various ways from the typical leaf. Generally they show some resemblance in their internal structure to mesophytic leaves, although often lacking differentiated palisade and spongy parenchyma tissue. They consist of ground parenchyma (often called mesophyll), a greatly reduced and branched vascular system, and epidermal layers on the adaxial (upper) and abaxial (lower) surfaces. The vascular supply (here termed veins and veinlets) usually bifurcates more noticeably at the ray ligule tips. Thick-walled supporting tissue often is found surrounding each veinlet. Furthermore, the vascular tissue often consists of several large veins and a system of smaller veinlets.

Perhaps the most striking anatomical feature of the ray ligules is the peculiar adaxial epidermal cells that bulge outward, and are modified into various sizes and shapes. The ray ligule adaxial epidermal surface is usually modified into the conical-papillate type as in *Erysimum cheiri* (Weston & Pyke 1999, light photomicrographs figure 1 B, C and figure 2 A SEM shows an adaxial conical-papillate type epidermis with a striated waxy cuticle epidermis) whereas, the cells of the abaxial epidermis in figure 2B, SEM shows lenticular cells with stomata. *Echinacea* closely parallels then the anatomy of most petals as all of the features mentioned previously are exemplified in its ray florets.

In some flowering plants both floral epidermal surfaces are papillose, but in *Echinacea* only the adaxial ligule epidermal surface exhibits this micromorphology. The inner tangential wall is often slightly convex. The outer wall, by comparison, is often more or less convex or papillose. However, in *Viola* and *Nasturtium*, for example, these cells are modified and bear one or more capitate or cone-shaped papillae (not illustrated in Esau 1958, p. 538). Similarly, in *E. angustifolia* "race *intermedia*" the adaxial epidermis of ray ligules partly consists

of cells enlarged basally appearing more bulbous, then capped by one or more (not more than three observed) catenulate, pyramidal-shaped cells. In many flowering plants the anticlinal walls appear either straight, wavy, or may bear internal ridges. The undulation and ridging varies widely in degree of expression in different species. Indeed, the epidermis is less simple than its foliar counterpart.

Due to the weak-walled complex nature of the ray ligule adaxial epidermis, a dovetailed arrangement seems to permit the greatest mechanical support. The functional importance of these highly modified cells is open to conjecture, but apparently they form a layer mechanically stronger than one of simpler form. Furthermore, in some plants epidermal anticlinal walls along the veins and at the base of the petal are usually straight, even if wavy elsewhere. In some cases variability in wall structure gives an assortment of shapes. In *Echinacea*, however, the size and shape of ray ligule adaxial epidermal cells remained somewhat constant for each taxon at least in the same capitulum. Stomata were not observed on the ray ligule adaxial epidermal cell surface suggesting these modifications in cell shape play a different functional role perhaps the attraction of insect pollinators. The ray ligule abaxial epidermis resembles the typical epidermal tissue having stomata, trichomes, and a heavy cuticular covering and internally often with dense contents consisting of chromoplasts and small particles. Chromoplasts occur in the cell sap with an array of colors as can be seen in the drooping or spreading ray florets.

RESULTS

Description of Ray Florets, Stem, and Petiole Anatomy in *Echinacea* Taxa

Microanatomy of the following *Echinacea* taxa is summarized in Table 2 (ray florets), Table 3 (stems), and Table 4 (petioles). Distribution maps (Figs. 1, 2) were created using ArcMap 10.2 showing counties in the U.S.A. shaded gray, indicating that the taxon occurs there (McGregor 1968; Kartesz 2013; USDA-PLANTS 2013).

1. Echinacea angustifolia DC. var. **angustifolia** Prodr. 5:554. 1836. (**Figs. 1A; 3A, B, E, I, J; 9B; 10A**). NARROW-LEAVED PURPLE CONEFLOWER, BLACK SAMPSON ECHINACEA, KANSAS SNAKEROOT.

This taxon includes a complex of taxa referred to by various names assigned by McGregor (1968) and followed during the course of this study (Keller 1962). Its distribution occurs throughout the high plains and drier prairie areas, barrens, and rocky to sandy-clay soils of Texas, Oklahoma, Kansas, and north to Canada. The westernmost extension of this taxon includes New Mexico, Colorado, Wyoming, and Montana (Fig. 1A). The strictly low habit (20–70 cm tall), mostly unbranched, moderately to densely hairy, tuberculate-hirsute to tuberculate-hispid stem with ray florets usually purplish to pink, rarely white, yellow pollen, and diploid chromosome number of $2n = 22$ characterize this taxon (Fig. 3A, B).

Ray ligule adaxial epidermal cell microanatomy has a length-width ratio that results in a dome shape with some cells slightly pinched in part way near the top (Fig. 3E). Secretory chambers of four to six epithelial cells are found on the abaxial side of the vascular traces (Table 2).

Stem microanatomy features nonglandular trichomes that thickly cover the stem. Epidermal stem cells have a wide range in length which tends to give an irregular pattern (Fig. 9B). Sclerotic cells with thickened walls occurring throughout the pith are easily seen in free hand transections but secretory canals are lacking (Fig. 3I, J). Stem interfascicular regions are sclerified but show no secondary growth (Fig. 10A). The cortex is largely made up of collenchyma tissue. The secretory system consists of 26 canals restricted to the cortex. Comparatively this taxon has one of the smaller stems in the genus at ~2 mm in diameter, including a pith diameter of ~1 mm (Fig. 10A; Table 3). The *E. angustifolia* complex of taxa all have sclerenchyma cells and lack secretory canals in the pith which differ from all other *Echinacea* taxa.

The petiole is smaller and somewhat V-shaped with sides not steeply inclined (Table 4). Outside the lateral vascular bundles an abrupt delimitation of fundamental tissue occurs, continuing as photosynthetic foliar tissue. The canal system is greatly reduced in number; thus only one canal 40 µm was seen beside the medial vascular bundle. The minimum of three vascular bundles traverse the petiole.

2. Echinacea angustifolia var. **angustifolia** "race **intermedia**" (**Figs. 1C; 3C, D, E; 10B**).

Table 2. Ray ligule micromorphology based on transections. Position of secretory chambers are relative to vascular traces.

	Adaxial epidermal cells					
Taxon	Mean height (µm)	Mean basal width (µm)	Overall shape	Ligule thickness (µm)	No. vascular traces	Secretory chamber position & diameter (µm)
E. angustifolia var. *angustifolia*	58	42	Dome with slight pinching at top	170	13	abaxial (38)
E. angustifolia var. *angustifolia* "race *intermedia*"	100*	45–54	Tapered conical; pyramidal catenulate	286	13	abaxial (31–40)
E. angustifolia var. *strigosa*	65	55	Conical with rounded corners	265	13	few to lacking
E. atrorubens	78	55	Broadened base with "nipplelike" apex	233	13	abaxial (55)
E. laevigata	47	33	Dome nearly isodiametric	184	15	lacking
E. pallida	72	48	Tapering upward to dome or conical	235	12	--
E. paradoxa var. *neglecta*	50	47	Variable tapering upward gradually to a rounded apex	--	--	abaxial & adaxial (42–81)
E. paradoxa var. *paradoxa*	74	52	Dome with parallel sides	312	13	abaxial & adaxial (42–70)
E. purpurea	65	48	Wide ball-like base tapers to rounded apex	--	31	--
E. sanguinea	115	69	Elongate "necked" papilla	267	13	lacking
E. simulata	105	75	Sharply pointed conical apex tapering upward from wide base	270	13	--

*multicellular (1–3 cells); 138–308 µm in total height

This name "race *intermedia*" was used by Keller (1962) but was not validly published. McGregor (1968) noted that crosses between *E. atrorubens* and *E. angustifolia* var. *angustifolia* produced populations of plants as a result of introgression that had many intermediate characteristics, hence the name *intermedia*.

The ray ligule adaxial epidermal cells observed were of two kinds (unicellular and multicellular) and described in detail and illustrated using light photomicrographs (Keller 1962). Unicellular cells have a tapered conical shape (Fig. 3D). These specialized multicellular structures appear in tiers mounted on an enlarged basal cell (Fig. 3C, D). The multicellular structures show a wider range of size: one pyramidal cell (basal cell 90 µm, terminal cell 48 µm, overall length 138 µm (Fig. 3C); two pyramidal cells (basal cell 70 µm, next cell 39 µm, terminal cell 41 µm, overall length 150 µm (Fig. 3C), and three pyramidal cells (overall length 308 µm), (Fig. 3C; Table 2). Ray ligule adaxial epidermal multicellular structures consist of an enlarged basal cell with a neck and a catenuliform series of one, two, or three discrete pyramidal cells (Fig. 3C, D). The multicellular epidermal cells are far fewer in number and scattered among the predominately papillose cells (Fig. 3C). These multicellular, adaxial epidermal cells appear unique and have not been described for any member of the Asteraceae or flowering plant and were not observed in any other *Echinacea* taxa (Table 2). Ray floret secretory chambers are present usually on the abaxial side of each veinlet. A ring of five elliptical epithelial cells surround the chamber lumen.

The stem is heavily covered with trichomes that are shorter, 0.5–1.5 mm, and stouter than in other taxa. Either two or three septa occur with conspicuous lenticular bumps in the trichome wall. A thick cuticle (9 µm) covers the outer tangential wall and cutinization is evident in the inner tangential wall. Prominent short, spike-like projections mark the surface of the cuticle. In surface view the epidermal cells appear rectangular and oblique walled. Stem diameter is ~2.6 mm, including a pith diameter of ~1.3 mm with sclerenchyma cells interspersed throughout. Elliptical pits densely occur in the walls. In transection dark streaks between adjacent sclerotic cells are the result of a black substance (probably phytomelanin seen in the roots) that occludes

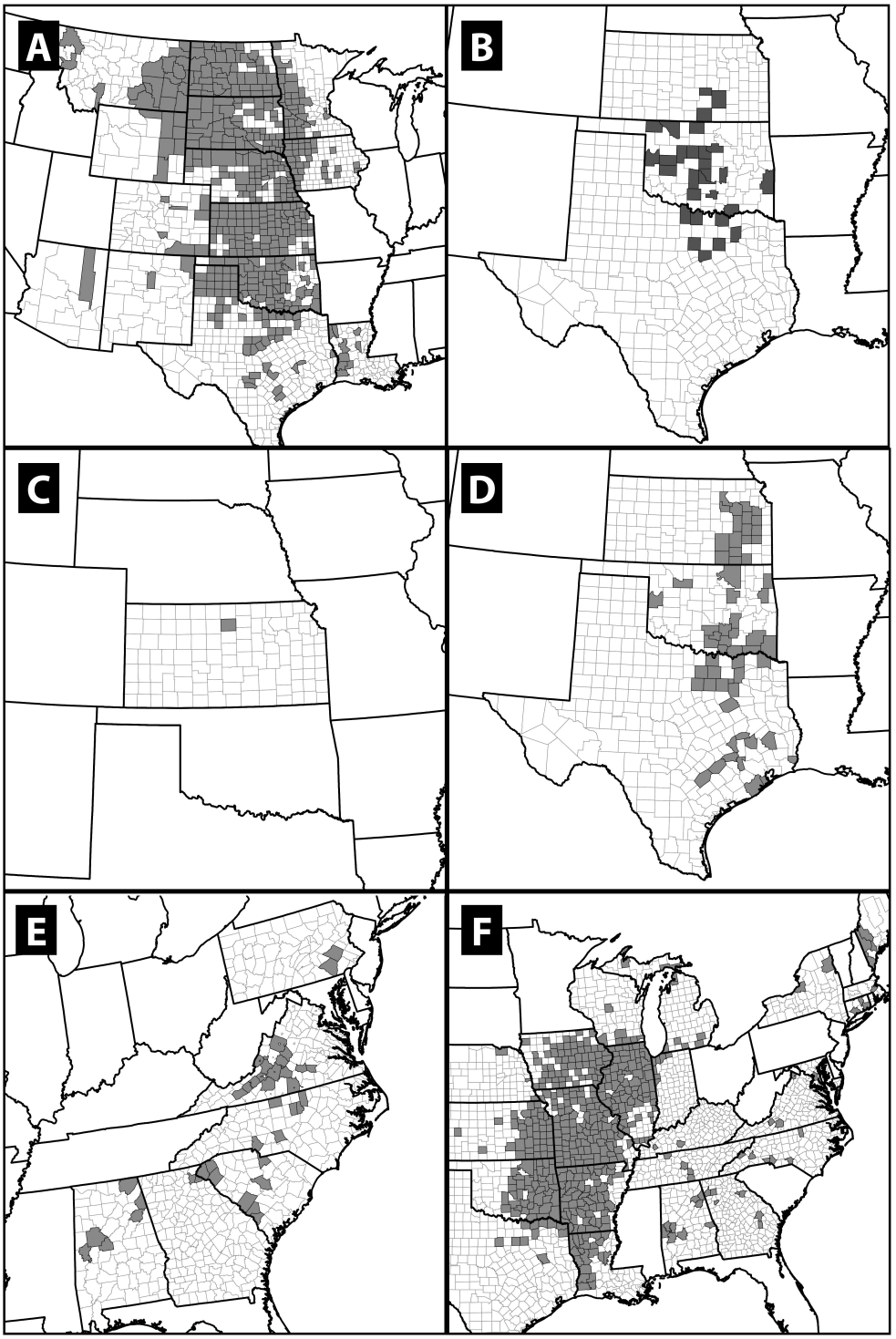


Fig. 1. Distribution maps. **A.** *Echinacea angustifolia* var. *angustifolia*. **B.** *E. angustifolia* var. *strigosa*. **C.** *E. angustifolia* var.*angustifolia* "race intermedia." **D.** *E. atrorubens*. **E.** *E. laevigata*. **F.** *E. pallida*. Maps were recreated from Kartesz (2013).

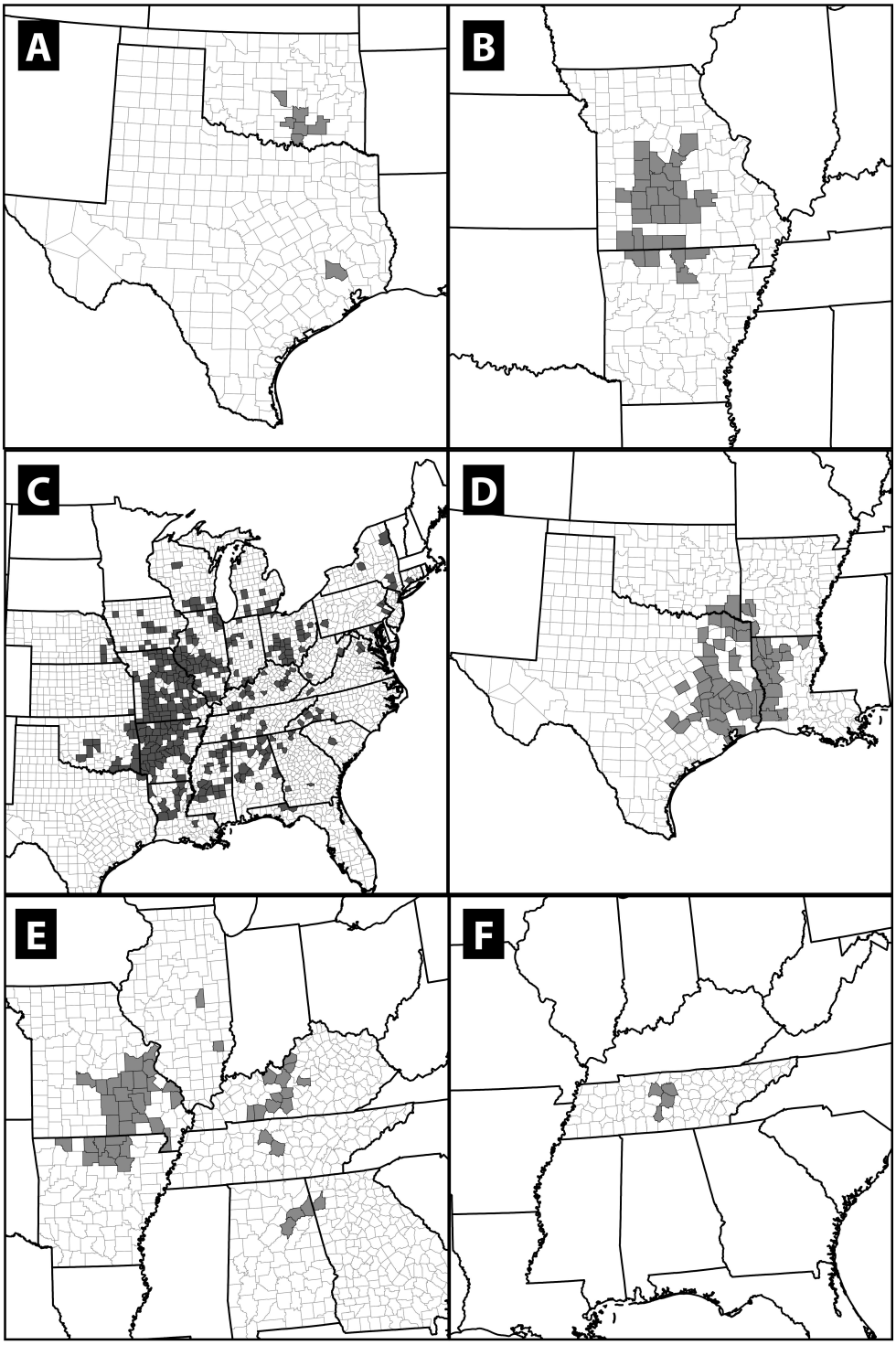
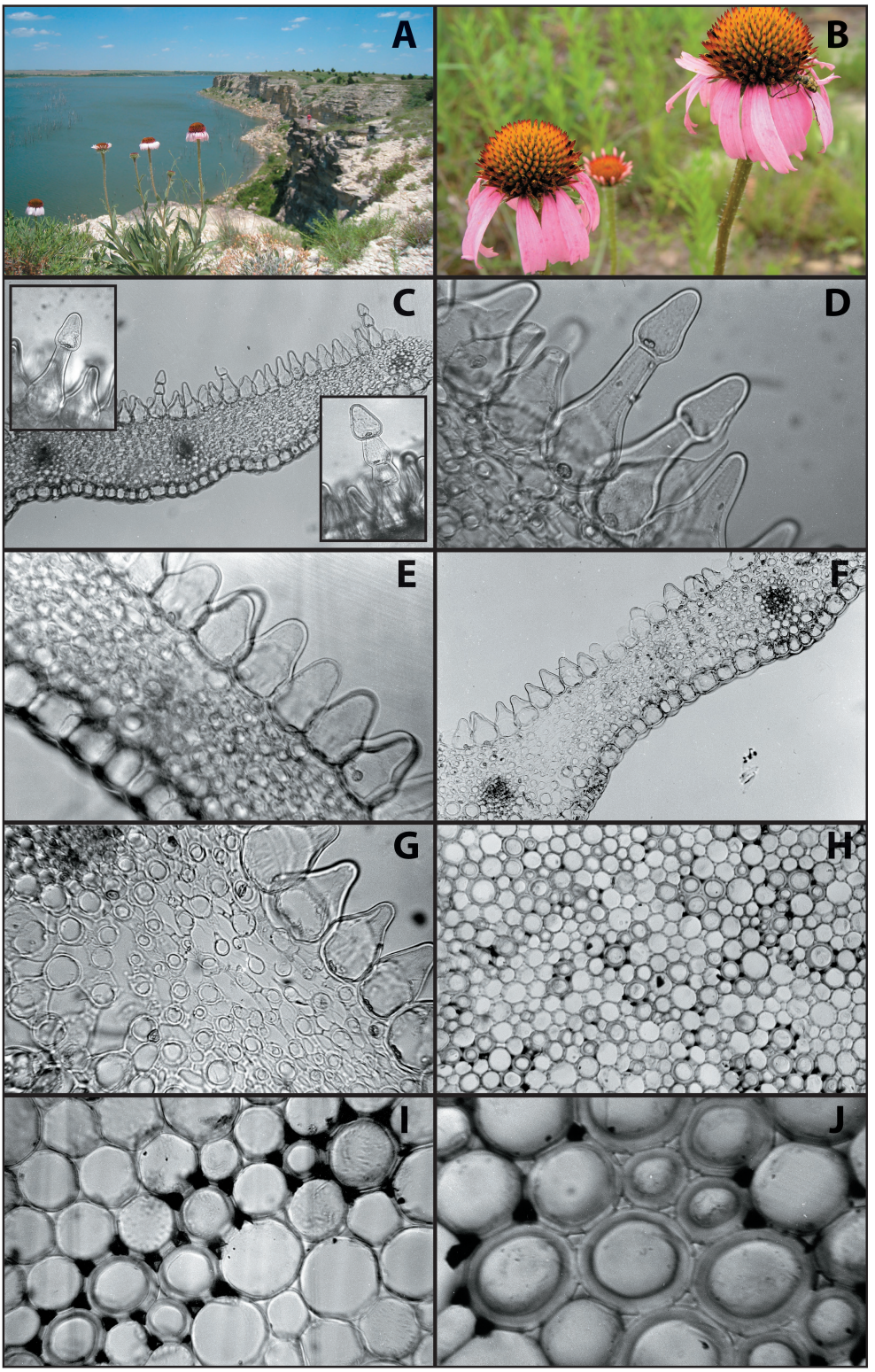


Fig. 2. Distribution maps. **A.** *Echinacea paradoxa* var. *neglecta*. **B.** *E. paradoxa* var. *paradoxa*. **C.** *E. purpurea*. **D.** *E. sanguinea*. **E.** *E. simulata*. **F.** *E. tennesseensis*. Maps were recreated from Kartesz (2013).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angular intercellular spaces (Table 3). A secretory canal system is absent in the pith but present in the cortex opposite the vascular bundle caps and interfascicular region. Five to seven celled epithelial rings surround these smaller canal cavities. Many vessels are tailed with oblique perforations. The vascular bundles are surrounded by a relatively large amount of fibrous tissue. Several vascular bundles seem to have undergone fusion undoubtedly caused by this fibrous tissue (Fig. 10B).

The petiole shows an abaxial convexity which tends to be horizontally flattened on the adaxial surface. A feature readily recognized is the large amount of collenchymatous supporting tissue occupying a position between the vascular bundles and abaxial epidermis. Three major vascular bundles are present in transection (Table 4)

3. Echinacea angustifolia DC.var. **strigosa** McGregor, Trans. Kans. Acad. Sci. 70:366–370. 1968. (**Figs. 1B; 3F, G, H; 9C; 10C; 11B**). STRIGOSE CONEFLOWER

McGregor (1968) recognized populations in the Arbuckle Mountains of Oklahoma, extending as far north as Cowley County, Kansas, and as far south as extreme north central Texas as variety *strigosa* (Fig. 1B). Stems 30–60 cm in height, are frequently branched, flexuous and covered with strigose-hirsute trichomes. Variety *strigosa* hybridizes with variety *angustifolia* and *E. atrorubens* yielding morphologically intermediate populations which in some cases are tetraploids.

The ray ligule adaxial epidermal cells are slightly modified into various shapes. Generally these cells tend to be conical with round corners. The outer tangential wall is slightly drawn out into a papilla. Note the lattice-work arrangement of the mesophyll tissue and air spaces between cells (Fig. 3F, G). Trichomes are present on the lower surface of the ray floret. In *Echinacea* the ground (mesophyllous) tissue is homogeneous and simple in structure. The thin-walled mesophyllous cells have a central cavity with radiating interconnected arms. These cells are loosely arranged into a meshwork of lacunose tissue (Fig. 3G). In transection the mesophyllous cell cavity is cylindrical and elongated in a horizontal plane (running parallel with the vascular system). These mesophyllous cells have outgrowths (arms) that become septate at a point of juncture (Table 2).

Nonglandular trichomes thickly cover the stem. In surface view stem epidermal cells consist of relatively small rectangular cells (Fig. 9C). Secretory canals are only present in the cortex. Sclerenchyma cells occur in the pith (Fig. 3H). Unsclerified cells range in size from 38 to 88 µm (65 µm). Stem diameter is ~2.6 mm, including a pith diameter of ~1.6 mm (Fig. 10C). The discrete vascular bundles are widely separated by an interfascicular region (Table 3).

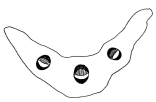
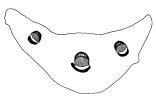
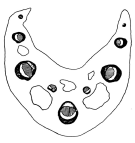
This smaller petiole tends to have the outer margins swinging upward slightly. The "wings" show a leaf-like anatomy made up largely of spongy photosynthetic tissue. Only three vascular bundles supply the petiole. Secretory canals are relatively small, 30 µm, and are sometimes absent from lateral vascular bundles (Table 4).

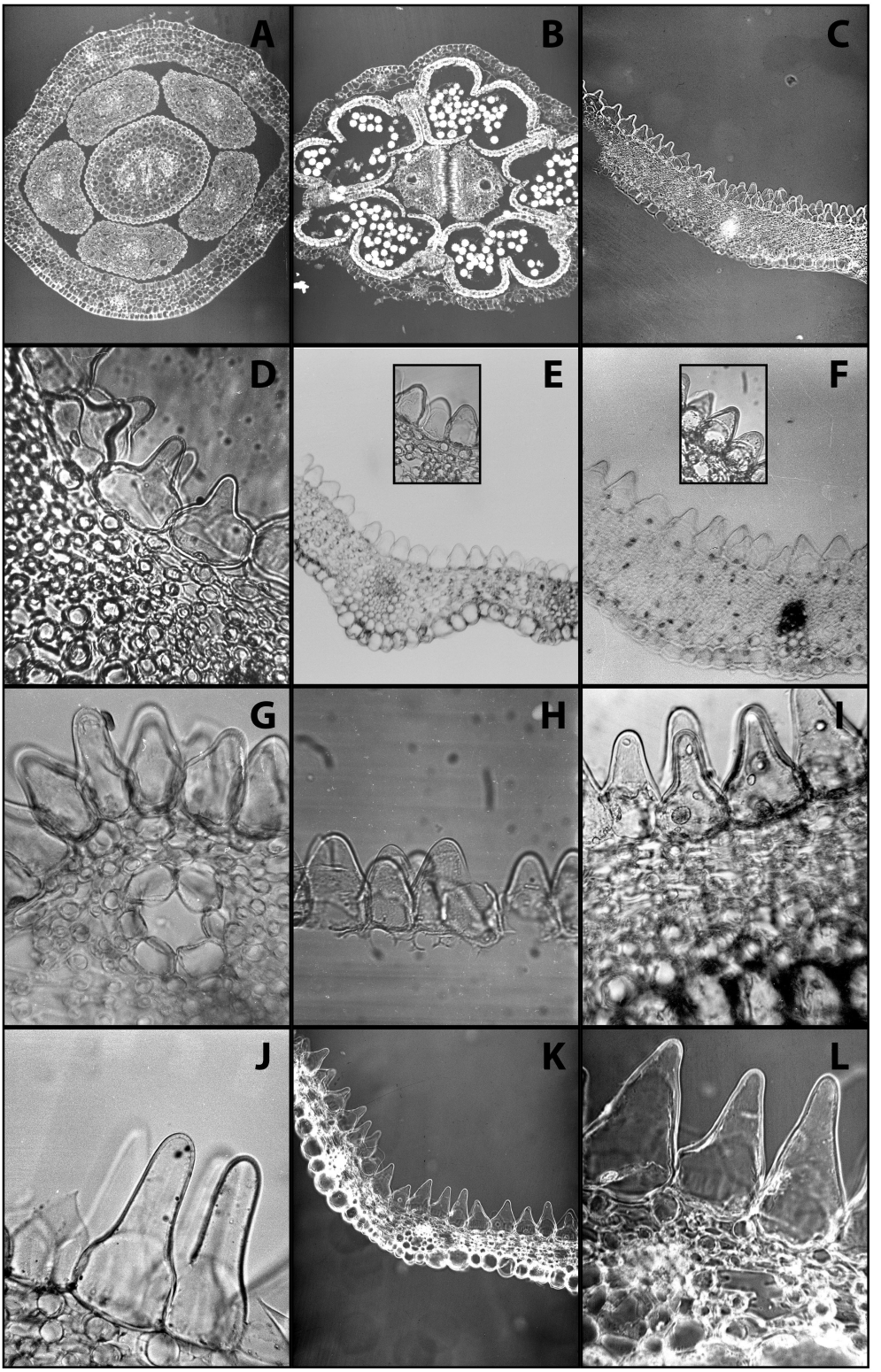
Fig. 3. A,B. ***Echinacea angustifolia*** **var.** ***angustifolia*****. C, D. E.** ***angustifolia*** **var.** ***angustifolia*** **"race intermedia" (free hand transections). Kansas: Mitchell County. Ray ligule showing adaxial epidermal cells of two types (unicellular and multicellular). A. Kansas: Trego County, Cedar Bluff Reservoir, on rocky ledge of limestone cliffs. This site has shallow soil where roots find permanent moisture several feet below, 17 Jun 2010. Note the whitish ray flowers reflexed vertically downward surrounding stem. B. Kansas: Riley County, Fort Riley Military Reservation on rocky, upland, tallgrass prairie growing in shallow, calcareous soil, 30 Jun 2003. Note shorter and downwardly reflexed pinkish ray florets with two or three notched ends and insect lower right (order Coleoptera, family Cerambycidae, subfamily Lepturinae (flower longhorn beetles),** ***Typocerus octonotatus*****), crawling over the cone of sharply pointed paleae. C. Lower magnification showing section of ray ligule with specialized upper epidermis and more typical leaf-like lower epidermis (×53). Close-up insets upper left showing one pyramidal cell and lower right three pyramidal cells. D. Close-up of single pyramidal cell atop enlarged bulbous basal cell (×232). E.** ***E. angustifolia*** **var.** ***angustifolia*****. Kansas: Comanche County. Dome-shaped adaxial ray ligule epidermal cells (×200). F.** ***E. angustifolia*** **var.** ***strigosa*****. Oklahoma: Murray County. Note modified nearly conical adaxial epidermal ray ligule cells in contrast to the isodiametric shape of abaxial cells more typical of leaf epidermis (×62). G.** ***E. angustifolia*** **var.** ***strigosa*****. Oklahoma: Murray County. Ray ligule adaxial epidermal cells showing conical shape with rounded corners and lattice-work arrangement of the mesophyll tissue with air spaces between cells (×200). H.** ***E. angustifolia*** **var.** ***strigosa*****. Oklahoma: Murray Country. Stem microtome transection showing most of pith region with sclerotic cells. Note the black phytomelanin substance deposited in the angles (intercellular spaces) between cells (×70). I.** ***E. angustifolia*** **var.** ***angustifolia*****. Kansas: Comanche County. Stem microtome transection showing thinner walled pith parenchyma cells lacking phytomelanin and the thicker walled sclerotic cells with discrete phytomelanin black deposits (×177). J.** ***E. angustifolia*** **var.** ***angustifolia*****. Kansas: Comanche County. Stem oblique free hand transection showing thicker sclerotic pith cells with variable wall thickness with some triangular intercellular spaces free of black phytomelanin and some with heavy deposits filling spaces (×292). Photo credits: Quinn Long (A), Craig Freeman (B).**

TABLE 3. Stem micromorphology based on transections.

Taxon	Nonglandular trichomes	Epidermal cell mean L/W (µm)	Epidermal cell shape	Stem diam (mm)/ size rank	Pith diam (mm)/features	Meta-xylem rows	Proto-xylem points	Vascular bundle width (µm)	Interfascicular region	Secretory canals (µm)
E. angustifolia var. *angustifolia*	hairy tuberculate, hirsute/hispid	115/34	irregular rectangular	2/10	1/sclerenchyma fibers throughout	3–5	21	350–450	sclerified; no secondary growth	cortex only; ~26 total; 38–43 diam
E. angustifolia var. *angustifolia* "race *intermedia*"	short and stout, 0.5–1.5 mm, 2–3 septa	103/33	Rectangular-oblique walls	2.6/9	1.3/sclerenchyma fibers throughout	3–4	24	450–550	fibrous tissue causes bundle fusion	cortex only; 26–28 total; 33–48 diam
E. angustifolia var. *strigosa*	strigose abundant	49/33	smaller rectangular	2.6/8	1.6/sclerenchyma fibers throughout	2–5	24	250–400	discrete; wide; lacking sclerified tissue	cortex only; ~26 total; 38–55 (43) diam
E. atrorubens	sparsely present	77/38	rectangular iso-diametric	4/5	3/parenchyma cells throughout	3–4	38	300–550; prominent bundle caps	discrete; narrow; no sclerification	pith/cortex; ~20 in pith 45–60 (54) diam and cortex 40–52 (46) diam with conspicuous cavity
E. laevigata	glabrous/ glaucous	150/38	largest trapezoidal irregular oblique-curved walls	5.2/1	4.2/parenchyma cells throughout	--	44	400–575; prominent bundle caps	discrete, narrow, little sclerification	pith/cortex; ~58 in pith and ~48 in cortex; pith 34–50 (45) diam; cortex 40–57 (50) diam
E. pallida	hirsute, 3–5 septa	113/41	rectangular-angulate, trapezoidal-oblique walls	5/3	3.2/parenchyma cells throughout	3–9	34	500–750	sclerification and secondary growth unite vascular bundles into continuous ring	pith/cortex; pith 32–45 (40) diam; cortex 35–46 diam
E. paradoxa var. *neglecta*	sparsely/ densely strigose	80/33	smaller rectangular-oblique walls	3.8/7	3/parenchyma cells throughout	3–5	42	250–450	highly sclerified; no interfascicular region; no secondary growth	pith 30–70 (55) diam; cortex 45–75 (60) diam
E. paradoxa var. *paradoxa*	sparsely/ densely strigose	90/30	rectangular-straight walls	4/6	3/parenchyma cells throughout	4–8	32	300–600	highly sclerified; no interfascicular region; partial secondary growth	pith 30–65 (50) diam; cortex 40–65 (60) diam
E. purpurea	hirsute/ glabrous	115/30	rectangular-angulate, trapezoidal-oblique walls	5/2	3.6/parenchyma cells throughout	5–8	42	250–550; prominent bundle caps	discrete; narrow; little sclerification	pith/cortex; pith ~34; cortex ~48; pith/cortex 34–51 (41) diam
E. sanguinea	hirsute/ glabrous	120/42	mostly rectangular straight walls	1.7/11	0.9/parenchyma cells throughout	3–5	18	200–300	discrete; wide; little sclerification	cortex only; 13–15; 40–48 diam
E. simulata	sparsely/ densely hirsute, 3/5 septa	104/38	rectangular-angulate, trapezoidal-oblique walls	4/4	3.3/parenchyma cells throughout	3–7	31	240–500	sclerification and secondary growth unite vascular bundles	pith/cortex; pith ~36; cortex 20–24; pith 24–50 diam; cortex 41–48 diam

Table 4. Petiole micromorphology based on transections.

Taxon	Shape outline	Shape description	No. vascular bundles	Secretory canals	Canal diam. (µm)	Notable features
E. angustifolia var. *angustifolia*		Somewhat v-shaped	3 throughout	Few (only 1 observed)	40	Sides not steeply inclined
E. angustifolia var. *angustifolia* "race *intermedia*"		More flattened adaxially; abaxially convex	3	Medial	(38)	Collenchyma patches between vascular bundles and abaxial epidermis
E. angustifolia var. *strigosa*		Outer margins swinging upward to become "wings"	3	Small	(30)	"Wings" largely made up of spongy mesophyll
E. atrorubens		Rounded to horse shoe with convex depression	9 (6 minor)	Paired, adjacent to each vascular bundle	--	3 large lacunae running length of petiole from axil to leaf base
E. laevigata		Horseshoe with deep adaxial convexity	5	Poorly developed, medial	(42)	Leaves with sheathing bases account for lengthy "wings"
E. paradoxa var. *neglecta*		Crescent/lunate/ bow	7 (4 minor)	Relatively large, numbering 14	64–84	Brachysclerids (stone cells) scattered throughout fundamental tissue
E. paradoxa var. *paradoxa*		Thickest with nearly flat adaxial surface and exaggerated abaxial curvature	5 (2 minor)	Paired, numerous	40	Chlorenchymatous pockets toward abaxial margin
E. purpurea		Thickened through medial sector with gradual flaring and ascending at ends	5 (2 minor)	Prominent, adjacent to medial vascular bundle	45	Scattered chlorenchymatous tissue creates abaxial bulge
E. sanguinea		Horseshoe nearer stem with ends folded together, unfolding near leaf base	7 (4 minor)	One on each side of abaxial bundle cap	30	Smallest overall size
E. simulata		Lateral margins arch upward, more or less lunate in outline	7 (4 minor)	Few, poorly developed	32	Fan-shaped medial vascular bundle with wide fibrous cap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4. Echinacea atrorubens (Nutt.) Nutt., Trans. Amer. Philos. Soc., n. ser. 7:354. 1840. (**Figs. 1D; 4A, B, C, D; 9D; 10D; 11D, E; 12A**). TOPEKA PURPLE CONEFLOWER.

Plants 50–100 cm tall, mostly unbranched, glabrous below more strigose above, basal leaves petiolate, with the leaf blade oblong-lanceolate. This taxon is found in a narrow band from Houston, Texas, to Ardmore, Oklahoma, northward to Topeka, Kansas (Fig. 1D) (McGregor 1968). It occurs on dry limestone or sandstone outcrops and prairies and is distinguished from *E. paradoxa* var. *paradox*a and *E. paradoxa* var. *neglecta* by its dark purple to dark red and sharply reflexed ray florets that curve inward to a point where they touch the stem.

Disc florets have the typical Asteraceae floral arrangement. Microtome transections near the base show the style, five stamen filaments, surrounded by a corolla tube (Fig. 4A). Another disc floret transection nearer the top shows the mature five bilocular, united anthers with stained pollen grains that surround the two-parted style. The gamopetalous corolla encloses these reproductive structures (Fig. 4B). Transections were made in June at the height of floral anthesis.

Ray ligule adaxial epidermal cells separate this taxon from all other *Echinacea* taxa. The distinctive squatty shapes feature an adaxial epidermal cell with a broadened base and a short "nipple-like" papilla (Fig. 4C, D). Vascular traces are accompanied by very few abaxial secretory chambers (Table 2).

Stem trichomes are sparsely present and often wanting on portions of the stem. Paradermal sections were peeled easily unlike many other species. The epidermal pattern consisted of rectangular, nearly isodiametric cells, with mostly straight end walls (Fig. 9D). Some walls are slanted so these areas may be more irregular. Cortical tissue consists of an outer collenchymatous zone intergrading into an inner parenchymatous region that extends from 350 to 400 µm. The phloem zone ranges from 42 to 56 µm in radial extent. The pith has parenchyma cells 92 µm in length (68 µm) and widths range from 41 to 110 µm (78 µm). The secretory system consists of canals present in both the pith and cortex (Fig. 11D, E). Moreover, an area of thin-walled accessory tissue is arranged above canals, surrounding canals, and not infrequently in the same morphological positions of the canals. Epithelial rings contain four to eight rectangular or diamond shaped cells (Fig. 11 D, E). Tangential divisions occur halfway around the canal to give a partial double ring. Usually a single well-defined canal is located opposite the protoxylem points and other canals originate opposite the interfascicular region. The stem diameter of ~4 mm includes a pith diameter of ~3 mm (Fig.10D). The stellar pattern has numerous discrete vascular bundles with prominent bundle caps separated by narrow interfascicular regions (Table 3). Sclerified intervening cells between vascular bundles and secondary xylem is lacking as shown in the tissue map (Fig. 10D).

Petiole outline is more or less rounded to horseshoe shaped except for a convex depression on the abaxial side. Each major bundle is associated with two resin canals located one on each side adjacent to the abaxial fibrous cap. The canals that accompany the central vascular bundle are relatively large, 40 µm, with five epithelial cells. Consequently they are conspicuous and well differentiated from the surrounding cells. The vascular bundles are almost perfectly rounded and completely ensheathed by fibrous tissue. Three air spaces or lacunae form passageways throughout the length of this petiole (Fig. 11D; Table 4). Serial sectioning demonstrated air spaces that begin near the stem axis ultimately terminating at the leaf base (Fig. 11D). Although *E. paradoxa* var. *paradoxa* has air spaces, they appear close to the leaf base but beyond the point where sections were made.

FIG. 4. **A, B.** Microtome transections of *Echinacea atrorubens* capitulum (×50). **C–L.** Free hand transections of ray ligules showing shapes of adaxial epidermal cells. **A.** Disc floret nearer the base showing the style, the five-parted stamen filaments, surrounded by corolla tube. **B.** Disc floret nearer the top showing the two-parted style surrounded by mature five-bilocular, united anthers with stained pollen grains and gamopetalous corolla. Note absence of sclerotic tissue. This floral arrangement is typical of the Asteraceae. **C.** *E. atrorubens* at low magnification showing nipple-like apex in upper left and more variable cells in lower right (×50). **D.** *E. atrorubens* at high magnification of C showing nipple-like apex and broadened base (×200). **E.** *E. laevigata* showing dome shape, undifferentiated mesophyll, lack of secretory chambers, and uniform isodiametric abaxial cells (×50). **F.** *E. pallida* showing dome-shape (×50) and close up inset. **G.** *E. paradoxa* var. *neglecta* tapering upward to rounded apex with secretory chamber below in mesophyll tissue (×200) and close up inset. **H.** *E. paradoxa* var. *paradoxa* showing dome shape (×200). **I.** *E. purpurea* showing ball-like base tapering to rounded apex (×200). **J.** *E. sanguinea* showing elongate "necked" apex and broader base (×200). **K.** *E. simulata* showing sharply pointed apex from a wider base (×50). **L.** *E. simulata* higher magnification detail showing conical cells largest in *Echinacea* (×200).

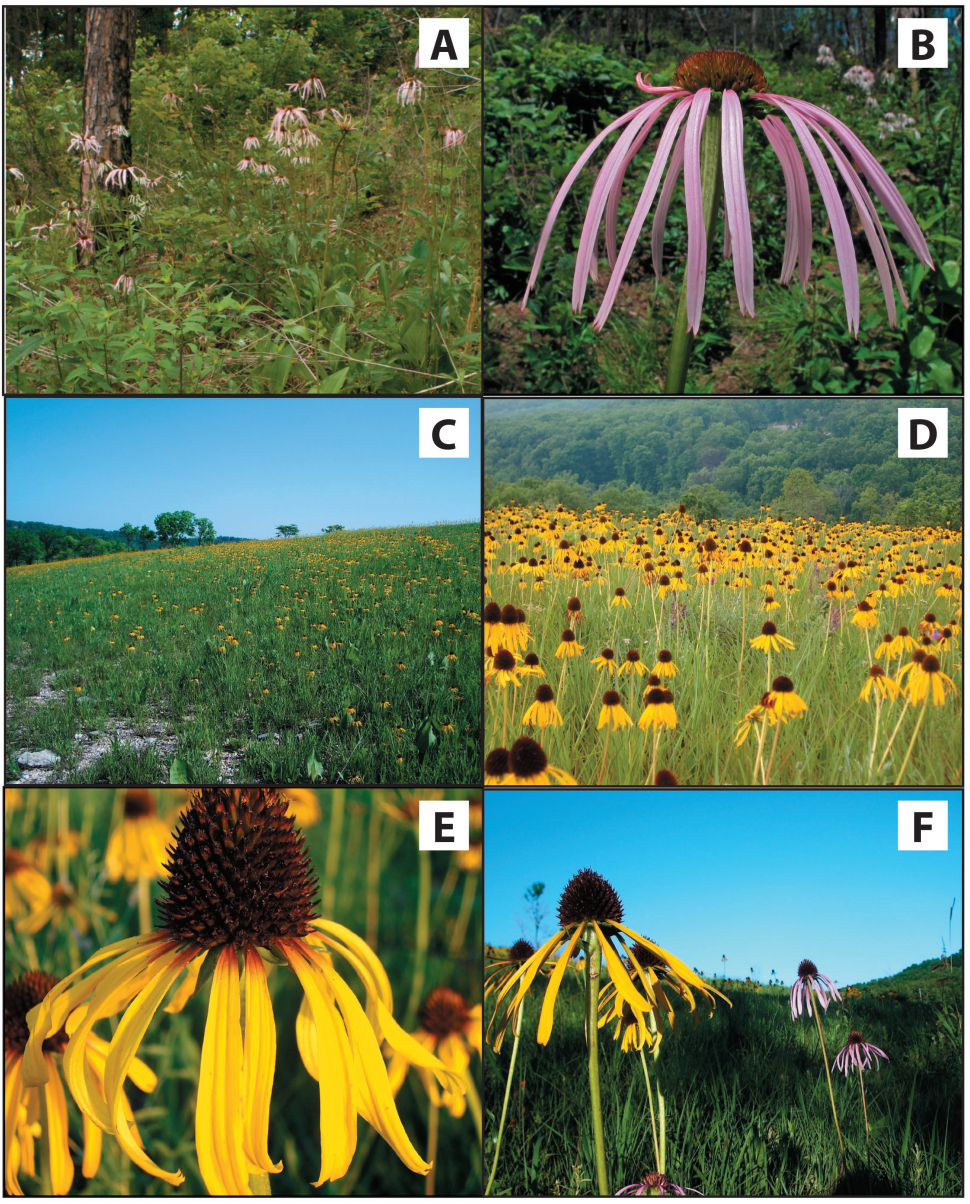

Fig. 5. **A, B.** *Echinacea laevigata* in sunny natural habitats of North Georgia, 28 May 2008. **A.** Stephens County, Chattahoochee National Forest at Currahee Mountain. The plants shown here occur in small groups scattered among other forbs growing in open forest areas on rocky soil mostly of amphibolite. Note the long, narrow purplish to white reflexed ray florets. **B.** Same site as A, showing close up of glabrous stem and long, narrow, drooping, purplish ray ligules with bifid tips. **C–F.** *E. paradoxa* var. *paradoxa* natural habitats include open sunny glades at Ha Ha Tonka State Park, Camden County, Missouri. **C.** Heimbeaugh Hill Glade. This area historically has been maintained with fire and current management includes the removal of *Juniperus virginiana* trees and prescribed burns. This more xerophytic west-southwest facing site is characterized by multiple layers of dolomite dating from the early Ordovician period and these areas are sometimes referred to as "balds" or "barrens." Note that yellow coneflower predominates as a single taxon carpeting the entire area in late May and early June. **D.** Close up of previous site from different direction showing synchronous blooming of the taller *Echinacea* plants forming a canopy over the shorter prairie grasses with tree line in background. **E.** Close up of capitulum with prominent raised cone of paleae and yellow reflexed ray florets with notched ends and crimson coloration at point of insertion shown later in blooming period. **F.** *E. pallida* at glade edge. It typically grows here near the edge or margins of glades with yellow coneflower. Photo credits: Hugh & Carol Nourse (A, B), Christopher D. Crabtree (C–F).

5. Echinacea laevigata (C.L. Boynton & Beadle) S.F. Blake, J. Wash. Acad. Sci. 19:273. 1929. (**Figs. 1E; 5A, B; 9E; 10E**). SMOOTH PURPLE CONEFLOWER.

Stems erect, usually unbranched and glabrous, 55–110 cm high, leaves petiolate, ray florets purplish or light pink to whitish, spreading to reflexed. A distinct taxon found outside the distribution range of most *Echinacea* taxa in open woods of Pennsylvania scattered in the Piedmont area south to the mountains of Georgia. Natural habitats occur in sunny openings in forested areas maintained by wildfires and grazing animals. Thus preferred sites are open woods, glades, cedar barrens, roadsides, clear cuts, dry limestone bluffs, and power line rights-of-way associated with soils rich in magnesium, calcium, dolomite or limestone and rocky substrates of amphibolite (Fig. 5A, B). It is most closely related to *E. purpurea* but differs in having a vertical caudex and taproot, unbranched glabrous and glaucous stems, and leaf blades broadly lanceolate or elliptical mostly glabrous, with ray florets longer and more slender (Fig. 5B). This is the second taxon of *Echinacea* that was Federally listed as an Endangered Species (USFWS 1992).

Ray florets are pale pink to purple and lack trichomes, demonstrating the glabrous character of this taxon. The venation pattern consists of 15 vascular traces in transection supported by abundant thick-walled tissue. The uniformity in size and dome-shape of the ray ligule adaxial epidermal cells is the most obvious character. Secretory chambers are apparently lacking (Table 2).

The smooth and glaucous characters of the stem distinguish this taxon from *E. purpurea* and other taxa of *Echinacea*. In every dimension the epidermal cells are larger than in other species. Moreover, a striking pattern of straight, oblique, and curved walls demonstrates the irregularity in cell shape (Fig. 9E). Only the outermost layers of the cortex consist of typical collenchyma. The greater part of the cortex is parenchymatous tissue with large intercellular spaces (10 µm). The pericyclic fibers give a weak phloroglucinol test. They are mostly thin and needlelike in structure and are easily broken into fragments. The end walls taper to a sharp point with lengths that vary from 360 to 1150 µm (794 µm) and widths from 8 to 20 µm (13 µm). The phloem zone is 60–70 µm in radial extent. Macerated tissue had vessels that range in length from 159 to 450 µm (302 µm) and widths range from 23 to 34 µm (29 µm). Some of the scalariform vessels are tailed with oblique perforation plates. Little sclerification occurs between vascular bundles (Fig. 10E) and none was present in the pith. Measurement of stem diameter was ~5.2 mm, including the pith (~4.2 mm) with pith cell lengths that range from 60 to 174 µm (121 µm) and widths from 60 to 97 µm (80 µm) (Table 3).

The secretory system was represented by canals in the cortex and pith. Those of the cortex are relatively abundant and large with usually five to eight epithelial cells originating opposite the vascular bundles and/or interfascicular region. About half of the vascular bundles have single canals and the others occur in pairs. Canals in the pith form double rings of epithelial cells as a result of a second periclinal division with an outer ring of elliptical cells and inner one of rectangular cells. Pith canals have five to eight epithelial cells that surround the canal cavity (Table 3).

The petiole in transection appears horseshoe-shaped. Leaves of this taxon tend to have sheathing bases and this accounted for the lengthy "wings" (Table 4). Fundamental tissue extends to the very end of each wing which fails to show a graduation into foliar anatomy. A uniseriate layer of cutinized cells underlies the epidermis that is structurally indistinguishable from it. This double-layered tissue forms a definite boundary at the periphery of the petiole. Five vascular bundles are seen in transection (Table 4).

6. Echinacea pallida (Nutt.) Nutt., Trans. Amer. Philos. Soc. II. 7:354. 1840. (**Figs. 1F; 4F; 5F; 6E; 9A (h); 10F; 11C**). PALE PURPLE CONEFLOWER.

This taxon is an apparent segmental allotetraploid possibly derived from a hybrid between *E. simulata* and *E. sanguinea* with a doubling of the chromosome number to $2n = 44$. It has white pollen (Fig. 6E) and the largest pollen grains in the genus, 24–28.5 µm in diameter (26.1 µm) when compared to the most closely related *E. simulata* with pollen grains 22.5–24.5 µm in diameter (24.2 µm) and other *Echinacea* taxa with much smaller pollen grains (McGregor 1968). This taxon flowers in late spring and early summer in rocky prairies, open wooded hillsides, savannas, and glades concentrated in eastern Kansas and Oklahoma, western Arkansas, and throughout Missouri. Plants are rarely branched, 40–90 cm up to 140 cm high, stem trichomes hirsute below

Fig. 6. *Echinacea purpurea* (A–D), *E. pallida* (E), and *E. simulata* growing in natural habitats in Arkansas. **A.** Saline County, open woodland growing in a clustered group, 16 Jun 2003. **B.** Saline County, Ouachita National Forest, open woodland along creek in shale barrens growing with *Rudbeckia grandiflora* var. *grandiflora*, 3 Jul 2003. **C.** Clark County, Terre Noire Natural Area, 6 Jul 2004, close up of capitulum showing brightly colored paleae, broad ray ligules with complex venation pattern, and bifid notched ends. **D.** Saline County, close up of capitulum showing visit of Zebra Swallowtail Butterfly, order Lepidoptera, family Papilionidae, genus *Protographium*, Jun 2001. **E.** *E. pallida*, Arkansas, Boone County, open areas on acidic chert, Baker Prairie Natural Area, 8 Jun 2013. Note bumblebee (*Bombus* sp.) on dark purplish paleae with contrasting traces of white pollen indicative of this tetraploid. **F.** *E. simulata*, Arkansas, Boone County, dolomite glades, Jun 1996. Note the yellow pollen adhering to the paleae and also the hirsute stem. Photo credits: John Pelton (A, B, D, F), Mark Clark (C), Joan Reynolds (E).

and more dense above, ray florets reflexed, sparsely hairy abaxially (McGregor 1968; Urbatsch et al. 2006). Ray florets vary from almost entirely dark rose red to reddish purple in eastern Kansas and throughout Missouri to whitish that predominates in eastern Oklahoma and western Arkansas and farther south to Texas and Louisiana (McGregor 1968). An excellent current source of general macromorphological and medical information is available for *E. pallida*, including color photographic images of habit and root microanatomy (Upton 2010).

Some irregularity in shape was apparent in the ray ligule adaxial epidermal cells, intergrading from a short bullet shape to a more conical or slightly dome-shaped papillose cell (Fig. 4F). Noticeable microscopically were trichomes of various lengths on the abaxial surface. A large amount of thick-walled supporting tissue enveloped the 13 vascular traces (Table 2).

Trichomes are moderately scattered over the stem surface described by McGregor (1968) as "hirsute on both surfaces." Three to five septa divide each trichome into four or five cells at irregular intervals (Fig. 9A, h). Generally the trichome is slender and tapered to a sharp point with lengths that range from 1 to 2 mm. In surface view the epidermis has angulate and rectangular cells; this is expressed in transection notably in the irregularity of epidermal cell size and shape (Fig. 9F). Paradermal sections were difficult to make due to the grooves and thick cuticle. The cuticular layer was 7.0 µm thick, bearing tiny spine-like projections on the surface. The inner tangential walls were heavily cutinized (Table 3).

The cortical zone including the endodermis and epidermis measures 180 µm at the narrowest point and 430 µm at the broadest. Collenchymatous tissue makes up most of the cortex. In macerated preparations collenchyma cells range in length from 110 to 280 µm (189 µm) and in width from 35 to 46 µm (45 µm). Pericyclic fibers as seen in macerated preparations range in length from 450 to 1450 µm (1056 µm) and in width from 40 to 18 µm (27 µm) with a lumen diameter of 8–16 µm. Xylem elements consist of vessels, fibers, and xylem parenchyma. Macerations of vessel elements ranged in length from 215 to 610 µm (378 µm) and in width range from 40 to 51 µm (48 µm). No annular vessels or tracheids are present. All vessels have simple perforation plates. The protoxylem consists mainly of spiral vessels, and the metaxylem, with scalariform to definite reticulate pits, seems to be developmentally more advanced than in *E. simulata*. Pith diameter is ~3.2 mm with no sclerenchyma cells present (Table 3). The stem tissue map shows cambial activity that unites the collateral vascular bundles into a mostly solid ring due to some secondary growth in the interfascicular regions (Fig. 10F). The pith consists wholly of parenchymatous tissue with length of cells ranging from 74 to 135 µm (101 µm) and widths that range from 72 to 110 µm (105 µm) (Table 3).

Secretory canals are present in both cortex and pith. Undeveloped canals have approximately four epithelial cells and a small canal cavity. These arise through radial (anticlinal) divisions sometimes followed by tangential divisions that give a rectangular shape to epithelial cells. With the completion of these divisions, the epithelial ring consists of at least six cells with an enlarged cavity. Canals of the pith appear singly, in pairs, or in triplets opposite the protoxylem points. Three to seven epithelial cells delimit the pith canals with small patches of thin-walled cells (11–15) designated as accessory tissue staining green with fast green stain. The pith canals in this species tend to anastomose, still, however, maintaining their individual identity. Cortical canals are spherical in shape. Each bundle cap has one canal on each side lying adjacent to the endodermis in the interfascicular region.

7. Echinacea paradoxa (Norton) Britton var. **neglecta** McGregor, Trans. Kansas. Acad. Sci. 70:366–370. 1968. (**Figs. 2A; 4G; 10G; 11F–G; 12C–F**). BUSH'S PURPLE CONEFLOWER.

Plant stems 30–80 cm high, usually unbranched, yellowish green, sparsely to densely strigose similar to variety *paradoxa*. This taxon is easily distinguished from var. *paradoxa* by its rose-colored, purple or white ray florets and a geographic distribution confined to the Arbuckle Mountains of southwestern Oklahoma in rocky prairies and open, wooded hillsides (McGregor 1968).

Ray ligule adaxial epidermal cells have one to three secretory chambers arranged on both sides of the vascular trace and scattered throughout the mesophyll tissue. These cells have a wide range in size that nearly corresponds to those of the stem, leaf, and petiole secretory systems. Each secretory chamber has an interior cavity of approximately 50 µm surrounded by three to seven epithelial cells. This is similar to the canals of the

stem and petiole, (Fig. 4G). Ray adaxial ligule epidermal cells have outer tangential walls that result in sides that are parallel about three-fourths the way up, sloping gradually into a rounded apex (Fig. 4G). These cells are highly variable and in some cases distinctly papillate. The two varieties, *paradoxa* and *neglecta*, have adaxial ray ligule epidermal cells that are similar in size and shape (Table 2).

Trichomes are sparse or lacking but with distinctive epidermal patterns. In surface view epidermal cells are uniform in shape but significantly smaller in width. The breadth of cortical tissue extends from 230 to 500 µm. Outer portions of the cortex mostly consists of collenchyma while the inner portion intergrades into parenchymatous tissue. The phloem zone extends from 46 to 56 µm. Stem diameter is ~3.8 mm including a pith diameter of ~2.9 (Fig.10G; Table 3). Pith cells range from 137 to 257 µm (189 µm) with widths from 33 to 65 µm (47 µm) and in longisection are densely pitted.

Stem secretory canals are present in both the pith and cortex. Many canals are tangentially flattened especially the larger ones. Canals occur either in ones, twos, or threes opposite protoxylem points (Fig. 11D). Epithelial rings consist of 5–15 cells rectangular in shape. Accordingly the canal cavity is relatively large from 20 to 36 µm surrounded by 8–14 cells (Fig. 11G). Canals originate opposite both the vascular bundle caps and interfascicular regions. The newly formed canals are much smaller and confined to the endodermal layer; while those embedded in the cortical tissue are well developed and relatively large (Table 3).

A lunate to bow-shaped petiole is the result of a greater lateral overall thinness (Fig. 12C). Canals are well formed (distinct epithelial cells surrounding a large cavity) and larger than can be found elsewhere in the genus. Comparatively some of these canals are double the size of even the largest canals of other taxa. The total number of canals present (14) far exceeds counts made for other taxa. These canals are located typically on the abaxial side of the vascular bundles and some atypically on the adaxial side. The medial vascular bundles had pairs of canals on both the abaxial and adaxial sides. Well-formed canals have an extremely large cavity 31–43 µm in diameter surrounded by a well-differentiated ring of epithelial cells (Table 4). In other taxa canals are surrounded by four to six epithelial cells but here 5 to 15 cells make up the epithelial layer. Furthermore, the epithelial cells have a distinctive shape closely approaching a rectangular shape; while others found in different *Echinacea* taxa usually are more elliptic with curved anticlinal walls.

Apparently no taxon in the genus *Echinacea* has brachysclerids or stone cells in aerial plant parts except in the petioles of var. *neglecta* (Fig. 12D, E, F; Table 4). These sclerids occur as single, conspicuous cells with highly refractive thickened walls that make them appear as shiny, glistening structures reminiscent of the clustered stone cells in pear fruit mesocarp (Fig. 12E). More noticeable was the concentric layering of the thickened walls and the branched ramified pit canals revealed by a preferential stain. In this case the walls were heavily lignified (stained with safranin) throughout, including the thickened secondary walls (Fig. 12D). The sclerids were scattered throughout the fundamental tissue as seen in Fig. 12D, E, F). Similar brachysclerids also were found in roots of *E. angustifola* (Axentiev et al. 2010) and *E. pallida* (Upton 2010).

8. Echinacea paradoxa (Norton) Britton var. **paradoxa** in N.L. Britton and A. Brown, Ill. Fl. N. U.S. ed. 2, 3:476. 1913. (**Figs. 2B; 4H; 5C, D, E; 9G; 10H**). YELLOW CONEFLOWER.

This taxon is easily recognized by its bright yellow ray florets either drooping or reflexed (Fig. 5E). It differs in color from all other *Echinacea* species that have deep purple, pinkish, to white ray florets. General habit of the plant is 40–90 cm tall, stems usually unbranched, yellowish green, sparsely to densely strigose (McGregor 1968). A relatively small distribution area is restricted to rocky and upland prairies, limestone and dolomite cedar glades, savannas, bald knobs and also roadsides of west-central and southern Ozarks of Missouri and north central Arkansas (Yatskievych 2006). Native wild populations of strikingly beautiful yellow coneflowers sometimes dominate the open field landscape in the Ozark cedar glades, especially in Ha Ha Tonka State Park, Missouri (Fig. 5C, D; Crabtree 2008; Richter 2013; H.W. Keller, pers. obsv.).

Ray ligule adaxial epidermal cells are wide at the base with straight parallel sides that become convex at the apex (Fig. 4H). The overall dome shape and size are similar in both var. *neglecta* and var. *paradoxa*. One chromoplast occurs in each cell. A secretory system is well differentiated in size, frequency, and arrangement. Secretory chambers are associated with vascular traces and occur on both adaxial and abaxial sides; each

chamber is conspicuous mainly due to their large cavities and the three to seven peripheral epithelial cells (Fig. 9G). The ray floret secretory system is similar in size and number in both varieties but exceeds in size and greater numbers the canals in all other *Echinacea* taxa, including the closely related *E. atrorubens* (Table 2).

Only a few trichomes were observed. The stem lacks grooves at this position so the paradermal sections show more uniformity in cell shape. This stem morphology results in a fairly constant rectangular shape of the epidermal cells in surface view (Fig. 9G). In transectional view many cells are squarish instead of the typical tabloid epidermal cell. Anticlinal walls of epidermal cells are thin and straight. The cortex is made up of an outer collenchymatous zone and an inner parenchymatous tissue with large intercellular spaces. Lengths of collenchyma cells range from 90 to 228 µm (180 µm) and widths range from 28 to 61 µm (45 µm). Pericyclic fibers range from 475 to 200 µm (1190 µm) and widths range from 16 to 23 µm (20 µm). The average wall thickness is 6 µm with a lumen of 6–10 µm. The phloem zone is 50–95 µm in radial extent. Vessels range in length from 325 to 650 µm (485 µm) and widths range from 23 to 50 µm (33 µm). Some secondary growth was observed in the stem (Fig. 10H). Comparison of stem tissue maps for varieties *neglecta* (Fig. 10G) and *paradoxa* (Fig. 10H) appear almost identical. Vascular bundles are connected by interfascicular activity with some sclerification that results in a more or less solid vascular cylinder without any interfascicular areas (Fig. 10H).

Pith parenchyma cells are 83–215 µm in length (190 µm). No thick-walled sclerotized cells are present in the pith region. The secretory system is represented by canals in both the pith and cortex. Secretory canals occur in the pith next to the protoxylem points and in the cortex opposite the vascular bundle caps and interfascicular regions in both varieties. Secretory canals occur in the pith next to the protoxylem points and in the cortex opposite the vascular bundle caps and interfascicular regions in both varieties. Epithelial rings consist of three to six cells positioned to give an hourglass or star-shaped cavity usually much reduced in size adjacent to the endodermis. Cortical canals originate opposite vascular bundle caps and interfascicular regions. Epithelial rings consist of five to eight cells (Table 3).

This taxon has the thickest petiole studied with an exaggerated curvature on the abaxial side that is discernible even macroscopically. Canals of the secretory system are comparatively numerous occurring in pairs instead of singly. Toward the abaxial margin of the petiole abundant chlorenchymatous pockets are interspersed among the collenchyma tissue. Five vascular bundles are noted in transection (Table 4).

9. Echinacea purpurea (L.) Moench., Methodus 591. 1794. (**Figs. 2C; 4I, 6A, B, C, D; 9H; 10I**). ($2n = 22$). EASTERN PURPLE CONEFLOWER.

This taxon has a deep fibrous root system that distinguishes it from all other *Echinacea* taxa. Distribution is more widespread but concentrated in the central plains states and then north and southeasterly in rocky open woods, thickets, and prairies (Fig. 2C; McGregor 1968). It is the most widely distributed taxon and characterized by its tall branching habit of 60–180 cm; awns of paleae as long as body; lower leaves ovate and often toothed; and dull purple to pinkish ray florets, spreading to recurved, 3–8 cm long 7–14 (–19 mm) wide (Fig. 6A–D). The larger colorful ray florets have encouraged the development of hybrids and cultivars grown commercially and as ornamentals in gardens.

Externally the gross morphology of the ray florets reflects the greater size in breadth but internally size is expressed by the increased degree of venation and the 31 vascular traces in transection. Comparatively *E. purpurea* has the broadest ray florets of all the taxa studied (Fig. 6C). Secretory chambers measure 40 µm in diameter with each canal surrounded by five epithelial cells.

Ray ligule adaxial epidermal cells have a wide ball-like base that gradually taper to a rounded apex (Fig. 4I). This shape contrasts with the shorter, convex shape of *E. laevigata* adaxial epidermal cells. In addition the broader ray florets of *E. purpurea* have a more complex venation pattern that differs from *E. laevigata* with more narrow ray florets and venation greatly reduced (Table 2).

Nonglandular trichomes thickly cover the stem. The base of the trichome is swollen and accentuated by an outgrowth of surrounding epidermal cells. Repeated sectioning was required to get thin paradermal peels. Heavy pubescence along with grooved stems undoubtedly caused this difficulty. In surface view the margin of the cuticle is wavy and rough in outline. Striae or rod-like bodies decorating the surface of the epidermis were

Fig. 7. ***Echinacea sanguinea*** **in sunny open field habitats. A. Wood County, Texas, 3.2 miles southeast of Winnsboro along State Highway 37 at edge of field near roadside ditch. Scattered groups of plants growing on sandy soil known geologically as the Queen City Sand, 22 May 1989. B. Nearby site 9.4 miles southeast of Winnsboro along State Highway 37 showing four capitula with whitish ray flowers and hirsute stems. Note the smallest stem diameter found in** ***Echinacea*** **and spindly habit. C. Same site as B showing close up of a single capitulum in early anthesis with spreading white ray ligules notched at tips. D. Same site as B showing close up of later stage anthesis. Note the reflexed ray florets and faint purplish colors at point of insertion. Photo credits: Bob O'Kennon.**

ascribed to cuticle depositions. Epidermal cells have some rectangular end walls which are usually oblique and angulate (Fig. 9H; Table 3).

The cortex contains an outer, several-layered, narrow zone of collenchyma cells and an inner, many-layered, loosely arranged broad zone of parenchymatous tissue. Collenchyma cell lengths range from 111 to 157 µm (131 µm) and widths from 21 to 37 µm (30 µm). Pericyclic fibers give a moderate phloroglucinol test as shown by the bundle caps. Fibers range in length from 663 to 2400 µm (1288 µm) with widths from 8 to 22 µm (15 µm). The average wall thickness is 5 µm and lumen size is 7 µm. The phloem zone radially extends from 59 to 73 µm. Vessel lengths range from 232 to 680 µm and widths from 20 to 42 µm. Pith diameter is ~3.6 with parenchymatous cell lengths from 92 to 130 µm with widths from 93 to 110 µm. No sclerids are present (Fig. 10I).

The secretory system consists of canals in both the pith and cortex. Those of the cortex tend to be tangentially flattened and are located opposite both vascular bundle caps and interfascicular regions. Epithelial cells are in direct contact with the endodermis and are not well differentiated from surrounding parenchymatous tissue. Epithelial rings of five to eight cells surround canals. Cortical canals number more than 48 in compari-

son to the less numerous pith canals (~34) centric or excentric in position. Epithelial cells vary from elliptical to subspherical shapes with no tangential divisions. The vascular stele is broken up into a dictyostele. Medullary rays pass out into the cortical tissue (Table 3).

Photosynthetic tissue is scattered among the parenchyma cells that make up the abaxial bulge of the petiole. Secretory canals are prominent when found adjacent to the medial vascular bundle, becoming progressively smaller as the lateral vascular bundle size decreases. Five vascular bundles are evident in sectional views; two more are diminutive but recognizable. The central vascular bundle lacks fibrous tissue on the abaxial side (Table 4)

10. Echinacea sanguinea Nutt., Trans. Amer. Phil. Soc. n. ser. 7:354. 1840. (**Figs. 2D; 4J; 7A–D; 9A-i–j, I; 10J; 12B**). SANGUINE PURPLE CONEFLOWER.

This is a distinct taxon often confused in the past with *E. pallida*. However, it differs in having hemispheric capitula, slender and more spindly stems, 40–90 cm high and sometimes reaching 122 cm in roadside ditches of East Texas (Fig. 7A–D; Bob O'Kennon pers. obsv.). It has narrower ray florets, elliptical leaves, yellow pollen, and is a diploid (n = 22). Distribution is more restricted to acidic, sandy soils, open pine barrens, woodlands, and prairies of southeastern Oklahoma, southwestern Arkansas, western Louisiana, and east Texas (McGregor 1968). It flowers in May in East Texas and gradually becomes a more rose color in a cline northward with later anthesis (Fig. 7A–D). Ray florets color and flowering times were genetically fixed from the original habitats while growing in the KU Experimental Gardens (McGregor 1968). This is the only taxon of *Echinacea* that has a more southerly distribution in the central states and thus differs from all other taxa.

Ray ligule adaxial epidermal cells have distinctive elongate, "necked" papillae that differ from all other taxa (Fig. 4J; Table 2). Trichomes are absent. No secretory chambers were present.

Short, stout trichomes thickly cover the stem. The trichome usually has fewer septa with the base heavily cutinized (Fig. 9A-i–k, I). Epidermal patterns consist of straight, mostly rectangular cells (Fig. 9I).

Collenchyma tissue makes up the greater part of the cortex except for several parenchymatous layers outside of the endodermis. Lengths in these elongated, tapered cells range from 151–373 µm (180 µm) and widths range from 40–45 µm (43 µm). Breadth of the cortical region ranges from 190–230 µm. Pericyclic fibers are not fragile or needle-like but are thicker with blunt or truncate ends with lengths from 300–1350 µm (850 µm), and widths 17–21 µm. The phloem zone in radial section is 22–45 µm (Table 3). Vessel lengths range from 275–950 µm (523 µm), and widths range from 20–35 µm (20 µm) (Fig. 11).

Echinacea sanguinea has the smallest stem diameter (~1.7 mm in width and pith diameter of ~0.9 mm) studied which reflects the spindly habit of this taxon (Fig. 10J). No sclerenchyma fibers are present in the pith (Table 3). Pith parenchyma cells ranged from 89–176 µm in length (137 µm), and widths from 41–105 µm (72 µm). The walls of the parenchyma cells are densely marked with elliptical and conspicuous primary pit fields.

The secretory system has canals that are interfascicular in origin with13–15 canals in the cortex. A brownish substance appears in some of the epithelial cells, making their presence conspicuous. Canals range from 40–48 µm with either a five or six-celled epithelial ring (Fig. 11A). The relatively small amount of vascularization reflects in part the spindly habit of the plant. Furthermore, interfascicular rays are sclerified, previously mentioned as a distinguishing character for other species (Table 3).

Gross morphology of the petiole varies based on the location either near or distant to the stem. Close to the stem the petiole is heart-shaped in transection with the ends folded together. Nearer the leaf base the petiole unfolds allowing reorientation of the margins. Secretory canals are relatively small arranged one on each side of the abaxial bundle cap (Fig. 12B). Four minor vascular bundles were present and the medial vascular bundle lacks an abaxial fibrous cap (Table 4).

11. Echinacea simulata McGregor, Sida 3:282. 1968. (**Figs. 2E; 4K–L; 6F; 9A-a–g, k, I; 10K; 11H**). WAVY-LEAF PURPLE CONEFLOWER.

This species was cited as *E. speciosa* McGregor by Keller (1962), but that name was never validly published. In the past it was included in *E. pallida*, but the pollen size in *E. simulata* is smaller and yellow as compared to

white in *E. pallida*. Stems 60–120 cm tall are mostly unbranched with sparsely to densely pustular based trichomes. It occupies a distinct geographic area restricted mostly to north central Arkansas, eastern Missouri, western Illinois, and west-central Kentucky (Fig. 2D). The diploid chromosome number of $2n = 22$ contrasts with *E. pallid*a, a polyploid with a chromosome number of $2n = 44$ (McGregor 1968).

This taxon has the largest adaxial epidermal ray ligule cells. The distinctive conical shape of the cell with a wide base and sides tapering upward that forms a sharp point, distinguishes this taxon from all other *Echinacea* taxa (Fig. 4K–L). Secretory chambers are present having either four or five epithelial cells. Thickness of the ray ligule is comprised mostly of the larger adaxial and abaxial epidermal cells. Trichomes are present on the abaxial surface and vascular traces number 13 in transection (Table 2).

Nonglandular trichomes are moderately scattered on the stem described by McGregor (1968) as "hirsute or somewhat tuberculate hirsute." These slender, uniseriate hairs gradually taper toward the apex. The terminal cell is highly variable and often structurally modified into a rounded or sharp point. Most of the trichomes have three septa, occasionally five in longer ones (Fig. 9A,-k). Marks sculptured in the trichome wall are lenticular in shape. The ontological sequence of the trichome conforms to the pattern in which only epidermal cells undergo division. However, the trichome is raised on a supporting base formed from both epidermal and sub-epidermal cells (Fig. 9A-a–g). In surface view they appear morphologically distinct from the surrounding epidermal cells and have a highly irregular outline, accounting for the wide range in size (Fig. 9J). This no doubt is due to the grooved surface of the stem which tends to give more rectangular shaped cells in the grooves, and more angulate cells on the ridges. In transection the cross diameter of epidermal cells is uniform, unlike the length, which varies considerably (Table 3).

The cortex has small pockets of thin-walled chlorenchymatous cells that underlie each sub-stomatal chamber. These fan out short distances around the stem and are interrupted at intervals by collenchyma which directly abuts the epidermis. Generally three types of collenchyma cells are recognized: angular, lamellar, and tubular. All three types often intergrade, but, the tubular type with intercellular spaces and angular thickenings is predominant in *E. simulata*. General shapes consist of elongate cells having unevenly thickened walls, with either rectangular, oblique, or tapering ends. A transitional region between cortical collenchyma and parenchyma occurs within one or two rows outside of the endodermis. Size and shape of collenchyma cells were studied from longitudinal sections and maceration: lengths range from 124 to 280 µm and widths range from 27 to 41 µm.

The uniseriate endodermis in transection is recognizable by the elliptic, thin-walled cells lacking pits. A starch test with a potassium iodide solution gave a positive reaction (a dark blue-black color) confined mostly to the endodermal layer. Instead of giving a blue ring, groups of two or three cells were filled with starch grains; then, for some distance, cells were void of starch. The starch granules measure 43 µm in diameter and appear roundish with a roughened surface.

The pericyclic fibers of the bundle caps in transection extended radially from 83 to135 µm (Fig. 4). Macerated tissue produced fibers between 322 and1341 µm (761 µm) in length and width 9.7–31 µm (16 µm) that varied in shape with some tapered to a sharp point, others blunt, and still others, truncate. Lumen diameter ranges from 4 to 24 µm (11 µm). All fibers have slit-like pits and give a strong positive phloroglucinol reaction. The phloem zone measures 51–75 µm in radial extent. No crystals or storage products are present. Stem transections show that the number of rows of metaxylem vessels ranges from three to seven with interspersed fibers (Table 3). Macerated preparations show vessel lengths vary from 197 to 644 µm. The end walls are completely dissolved, resulting in the simple perforation type in end view. Shapes vary from barrel-shaped with horizontal end walls while others are oblique and pointed. No annular vessels were observed and tracheids were absent. Vessel widths range from 24 to 42 µm with spirals either loose to close that range from 364 to 1008 µm and pitted vessels range from scalariform to elliptical.

Stem diameter was ~4 mm, including a pith diameter of ~3.3 mm, with intercellular spaces of 1 µm. Pith cells are nearly isodiametric, loosely arranged, and densely pitted. Cells in the center are larger, merging toward the periphery into smaller thicker-walled cells. The cells supporting vascular bundles tend to become

sclerotized, especially surrounding the resin canals. No sclerotic cells are present in the center of the pith. Secretory canals originate adjacent to and through the division of the endodermis forming within the interfascicular region, the exception being where surrounding cells of the vascular system undergo positional rearrangement, tending to relocate the canal along the ascending arc of the bundle caps. Canals also are found either singly or in pairs closely associated with the vascular bundle at the periphery of the pith (Fig. 11H). These appear to be an integral part of the vascular bundle, but they actually form outside of and opposite the protoxylem points, becoming surrounded by cells that are highly lignified (Table 3).

Pith canals number 36 with diameters from 24 to 50 µm. Such a great range can be attributed to the developmental stage of the canal. Smaller canals have reduced cavities, and larger, relatively fewer epithelial cells. The larger canals have an enlarged cavity and smaller, relatively more epithelial cells that range in size from 17 to 22 µm between anticlinal walls. The number of epithelial cells lining the cavity varies from four to seven. Epithelial cell shape varies from square to oblong rectangular, sometimes ovoid but always the walls form an oblique angle. Each epithelial cell has a dense cytoplasmic content with a conspicuous nucleus. When present in pairs they appear in a juxtaposition sometimes spatially separated by as much as 100 µm. Cortical canals tend to be crushed in sectional view and are not well differentiated from the surrounding cortical parenchyma.

Stem diameter is relatively large in part because of sclerification and continuous growth in the interfascicular region (Fig. 10K). Stellar configuration seems to follow a circular design with secondary growth in only a portion of the stem. It is interesting to note that both *E. pallida* and *E. simulata* have interfascicular growth to a degree not seen in other *Echinacea* taxa (Table 3).

A striking feature of the petiole not found in other species is the fan-shaped medial vascular bundle. It has a wide fibrous cap gradually sloping adaxially as the phloem and xylem diminish in lateral extent. Seven vascular bundles can be seen in transection. The canal system is not well developed; in fact, canals associated with the more lateral vascular bundles are reduced greatly in size. The tips of the petiole arch upward and give a more or less lunate-shape in outline. Some petioles have loosely arranged parenchymatous tissue orientated adaxially near the epidermis, however, in *E. simulata*, this area is compacted and constituent cells have smaller intercellular spaces (Table 4).

12. Echinacea tennesseensis (Beadle) Small, Man. Southeast Fl. 1421, 1509. 1933. (**Figs. 2F; 8A–F**). TENNESSEE PURPLE CONEFLOWER.

This taxon's microanatomy was not included here because live plants were not available in the KU Experimental Gardens. McGregor (1968) described its distribution as an endemic restricted to the "dry, gravelly hills and barrens near LaVergne, Tennessee" (Rutherford County). Specimens were only available from herbaria so McGregor (1968) confined his observations to the type specimen. He recognized *E. tennesseensis* as a distinct species most closely related to *E. angustifolia* var. *angustifolia,* noting that it was "a very rare or possibly extinct species endemic to the Cedar Barrens area of central Tennessee." However, it differs morphologically in the smaller stature, 10–50 cm tall, softer pubescence, smaller pollen grains, leafier stem, and ray florets ascending rather than drooping (Fig. 8A–F)

A review article by Walck et al. (2002) notes that *E. tennesseensis* was one of the first plant species listed on the Federal Endangered Species list in 1979. It was listed in Tennessee as endangered and protected under the Rare Plant Protection and Conservation Act of 1985. Five additional populations of this species were found in Davidson, Rutherford, and Wilson counties (Fig. 8A–F) in the vicinity of Nashville as part of the Central Basin region of Tennessee. Several population sites near Nashville were destroyed when land was cleared for housing developments. In these localities it occurs in a general area referred to as the Cedar Glades (*Juniperus virginiana* L.) on outcrops of Ordovician-age Lebanon Limestone. The United States Fish and Wildlife Service removed *E. tennesseensis* from the Federal List of Endangered and Threatened Plants delisted in the Federal Register 2 Sept. 2011 (USFWS 2011). The discovery and recovery of additional populations of this species represent the cooperative efforts of scientists, especially Elsie Quarterman, and conservation groups over the last 30 years.

Nonglandular trichomes are present on the stem either sparsely or thickly covering the surface in all *Echinacea*

Fig. 8. *Echinacea tennesseensis* in sunny, open natural habitats in Wilson County, Tennessee, on a site derived from soils of Ordivician age limestone (Lane Farm State Natural Area). These areas have shallow soils and more xerophytic conditions referred to as cedar (*Juniperus virginiana*) glades or barrens often interspersed with Little Bluestem grass. Photographs taken the evening of 21 Jun 2013 and the morning of 23 Jun 2013 at the peak of anthesis. **A.** Landscape panoramic view of glade where Eastern Red Cedar trees occur around the marginal edge and *Echinacea* dominates in patches backlit by the evening sun. **B.** Morning hours showing capitula facing the sun. **C.** Patchy distribution on gravelly, rocky site. **D.** Top view of capitulum showing 13 marginal ray florets ascending upward and not reflexed downward with two-notched tips. **E.** Underside view of capitulum showing the hairy whorl of phyllaries subtended by hairy fluted stem. **F.** Side view of entire flower and stem showing ascending ray florets with two and three notched tips. Photo credits: Todd Crabtree.

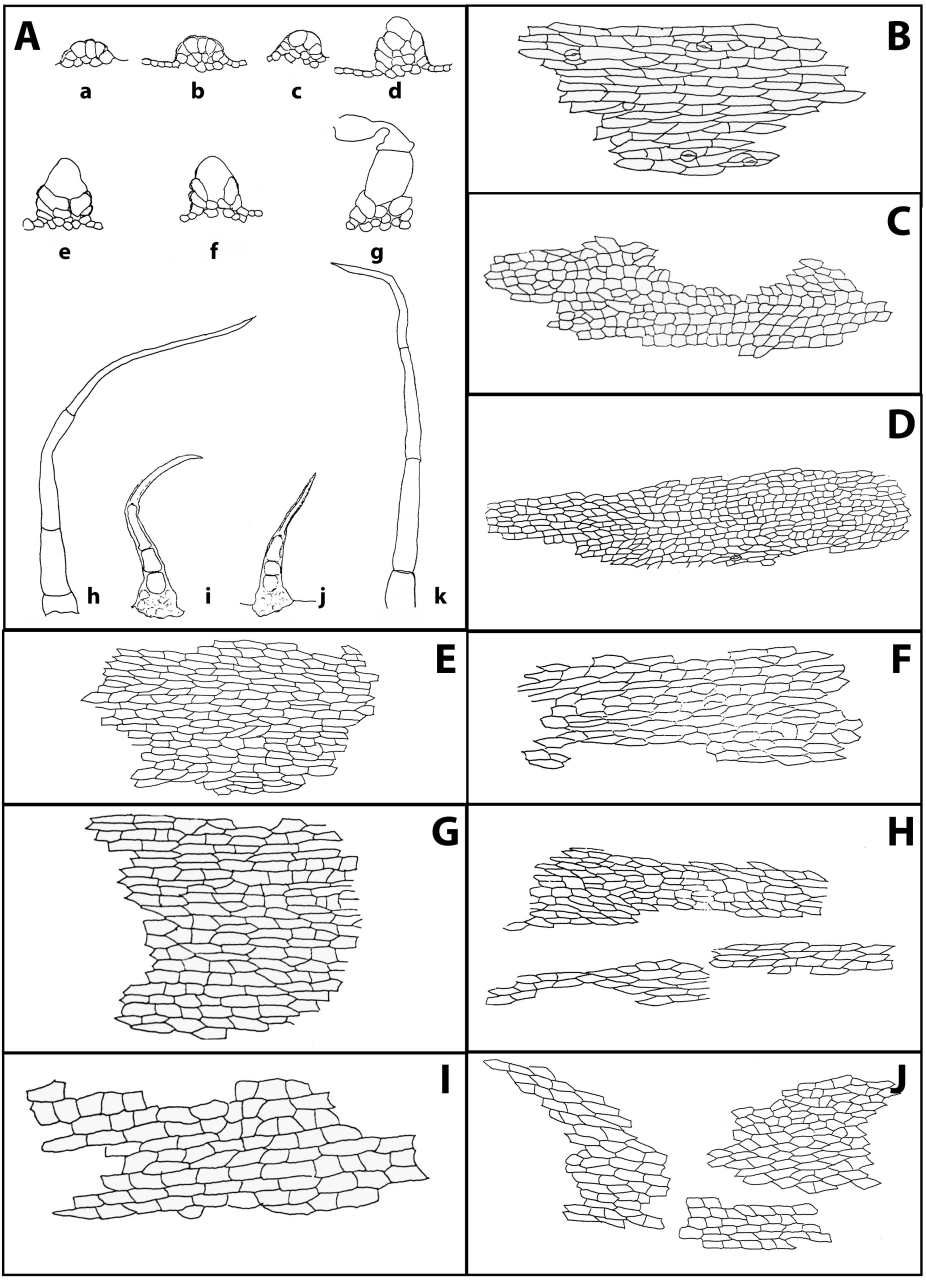

Fig. 9. Stem paradermal peels showing line drawing illustrations of trichomes. **A.** *Echinacea simulata* developmental sequence of nonglandular, uniseriate, multiseptate trichome (a–g). Note that the epidermal and sub-epidermal layers give rise to three to five septate trichomes with sharply pointed ends (h, *E. pallida* with four septa; i–j, *E. sanguinea* with three septa, greatly thickened walls and reduced lumen; k, *E. simulata* with five septa in longer flexuose trichome) (×78). **B–J.** Stem paradermal peels showing line drawing illustrations of epidermal cell shapes and sizes (all images ×30). **B.** *E. angustifolia* var. *angustifolia* showing irregular pattern with four anomocytic type stomata in field of view. **C.** *E. angustifolia* var. *strigosa* showing rectangular cells. **D.** *E. atrorubens* showing mostly rectangular cells with straight end walls in middle portion. **E.** *E. laevigata* showing the largest cells in irregular sizes and shapes with mostly oblique to curved walls. **F.** *E. pallida*. Note irregular angulate and rectangular cells. **G.** *E. paradoxa* var. *paradoxa*. Note mostly rectangular cell shapes. **H.** *E. purpurea*. Note oblique and angulate end walls. **I.** *E. sanguinea*. Note mostly rectangular cells. **J.** *E. simulata*. Note irregular outline from trapezoid to rectangular.

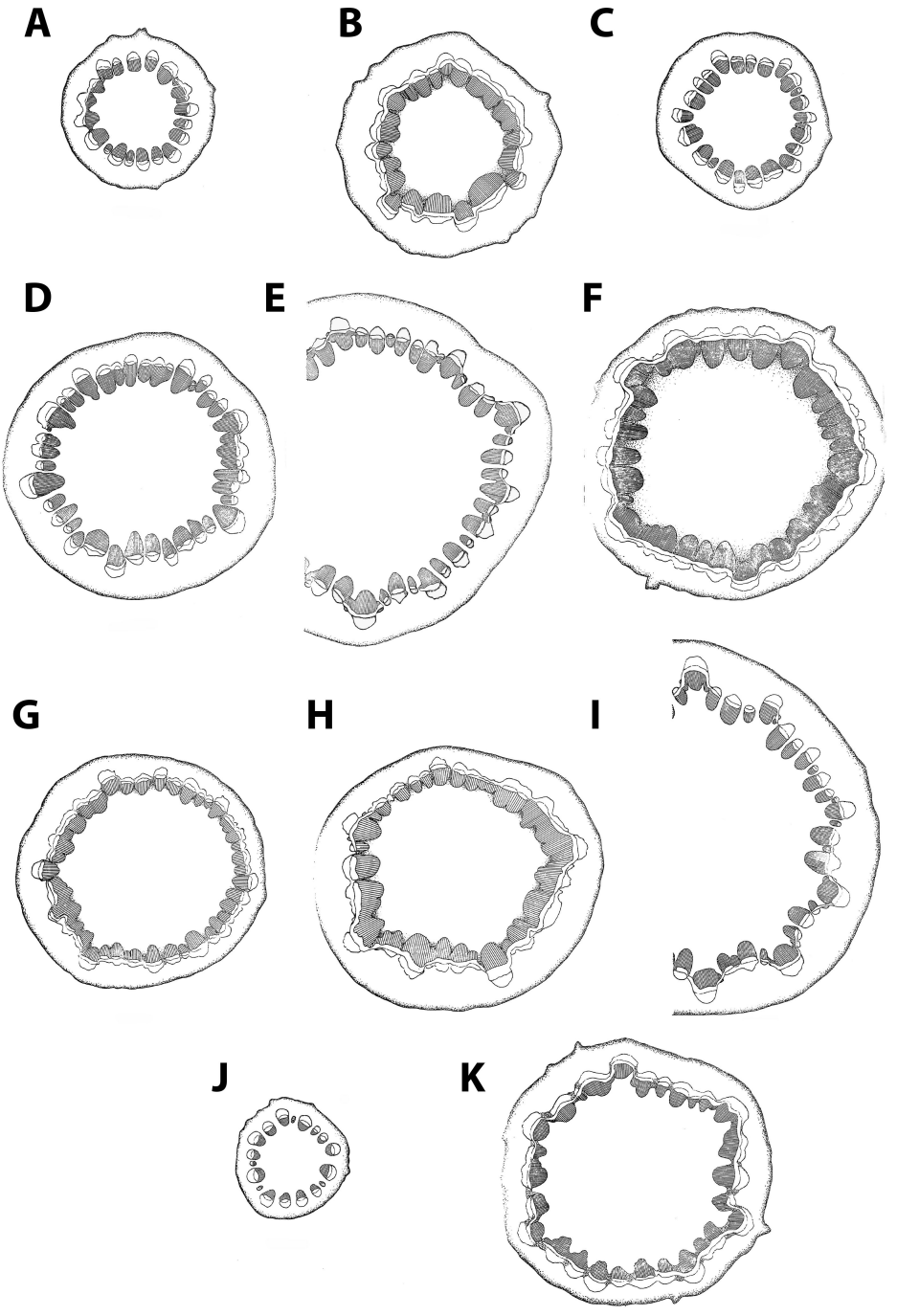

Fig. 10. *Echinacea* stem transections compared as tissue maps at same magnification (×8.8). **A.** *E. angustifolia* var. *angustifolia*. **B.** *E. angustifolia* var. *angustifolia* "race intermedia." **C.** *E. angustifolia* var. *strigosa*. **D.** *E. atrorubens*. **E.** *E. laevigata*. **F.** *E. pallida*. **G.** *E. paradoxa* var. *neglecta*. **H.** *E. paradoxa* var. *paradoxa*. **I.** *E. purpurea*. **J.** *E. sanguinea*. **K.** *E. simulata*.

taxa with the exception of *E. laevigata* where they are lacking. These trichomes are simple but variable in size with three to five septa and usually sharply pointed tips (Fig. 9A). Lenticular markings always appear in the trichome wall. All trichomes form on a morphologically distinct area of the epidermis (Fig. 9A, a–g). The grooved (fluted) stem is extended directly below the capitulum for variable distances in different taxa (Fig. 8E). This grooved surface area accounts in part for the irregular shapes and sizes of stem epidermal cells in paradermal peels (Fig. 9B–J).

Throughout the genus only anomocytic stomata occur which have an irregular outline surrounded by a limited number of cells which cannot be distinguished from other epidermal cells (Fig. 9B). The subsidiary cells usually found in other stomata types are absent. This type of stomata opening is also called the ranunculaceous type because it commonly is found in the family Ranunculaceae (Fig. 9B; Metcalfe & Chalk 1950).

Surface views of stem epidermal cells show more irregular patterns of straight, oblique, and curved cell wall shapes with overall larger dimensions as in *E. laevigata* (Fig. 9E). In striking contrast, *E. angustifolia* var. *strigosa* had the smaller stem epidermal dimensions and a more regular rectangular to squarish cellular pattern (Fig. 9C). *Echinacea paradoxa* var. *neglecta* had a distinctive pattern of rectangular to more trapezoidal-like shapes with slanted end walls (Table 3). Stem epidermal cells of all 11 *Echinacea* taxa studied were illustrated with line drawings by Keller (1962).

Cortical tissue in the near-surface region of the stem outside of the vascular bundles is modified into chlorenchymatous tissue with neighboring amounts of parenchymatous cells with much larger intercellular spaces. This photosynthetic tissue is manifest in the greenness of the stem. The cortical zone is comprised mostly of collenchyma with thickened cell walls that abut on the smaller intercellular spaces (Fig. 11B). All *Echinacea* taxa apparently have collenchyma in this region to strengthen and support the aerial system of the stem. Stem tissue systems in *Echinacea* lack crystals of any type as well as lacticiferous canals and mucilaginous cells. Secretory canals are present adjacent to and originate through the division of the endodermis. All species have secretory canals located either opposite the vascular bundle caps and/or interfascicular region. When secretory canals occur in the pith, they originate opposite the protoxylem points (Table 3).

All *Echinacea* taxa have a distinct, continuous endodermal layer or layers. Carbohydrates appear confined to the endodermal tissue as granular starch grains with roughened surfaces (Fig. 11C). Macerated stem tissues consisted of xylem elements either spiral, scalariform, and reticulate vessels, xylem parenchyma, fibers but no tracheids. Phytomelanin-coated sclerids found in the vascular tissue in the roots of *E. angustifolia* and *E. pallida* were not observed in the stem tissue preparations of the xylem and phloem.

GENERAL DICHOTOMOUS KEY TO *ECHINACEA*

This key to *Echinacea* taxa is based on macro- and microcharacters of ray florets, stems, and petioles. *Echinacea tennesseensis* is not included here and is only known from cedar barrens near Nashville, Tennessee.

1. Stem pith tissue with sclerotic cells scattered throughout ____ **E. angustifolia** var. **angustifolia, E. angustifolia** var. **strigosa,** and hybrids

1. Stem pith tissue lacking sclerotic cells.
   2. Secretory canals present only in cortex; stems slender, less than 2 mm diameter ____ **E. sanguinea**
   2. Secretory canals present in pith and cortex; stems stouter, more than 2 mm diameter.
      3. Stem diameters >4.5 mm; >42 protoxylem points.
         4. Ray ligules 5–6 mm; ~15 vascular traces; stems and leaves glabrous; plants with a taproot; distribution restricted to southeastern U.S.A. ____ **E. laevigata**
         4. Ray ligules 7–12(19) mm wide traversed by ~31 vascular traces; stems and leaves bearing trichomes; plants with fibrous root system and horizontal rhizome; distribution mostly in Missouri and Arkansas with scattered populations farther east ____ **E. purpurea**
      3. Stem diameters <4.5 mm; <42 protoxylem points.
         5. Petioles with stone cells ____ **E. paradoxa** var. **neglecta**
         5. Petioles lacking stone cells
            6. Ray florets bright yellow ____ **E. paradoxa** var. **paradoxa**
            6. Ray florets purple, red, pink, or white
               7. Petioles with three air spaces; ray ligules adaxial epidermal cells with a broadened base and a short "nipple like" apex ____ **E. atrorubens**
               7. Petioles lacking air spaces; ray ligules adaxial epidermal cells dome shaped to conical.

8. Ray ligules adaxial epidermal cells distinctly conical and largest in size (83–125 µm in height); pollen yellow _____ **E. simulata**
8. Ray ligules adaxial epidermal cells dome shaped and smaller in size (58–82 µm in height); pollen white ____ **E. pallida**

DISCUSSION

Ray ligules

Baagøe (1977a) assessed and discussed the functional role of ray ligule adaxial epidermal cells in the Asteraceae. Although *Echinacea* was not included in her study, the highly specialized adaxial epidermal cells occurring here and in a broad spectrum of unrelated taxa suggest that this epidermal type is a genetically fixed functional adaptation rather than ontogenetically developed structures based solely on growth rates and other factors.

There are two main morphological properties of ray ligule adaxial epidermal cells: their size and shape. Functionally, these relate to the absorption of visible or ultraviolet light that plays a role in insect pollination biology or the surface-to-volume ratio as the increased height increases the larger surface area to various external physical properties or physiological activity. It is likely that the interaction between these two selection pressures, namely pollination biology and physiological adaptations, play a major role in shaping adaxial ray ligule epidermal cells (Baagøe 1977a).

This was apparent when Noda et al. (1994) reported that petal conical cells in comparison to flat cells increased the proportion of incident light that entered epidermal cells, enhancing light absorption by the pigments (anthocyanins) and thus increasing the intensity of petal color. Experimental evidence came from a *mixta* mutant of *Antirrhinum majus* that had petals composed of flat hexagonal-based cells that changed the mutant cell morphology of petal color to a slightly paler and less velvety surface. Conical adaxial petal cells had more sparkle that attracted bumble bee pollinators and a velvety surface that facilitated clinging to the petal surface.

A more recent review paper by Whitney et al. (2011) noted the variation of conical adaxial epidermal cells of *Geranium procurrens*, *Helianthus annuus*, and *Hibiscus trionum*. These epidermal cells were considered as they relate to petal color, petal reflexing, petal scent production, petal wettability, and insect pollinator grip on the flower surface. They recognized at the outset that conical epidermal cells found on adaxial surfaces of flowers are a special feature of petals rarely found on leaves or any other plant surfaces. Floral conical cells had the ability to focus light into epidermal vacuoles that contain anthocyanins, increasing color saturation of the petal and, with the scattering effect from the mesophyll, was more even than found in flat cells and therefore resulted in a brighter sheen or velvety surface. A series of observations was made noting, (1) petal reflexing with conical cells that stand more upright and presented a larger surface or target area to attract pollinators (bumblebees), (2) flat-celled petals were more wettable than conical-celled petals, (3) conical cells were self-cleaning and aided in removal of dirt particles or potential fungal pathogenic spores, and (4) more recent convincing evidence shows that bumblebees can discriminate by tactile touch alone the difference between conical and flat-celled surfaces on petals but have a clear preference for the conical surface (Whitney et al. 2011).

Echinacea florets are visited and pollinated by bumblebees (*Bombus* spp., H.W. Keller, pers. obs.), butterflies, and longhorn beetles (Figs. 3B; 6D, E). The co-evolution of insect pollinators suggests there is selection pressure toward specialized ray ligule adaxial epidermal cells in *Echinacea* as observed in the variation of cell shapes and sizes. There are no flat adaxial epidermal ray flower cells in any *Echinacea* taxa. The presence of curious multicellular ray ligule adaxial epidermal cells found only in *E. angustifolia* "race *intermedia*," and lacking in the closely related varieties *angustifolia* and *strigosa*, merits special consideration because these anatomical structures previously were not included in publications. Their presence or absence is complicated by a limited sample size that may have overlooked their presence in other related taxa. Not knowing the cause or frequency of such a micromorpholgical character in a colony, hybridized population, or species makes it difficult to interpret its diagnostic significance and utility. A survey of the literature indicates that these trichomes are rarely found on the adaxial surface of petals. Their function may be protective against desiccation or predation, produce secretions, or as in this case of *Echinacea*, their function appears to be unknown. These cells are

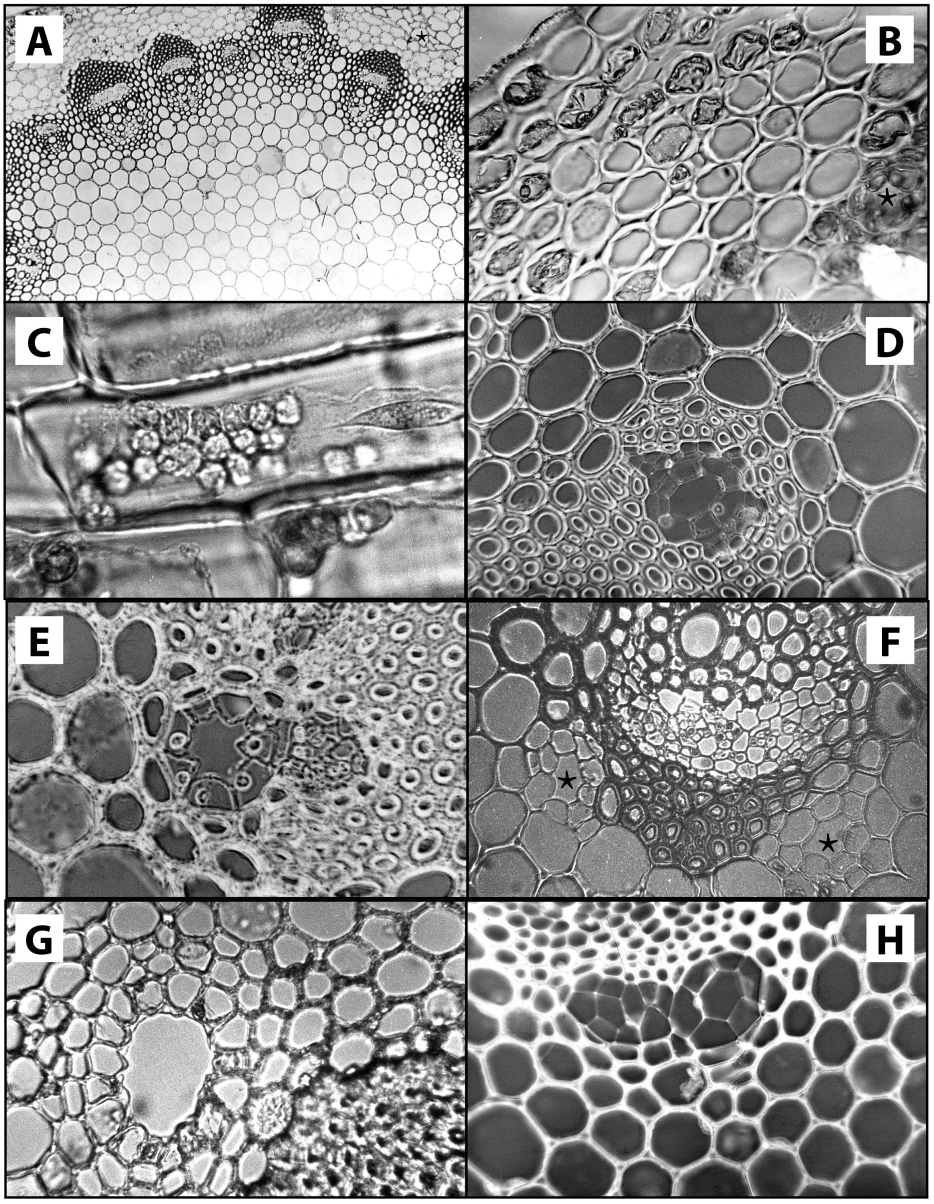

Fig. 11. Stem transections of *Echinacea* taxa. **A.** *E. sanguinea* showing portion of vascular system. Note the absence of secretory canals and sclerotic cells in the parenchymatous pith region, discrete collateral vascular bundles composed of primary tissue with fibrous bundle caps, partially sclerified interfascicular regions, one small secretory canal middle right in cortex composed mostly of collenchyma cells, and chlorenchyma cells underlying the epidermis. This microtome section was stained with safranin and fast green (×50). **B.** *E. angustifolia* var. *strigosa* free hand section showing typical thick-walled, elliptical collenchyma cells of outer cortical region (compare isodiametric parenchyma cells with intercellular spaces in pith see Fig. A). Note secretory canal in lower cortex (star) (×25). **C.** *E. pallida* showing endodermis cell in longitudinal section. Note numerous starch grains with roughened surfaces ~4.6 µm in diameter (×652). **D–H.** Stem transections showing secretory canals. **D.** *E. atrorubens* with secretory canal lumen surrounded by thin-walled, rectangular, eight-celled, epithelial ring embedded in thick-walled sclerified tissue next to the protoxylem points of the vascular bundle (×430). **E.** *E. atrorubens* with secretory canal embedded in vascular bundle cap (×430). **F.** *E. paradoxa* var. *neglecta* showing two secretory canals (stars) opposite the vascular bundle cap near the cortical region (×430). **G.** *E. paradoxa* var. *neglecta* with the largest secretory canal located near the protoxylem points in the pith with a 12-celled epithelial ring (×430). **H.** *E. simulata* with paired secretory canals opposite protoxylem points near the pith (×430).

widely scattered and therefore do not cover extensive areas nor do they appear to be secretory cells. Additional studies are justified to elucidate the occurrence and function of these cells.

Groups of *Echinacea* taxa may share a basic ray ligule adaxial epidermal shape. For example, the dome-shape adaxial epidermal cell with a rounded apex was observed in *E. laevigata*, *E. paradoxa* var. *neglecta*, *E. paradoxa* var. *paradoxa*, and more modified into a rounded but somewhat pointed apex in *E. angustifolia* var. *angustifolia*, *E. angustifolia* "race *intermedia*," *E. angustifolia* var. *strigosa*, *E. pallida*, and *E. purpurea*. These are more or less transitional shapes that could be considered the same basic epidermal cell type. In contrast the distinctive shapes of *E. atrorubens* (apex nipple-like), *E. sanguinea* (apex necked), and *E. simulata* (apex sharply pointed conical) appear different from the dome-shaped epidermal cell type. The question remains, however: how constant will these shapes remain over a broad range of habitats and given statistically significant sampled populations?

Echinacea simulata has the largest (length and width) ray ligule adaxial epidermal cells in the genus (Fig. 4K, L). The similarity between *E. pallida* and *E. simulata* based on macromorphological characters can be distinguished by adaxial ray ligule epidermal cells that appear to have distinctive shapes; the more dome shape in *E. pallida* in contrast to the more conical shape in *E. simulata*. This anatomical character alone distinguishes the two taxa even though the macromorphological characters have created confusion in the past.

Polyploidy, including tetraploids, with the increase in ploidy or sets of chromosomes, usually results in more robust, larger plants in overall size (stem height and diameter, leaf, flowers) and a proportional increase in cell size and volume. This increase in the component parts of the plant is called the "gigas effect." Comparison of ray floret anatomy of *E. pallida* (a tetraploid) with *E. simulata* (a closely related diploid with which it has been confused in the past) resulted in no obvious differences attributed to ploidy. For example, the ligule ray adaxial epidermal cells in *E. pallida* average 72 µm in length and 48 µm in width compared to *E. simulata* that average 105 µm in length and 75 µm in width. There are no other consistent micromorphological characters other than pollen size that suggest any differences in cell size between these two taxa.

Microanatomy of the ray florets of *E. purpurea* described by Upton (2007) noted (1) nonglandular trichomes similar to those on the leaf, (2) papillose epidermal cells of the ligule, (3) secretory ducts along veins, and (4) epidermis with wavy anticlinal walls, anomocytic stomata, and a light area indicating a secretory duct beneath a vein. This ray floret description fails to indicate adaxial and abaxial surfaces of the ray ligule, and indeed, the papillose surfaces in all the *Echinacea* taxa studied here were modified into various shapes and the abaxial surface had more epidermal-like trichomes. The line drawing illustration in 6f shows ray ligule trichomes similar to leaf trichomes (Upton 2007). Marginal ray florets in the Asteraceae usually have three teeth (notched) at the apex with veins outlining the three teeth (Carlquist 1976).

Internal ray floret anatomical characters, apart from epidermal cells—such as the number of vascular traces (usually 13), thickness of ray florets (170–312 µm), and number and location (adaxial or abaxial surfaces) of secretory chambers—are more or less constant for all *Echinacea* taxa except for *E. laevigata* and *E. purpurea*. *Echinacea purpurea* has the broadest ray florets of all the taxa and a more complex venation pattern that distinguishes *E. laevigata* and *E. purpurea* based on ray floret anatomy. Here the difference in the size of the ray floret width is reflected in the number of vascular traces (15 in *E. laevigata* and 31 in *E. purpurea*) and the abundant presence of secretory chambers in *E. purpurea* and apparent absence in *E. laevigata* (Table 3).

Stems

Rank ordering of stem diameters from the smallest to the largest was based on measurements in Table 3: *E. sanguinea*, *E. angustifolia* var. *angustifolia*, *E. angustifolia* var. *angustifolia* "race *intermedia*," *E. angustifolia* var. *strigosa*, *E. paradoxa* var. *neglecta*, *E. paradoxa* var. *paradoxa*, *E. atrorubens*, *E. simulata*, *E. pallida*, *E. purpurea*, and *E. laevigata* that follow a general trend of the higher the stem height the greater the stem width with the exception of *E. sanguinea* that has the smallest stem diameter and a more spindly habit (Fig. 10J; Table 3).

Samples were taken at the height of anthesis. Comparison of stem diameters of all *Echinacea* taxa shows a well-developed pith region usually occupying about 75 percent of the total stem diameter. This contrasts with the findings of Upton (2007) who reported in the general habit description of *E. purpurea* a stem diameter of

2–5 mm in transection with the pith hollow or solid. It is difficult to assess the importance and comparison of these characters with the present study. All of the stem tissue maps and measurements given here show an extensive pith region in all *Echinacea* taxa with no sign of tissue disintegration, which might occur later in the growing season and account for a hollow stem. Transections taken later in the growing season in July and August could possibly result in a hollow pith region and connected interfascicular regions with cambial activity and some secondary growth.

Perhaps the most striking micromorphological characters of the stem is the presence of sclerenchyma fibers (sclerotic cells with phytomelanin) in the pith and the absence of secretory canals in the pith and presence in the cortex of *E. angustifolia* var. *angustifolia*, *E. angustifolia* "race *intermedia*," and *E. angustifolia* var. *strigosa*. This distinguishes the *E. angustifolia* complex from all other *Echinacea* taxa.

The secondary xylem and phloem in roots of *E. angustifolia*, *E. atrorubens*, *E. pallida*, and *E. purpurea* have sclerids with associated phytomelanin deposition (see table 3; figs. 5d, e; 6a, b, c, g in Upton 2010). This is similar to the sclerotic cells with associated black substance in the stem pith tissue of *E. angustifolia* var. *angustifolia*, *E. angustifolia* var. *strigosa* and hybrids (Fig. 3H, I, J). Thus, in a review of all *Echinacea* taxa McKeown (1999) noted that *E. angustifolia* var. *angustifolia* has short plant height, short and broad reflexed ray ligules, yellow pollen color and is a selection candidate for cold hardiness because of adaptation to northern climates. It is also a potential candidate in breeding for stem strength when coupled with the sclerified pith tissue (McKeown 1999).

Stem diameters and secondary growth patterns based on stem anatomy tissue maps appear most similar when *E. pallida* and *E. simulata* are compared. Comparison of *Echinacea* root anatomy in *E. pallida* (Upton 2010, see Table 3) noted the presence of phytomelanin-coated sclerids in the secondary phloem and secondary xylem in roots of *E. angustifolia*, *E. pallida*, and *E. atrorubens*, and the rhizome of *E. purpurea* pith and secondary phloem. Another paper on the root anatomy of *E. angustifolia* (Axentiev et al. 2010, see Table 3) also noted the presence of phytomelanin in the same locations as in *E. pallida* with the added description that this black substance fills the triangular intercellular spaces around the sclerids, causing them to appear star-shaped not unlike the stem anatomy described here for the *E. angustifolia* complex (Fig. 3H–J). Interestingly, in my anatomical study of *E. atrorubens* stem transections, sclerotic cells were not observed, but this taxon is a good candidate to look for stem sclerotic cells since it hybridizes with *E. angustifolia* var. *angustifolia*.

Stem tissue maps for *E. laevigata* and *E. purpurea* show anatomical characteristics that distinguish these two species from other *Echinacea* taxa. Numerous secretory canals in the pith (~58) and in the cortex (~48) of *E. laevigata* compared to *E. purpurea* with (~34) in the pith and (~50) in the cortex are in contrast to all other *Echinacea* taxa with far fewer numbers. These secretory canals originate only opposite the vascular bundles and interfascicular region. Vascular tissue has 44 protoxylem points in *E. laevigata* and 42 protoxylem points in *E. purpurea,* far greater numbers than in all other *Echinacea* taxa (Table 3).

The largest stem diameter of ~5.2 mm with pith diameter of ~4.2 mm occurs in *E. laevigata* (Fig. 10E) compared to stem diameter of ~4.8 mm with pith diameter of ~3.6 mm in *E. purpurea* (Fig. 10I). These two taxa are the tallest and overall largest in the genus *Echinacea*. Stem tissue maps of these two taxa (Fig. 10E, I) are similar in vascularization and spatial arrangement of the secretory system in development, position, and size.

Cortical tissue in both taxa is composed primarily of parenchyma. The epidermis differs in the presence of trichomes in *E. purpurea* and absence in *E. laevigata*. The more robust habit of these two taxa would suggest *a priori* that activity in interfascicular areas would connect the vascular bundles with some degree of sclerification. However, vascular bundles are discrete, surrounded and separated by regions of parenchymatous tissue. Sclerotic cells are lacking in the pith of both species. It appears that macromorphological differences are in contrast to internal microanatomical similarities.

Petioles

In *Echinacea* the petiole is supplied by three major collateral vascular bundles, a manifestation of departing foliar traces from the stem. In addition petiole shape can be used as a taxonomic character. Transversely cut petioles can be recognized by shapes, for example, horseshoe-shaped, V-shaped, and cylindrical-shaped.

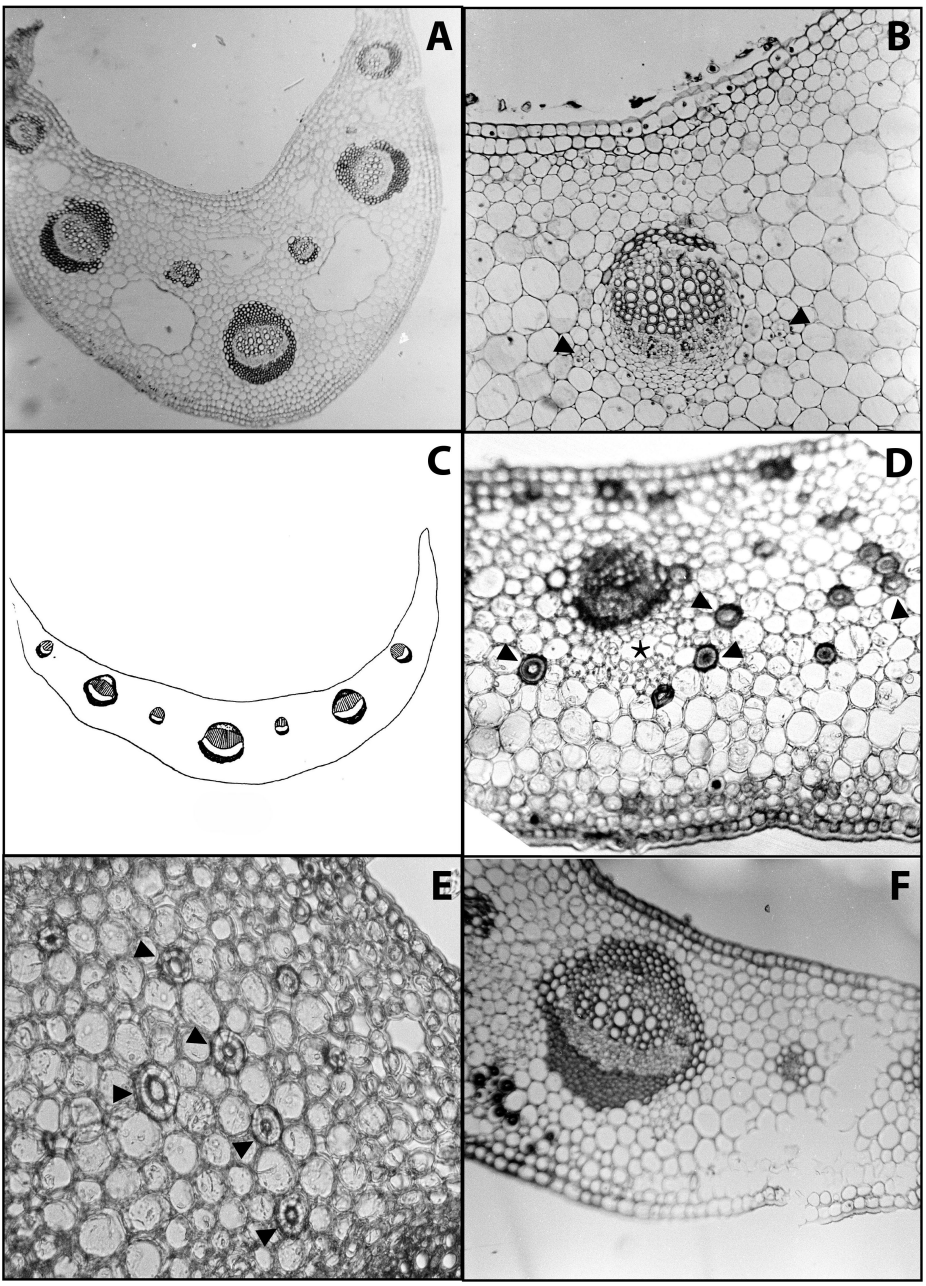

Fig. 12. Petiole transections. **A.** *E. atrorubens*, microtome section showing three major collateral vascular bundles ensheathed by fibrous tissue (vascular traces) typical for the Asteraceae. Note three air spaces that form passageways throughout the length of the petiole (×20). **B.** *E. sanguinea*, microtome section showing central major vascular bundle lacking fibrous tissue on abaxial side and two small secretory canals one on each side (arrows). Note the two-layered epidermis with similar cells and undifferentiated ground mesophyll (×17). **C.** *E. paradoxa* var. *neglecta*, lunate to bow-shape with venation illustrated by line drawing (×12). **D.** *E. paradoxa* var. *neglecta*, oblique microtome section showing brachysclerids (stone cells) as scattered single cells or in rows (arrows). Larger secretory canal (star) visible near vascular trace (×40). **E.** *E. paradoxa* var. *neglecta*, oblique microtome section showing single, isolated stone cells with highly refractive thickened cell walls with reduced central lumen. Note the primary pit fields that radiate like the spokes of a wheel (arrows) (×70). **F.** *E. paradoxa* var. *neglecta*, microtome section stained with red safranin and fast green showing central vascular bundle and cluster of stone cells at lower left (×14).

Moreover, secretory canals, universally present in the genus, differ in size, number and position in the petiole. The petiole contains the same tissues as the stems that include epidermis, collenchyma in varying amounts, and vascular bundles with associated fibrous sheaths. However, significant differences were observed for several taxa, for example, in *E. purpurea* and *E. sanguinea* the medial vascular bundle lacks a fibrous cap (Fig. 12B; Table 4).

Another apparently unique structure found in *E. paradoxa* var. *neglecta* is the brachysclerid or stone cell. They appear either isolated, clustered, or in rows (Fig. 12D–E). Structurally the petiole of *E. atrorubens* has three lacunae situated around the medial vascular bundle (Fig. 12A) and this differs from other *Echinacea* taxa.

General Habit

Echinacea has mostly a scapose habit with above ground stem that persists in to the fall along with the flower head that gradually dies and begins to undergo decay. Stems do not survive the winter in the high plains region of western Kansas due to freezing temperatures and heavy snows that cause lodging, and eventually aerial parts deteriorate into ground litter as part of the annual life cycle. The stems are not stout enough to withstand the elements of nature unlike species of *Yucca* where stems may survive for several years. In grassy habitats such as prairies and glades *Echinacea* populations can dominate the landscape, (Figs. 5C–D; 8A–C) representing the tallest elements not unlike the forest canopy of trees (Kilgore et al. 2009; Richter 2013). This standing cover of stems serves to intercept spores of windblown organisms such as myxomycetes (plasmodial slime molds).

The round spiny cone head also creates a surface area and landing platform for spores. Indeed, a recent study that is the first of its kind (Kilgore et al. 2009) found that *Echinacea* species collected from native prairies in Kansas and Missouri (*E. angustifolia*, *E. pallida*, and *E. paradoxa* var. *paradoxa*) have a distinct assemblage of myxomycete species that were isolated in moist chamber cultures. Reproductive structures of herbaceous plants had greater mean species richness than stems. This study proposed a new term (herbicolous myxomycetes) for herbaceous, perennial grassland plants associated with this group of myxomycete species (Kilgore et al. 2009). This above ground canopy of perennial plants should be explored for potential species diversity and possible myxomycete species new to science.

CONCLUSIONS AND FUTURE CONSIDERATIONS

This study took advantage of more than 15 years of research on the biology of *Echinacea* by Professor Ronald L. McGregor at the University of Kansas. His field experience collecting plants throughout the state of Kansas and as Coordinator of The Flora of the Great Plains research project (Great Plains Flora Association 1986) gave him a "trained eye" for habitats and species identification, especially *Echinacea* taxa involving hybrid swarms, introgressed populations, and pockets of typical taxa in part represented here. All of the plants selected for this study were identified personally by R.L. McGregor. Freshly collected plants were used for this anatomical survey not herbarium specimens. This avoided using material that often shows shrinkage and distortion of cells. Therefore, the names and collections used here carry the accuracy and weight of McGregor's field experience.

All of the plants studied here were collected within a few days at the height of anthesis and for the most part in the same place. This part of the sampling protocol assures that possible variables were at least partially controlled. Nevertheless, the limited sampling lacked a statistically significant number of plant individuals, different populations from different natural habitats, and ray florets from different capitula. The sample size places limitations on the use of anatomical characters in dichotomous keys, and therefore, the combination of macro- and microcharacters used in couplets here. Furthermore, the presence of anatomical characters, while potentially important in separating taxa—for example, sclerotic pith cells, brachysclerids in petioles, and differences in shapes of adaxial ray ligule epidermal cells—suggests that special caution should be used in the assumption that these characters are consistent within and across *Echinacea* taxa. This is especially true of the multicellular, catenulate, adaxial ray ligule epidermal cells observed in the hybrid population of *E. angustifolia* var. *angustifolia* "race *intermedia*" that are present but scattered in small numbers. These epidermal cells were

not observed in either of the closely related *E. angustifolia* var. *angustifolia* or *E. angustifolia* var. *strigosa*.

It is apparent that *E. laevigata* and *E. purpurea* are closely related in anatomical characters that include: stem diameter, cortical breadth comprised mostly of parenchyma cells, vascularization and spatial distances of tissue systems, diameter of pith, and position, number and size of secretory canals. In contrast ray florets differ in the number of veins and secretory chambers, but adaxial epidermal cells have similar shapes and sizes noted previously.

Future studies should concentrate on fewer taxa and more critical sampling to more accurately assess the value of anatomical characters useful in the identification of *Echinacea* taxa. Nevertheless, this anatomical study of *Echinacea* aerial plant parts contributes to a better understanding of structure and function that relates to similarities and differences in this medically important herbaceous perennial prairie plant.

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