A STUDY OF OLEORESIN CANALS IN *ECHINACEA PURPUREA* (L.) MOENCH

Jon C. Caster and Gerald A. Myers
Department of Biology
South Dakota State University
Brookings, South Dakota 57007

ABSTRACT

The purple coneflower, *Echinacea purpurea* (L.) Moench, produces a naturally biodegradable insecticide, echinacein, which also displays various medicinal properties. These plants also contain oleoresin throughout a network of oleoresin canals. The function of oleoresin and oleoresin canals has not been determined, nor has an association been made between echinacein and oleoresin.

The objectives of this study were to locate synthesis, transport, and storage sites of oleoresin, identify organelles responsible for oleoresin synthesis and establish a relationship between echinacein and oleoresin.

Taste tests, performed on various parts of the plant to determine the location of echinacein, indicated the presence of echinacein in the achenes (fruit coats) and roots. Light microscopic study of tissue samples revealed the presence of oleoresin canals in the stem, receptacle, floral bracts, and achenes. Electron microscopic study revealed the presence of oil bodies in the cytoplasm of cells forming the canal walls.

The high concentration of oleoresin canals in the placental region of the receptacle, which continued into the floral bracts and achenes, indicated a correlation between the occurrence of echinacein and the presence of oleoresin canals. The presence of oil bodies in canal cells supported the assumption that these cells produce oil, possibly echinacein.

LITERATURE REVIEW

The plant genus *Echinacea* includes 9 species and 2 varieties (McGregor, 1968) containing pest-resistant plants. These plants were used by various Indian tribes of the midwestern United States for antiseptic and analgesic applications, and root extracts have been marketed by pharmacists for various medicinal purposes (Jacobson et al., 1974). In the 1940's and 1950's, extracts from the root of *Echinacea* were reported to have anti-bacterial properties (Stoll et al., 1950). It was found to be highly toxic to the adult housefly, mosquito larvae, the German cockroach, and the yellow mealworm (Jacobson, 1954). Bame (1984) found the echinacein extract toxic to the western corn rootworm larvae. A chemical structure for a compound believed to be responsible for these prop-

Submitted as an Agricultural Experiment Station Journal Article # 2253.

erties of *Echinacea* was published and this compound was named "echinacein" (Jacobson et al., 1974). There is now some question of the exact chemical structure of echinacein.

Past studies have revealed the presence of an amber-colored substance of oily or resinous consistency, oleoresin, (Kraemer and Sollenberger, 1911) in a network of oleoresin canals (Boodle and Fritsch, 1908). Both schizogenous and lysigenous canals have been observed in the leaves, stems and roots of some species of *Echinacea* (Metcalfe and Chalk, 1958). Canal locations are assorted but predictable. In the root, canals are generally of endodermal origin, sometimes occurring in the cortex near vascular strands. In stems and leaves, canals normally lie opposite the vascular bundles, sometimes between them (Boodle and Fritsch, 1908).

The function of oleoresin and oleoresin canals has not been determined, nor has an association been made between echinacein and oleoresin. The objectives of this study were: (1) to locate synthesis, transport and storage sites of oleoresin; (2) identify organelles responsible for oleoresin synthesis; and (3) establish a relationship between echinacein and oleoresin.

MATERIALS AND METHODS

Plants of *Echinacea purpurea,* the purple coneflower, were grown in the greenhouse, and various developmental stages of flowering plants were selected for study. Taste tests were performed on the roots, leaves, seeds, floral bracts, and achenes of these plants. Tissue samples from the roots, stems, leaves and heads were killed, fixed, dehydrated, embedded and stained according to the technique outlined by Sass (1964). Eight um thick sections of these tissues were made and studied with a Leitz Ortholux microscope. Photographs were taken using a Leitz Orthomat automatic camera. Hand sections were made of achenes, stained with Sudan IV and observed.

Tissue samples from the root, leaf, bract and the placental receptacle region were prepared according to the technique outlined by McMullen et al. (1977), for thin sectioning in plastic. One um sections of these tissues were stained with safranin and crystal violet, mounted in immersion oil, and photographed using the Leitz Ortholux microscope and the Orthomat automatic camera.

Those tissues determined to contain canals were then prepared for transmission electron microscopy (TEM) according to techniques outlined by McMullen et al. (1977). Canal cells in receptacle and bract tissue were studied and photographed using a Hitachi HU-12 transmission electron microscope. Sections of root, floral bract and receptacle tissue were also prepared for scanning electron microscopy and were observed and photographed using the ISI Super IIIA scanning microscope (SEM).

RESULTS

Upon chewing achenes and roots, numbness quickly occurred on the tongue and lining of the mouth, indicating the presence of echinacein in these areas. The older the tissue tested, the more pronounced was the effect.

Examination of thick sections with a light microscope revealed canals in the leaves, stem, receptacle, floral and involucral bracts, and achenes. Canals in the leaves were always associated with a vascular bundle. Canals in the stem were opposite the phloem in the cortex, or opposite the protoxylem in the pith. Canals in the receptacle occurred with no definite orientation pattern and were most numerous in the placental region of the receptacle, usually close to a procambial strand (Figure 1). These canals continued into the floral and involucral bracts and into the achene walls, which were attached to the receptacle at the placental region. Scanning electron micrographs showed the cells forming the walls of the canal were elongated parallel to the longitudinal axis of the canal (Figure 2).

Thin sections stained with safranin and crystal violet and mounted in immersion oil, displayed brilliant color contrasts without fading (Fgure 1).

Observations of canal cells with the TEM revealed oil bodies in the cytoplasm (Figure 3). These oil bodies were not membrane-bound (Figure 4).

DISCUSSION

Taste tests indicated that the highest concentrations of echinacein were in the achenes and roots and that the numbing sensation increased as the plant part got older. Microscopic study indicated that the highest concentraion of oleoresin canals occurred in the placental region of the receptacle. Many of these canals continued into the bracts and achenes and became larger and more frequent as the flower head matured. From these results a correlation may be inferred between the occurrence of echinacein and the presence of oleoresin canals. In this study, no canals were observed in the roots, possibly because of poor preparations of root tissue.

Boodle and Fritsch (1908) observed interfascicular canals in the stem and in the leaves. In this study, however, the only canals observed in the stem and leaves were opposite the vascular bundles, some near the protoxylem and some near the phloem.

Thin sections were made in order to determine which tissue samples contained oleoresin canals. Those samples which contained canals were then further studied using the TEM. Jensen

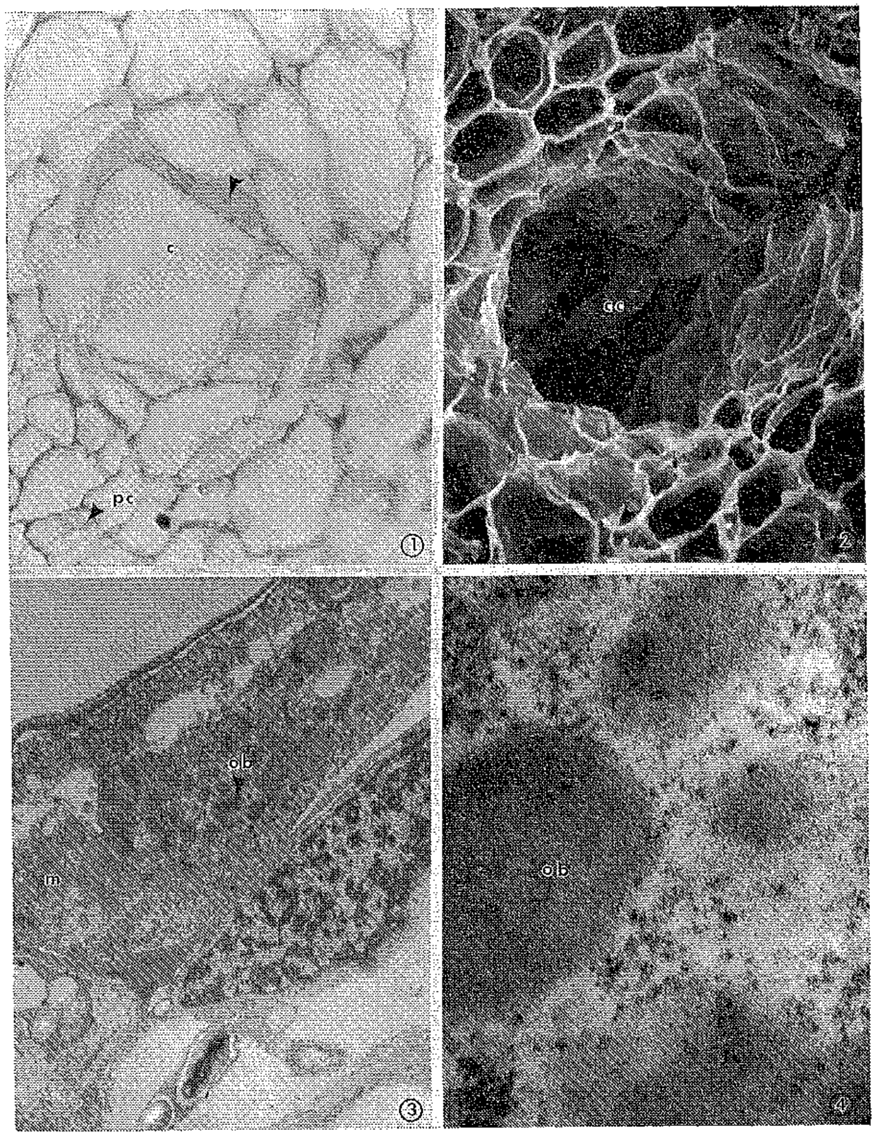

Figure 1. One um cross section of a canal in receptacle tissue, showing close association of canal with procambial tissue. Arrow indicates high protoplast density. pc = procambium; c = oleoresin canal (X 1000).

Figure 2. Scanning electron micrograph of oleoresin canal (X 570). cc = canal cell.

Figure 3. Transmission electron micrograph of a canal cell containing oil bodies and several mitochondria. ob = oil body; m = mitochondria (X 10,000).

Figure 4. Transmission electron micrograph of a non membrane-bound oil body in a canal cell. ob = oil body (X 150,000).

(1964) suggested that triglycerides are synthesized in mitochondria. Oil in the canal cells occurs as non-membrane bound globules and mitochondria are numerous in these canal cells as compared to the surrounding cells. This supports the assumption that the oleoresin canal cells are oil producing cells.

If cells forming the walls of the canals in *Echinacea* are oil synthesis sites, then genes that control the production of oil and possibly echinacein, are expressed in these cells. This gives support to the idea that transfer of the gene(s) controlling synthesis of echinacein into sunflower for the control of insect pests is feasible.

RECOMMENDATIONS

Studies of the canal cell contents in the receptacle, floral bracts, and achenes should be carried out using silica gel chromatography to isolate echinacein. Mass spectra and nuclear magnetic resonance spectrum of the isolated substance should determine the actual structure of echinacein and see if oleoresin in the canals is indeed an echinacein carrier.

LITERATURE CITED

Bame, G. A. 1984. Biological and chemical analysis of **Echinacea pallida** (Nutt.) Nutt. MS thesis, South Dakota State University.

Boodle, L. and F. Fritsch. 1908. **Solereder's Systematic Anatomy of the Dicotyledons.** Clarendon Press. Oxford 1:457-468.

Hartzell, A. and F. Wildoxon. 1941. Contrib. Boyce Thompson Inst. 12.

Jacobson, M. 1954. Occurrence of a pungent insecticidal principle in American Coneflower roots. Science 120:1028-1029.

Jacobson, M., R. E. Redfern and D. J. Voaden. September 1974. American coneflower roots: a pesticidal cornucopia. A paper presented at the 168th American Chemical Society meeting, Atlantic City, New Jersey.

Jensen, W. 1964. The Plant Cell. Wadsworth. Belmont, CA.

Kraemer, H. and M. Sollenberger. July 1911. The pharmacognosy of **Echinacea.** Amer. Jour. Pharm. 315-324.

McGregor, R. L. September 1968. The taxonomy of the genus **Echinacea** (Compositae). Univ. of Kansas Sci. Bull. 48:113-142.

McMullen, C. R., W. S. Gardener and G. A. Myers. 1977. Ultrastructure of cell-wall thickenings and paramural bodies induced by barley stripe mosaic virus. Phytopathology 67:462-467.

Metcalfe, C. R. and L. Chalk. 1958. **Anatomy of Dicotyledons.** Clarendon Press. Oxford.

Sass, J. E. 1964. **Botanical Technique, 3rd Ed.**

Sorokin, H. P. 1967. The spherosomes and the reserve fat in plant cells. Am. Jour. Bot. 54:1008-1016.

Stoll, A., J. Renz and A. Brack. 1950. Antibacterial substance II. Isolation and constitution of echinocide and the glycosides of **Echinacea angustifolia.** Helv. Chim. Acta. 33:1877-1893.