

STOMATA ON THE FRUITS AND SEEDS OF ESCHSCHOLZIA (PAPAVERACEAE)¹

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ABSTRACT

Stomata are present on the outer and inner fruit walls and seed coats of *Eschscholzia californica*, *E. covillei*, *E. glyptosperma*, *E. lemmonii* and *E. minutiflora*. The stomata on the inner fruit wall and seed coat remain constantly open, even under plasmolyzing conditions, whereas those of the outer fruit wall are able to open and close. This allows for gas exchange in these chlorophyllous structures. Fibrous bundle caps in the costal regions of the fruit act as windows allowing light transmission to the photosynthetic seeds within. Preliminary results show that the total photosynthesis by the fruits and seeds of *Eschscholzia californica* together appears to at least balance respiratory losses, and under favorable conditions might significantly contribute to seed and fruit development.

STOMATA are known to occur on the seeds of species in a number of apparently unrelated families: Amaryllidaceae, Bixaceae, Bombacaceae, Cannaceae, Cappariaceae, Dipterocarpaceae, Euphorbiaceae, Fagaceae, Flacourtiaceae, Geraniaceae, Iridaceae, Juglandaceae, Krameriaceae, Liliaceae, Magnoliaceae, Malvaceae, Meliaceae, Myrsinaceae, Polygalaceae, Rosaceae, Sterculiaceae, and Violaceae (Macloskie, 1884; Netolitzky, 1926; Corner, 1976). In the Papaveraceae, three genera, *Argemone* (Sachar, 1955), *Eschscholzia* (first reported by Meunier, 1891), and *Bocconia* (our observations of *B. frutescens*) are known to have seed coat stomata.

Considerations of the function of seed coat stomata have appeared in the literature over the years. Netolitzky (1926) suggested that seed coat stomata function in some species in water uptake by the seed during germination, rather than in gas exchange. However Flint and Moreland (1943) found considerable photosynthetic activity in the seeds of *Hymenocallis occidentalis* after they are shed from the fruit and inferred that seed coat stomata allow for gas exchange within this green seed.

Our work has led us to believe that the seed coat stomata in *Eschscholzia* function in photosynthetic gas exchange by the seed while it is

still contained within the fruit. In this paper we will (1) describe the structure of *Eschscholzia* fruits and seeds with particular attention to features that are important for photosynthesis and gas exchange, and (2) provide preliminary data showing the occurrence of photosynthesis in the fruits and seeds.

MATERIALS AND METHODS—For SEM studies fresh immature seeds and fruits of *Eschscholzia californica*, *E. glyptosperma*, *E. minutiflora*, *E. lemmonii* and *E. covillei* were fixed in FAA, dehydrated in an ethanol series, dried using the critical point method, attached to aluminum stubs with silver conductive paint and coated with gold in a vacuum evaporator or sputter coater. Unimbibed mature seeds of these and the remaining eight species in the genus were likewise attached to stubs and coated with gold. Specimens were observed with a Cambridge Stereoscan S4 scanning electron microscope. Fresh material was hand-sectioned for anatomical study and observed either unstained or stained with 0.25% toluidine blue O. Unstained guard cells were plasmolyzed with glycerine. Additional material was fixed in FAA, dehydrated through a tertiary butyl alcohol series, embedded in "Paraplast," sectioned at 10 μ m, mounted and stained with safranin, fast green and crystal violet.

Using an infra-red gas analyzer, we measured photosynthesis and respiration as changes in CO₂ concentration in a reference gas mixture as it passed through a chamber enclosing several capsules attached to a single plant of *E. californica*. The temperature was 24–25 C, and the experiments were repeated at various light intensities. After completion of measurements the seeds

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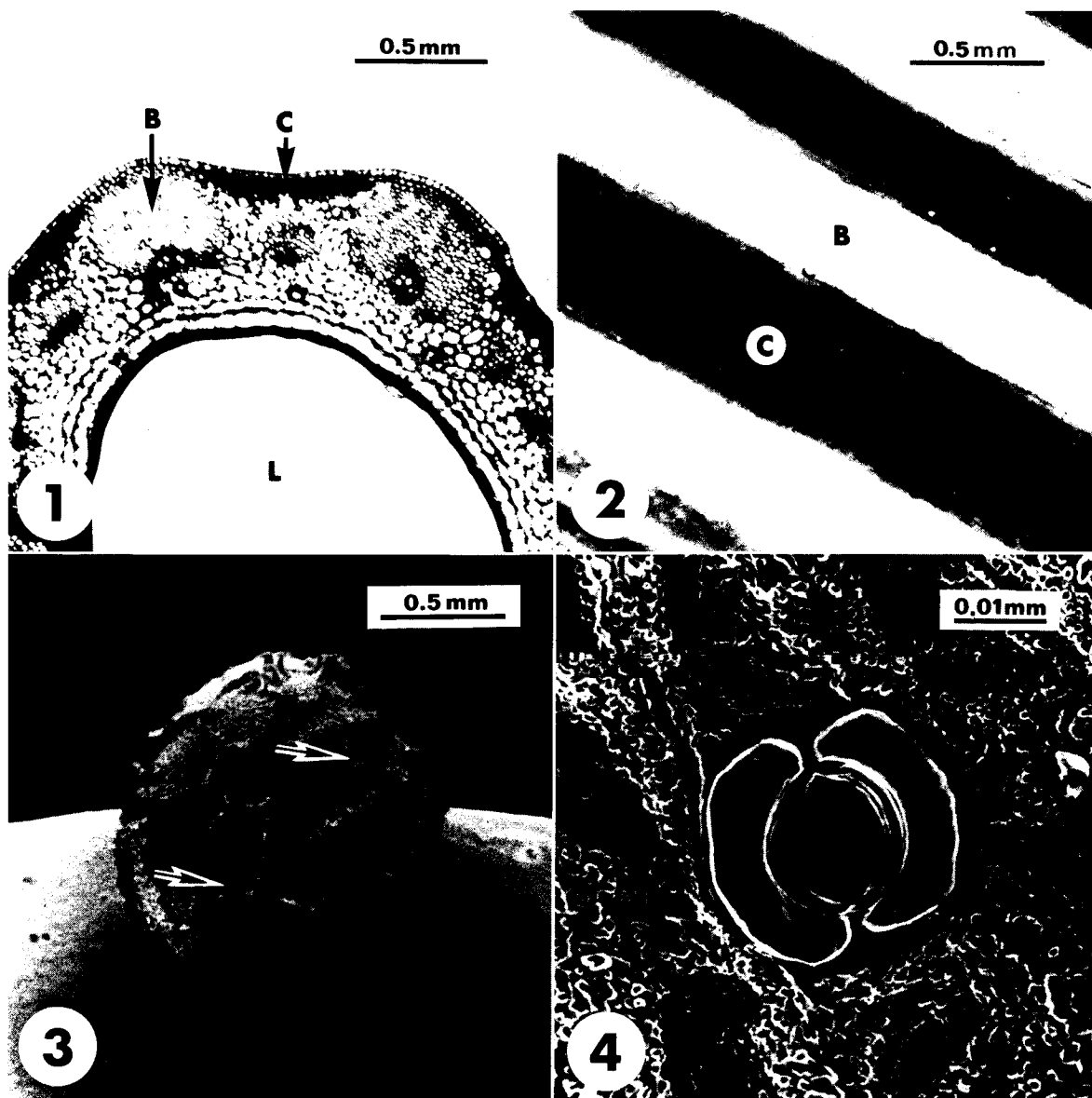


Fig. 1-4. 1. Transverse section of living fruit of *Eschscholzia californica* showing lumen (L), fibrous bundle cap (B) and chlorenchyma (C). 2. Living fruit of *E. californica* split longitudinally, viewed from inside with transmitted white light. Dark regions (C) are chlorenchyma, light regions (B) are bundle caps. 3. Seed of *E. californica*; arrows point to stomata, others visible as small black dots scattered over surface. 4. Stoma on mature seed of *E. californica*.

were removed from the capsules, and capsule and seed respiration and photosynthesis were measured separately in the same chamber. The same measurements were also made with leaves and stems together. The method was described by Flinn and Pate (1970). The data are expressed in Table 1 as rates of net carbon dioxide uptake by the tissue ($\text{mg CO}_2/\text{g/hr}$). The results shown are of representative experiments at comparable levels of irradiation for each of the tissues.

RESULTS AND DISCUSSION—Capsules of *Eschscholzia* are bicarpellate, with two parietal placentae bearing numerous anatropous ovules. The capsules are typically 10-ribbed or nerved, and dehisce septicidally in two valves from base to apex.

The inner and outer epidermis of the fruit wall both bear stomata and enclose parenchyma in which vascular bundles are distributed (Fig. 1). The ten major vascular bundles of the fruit are

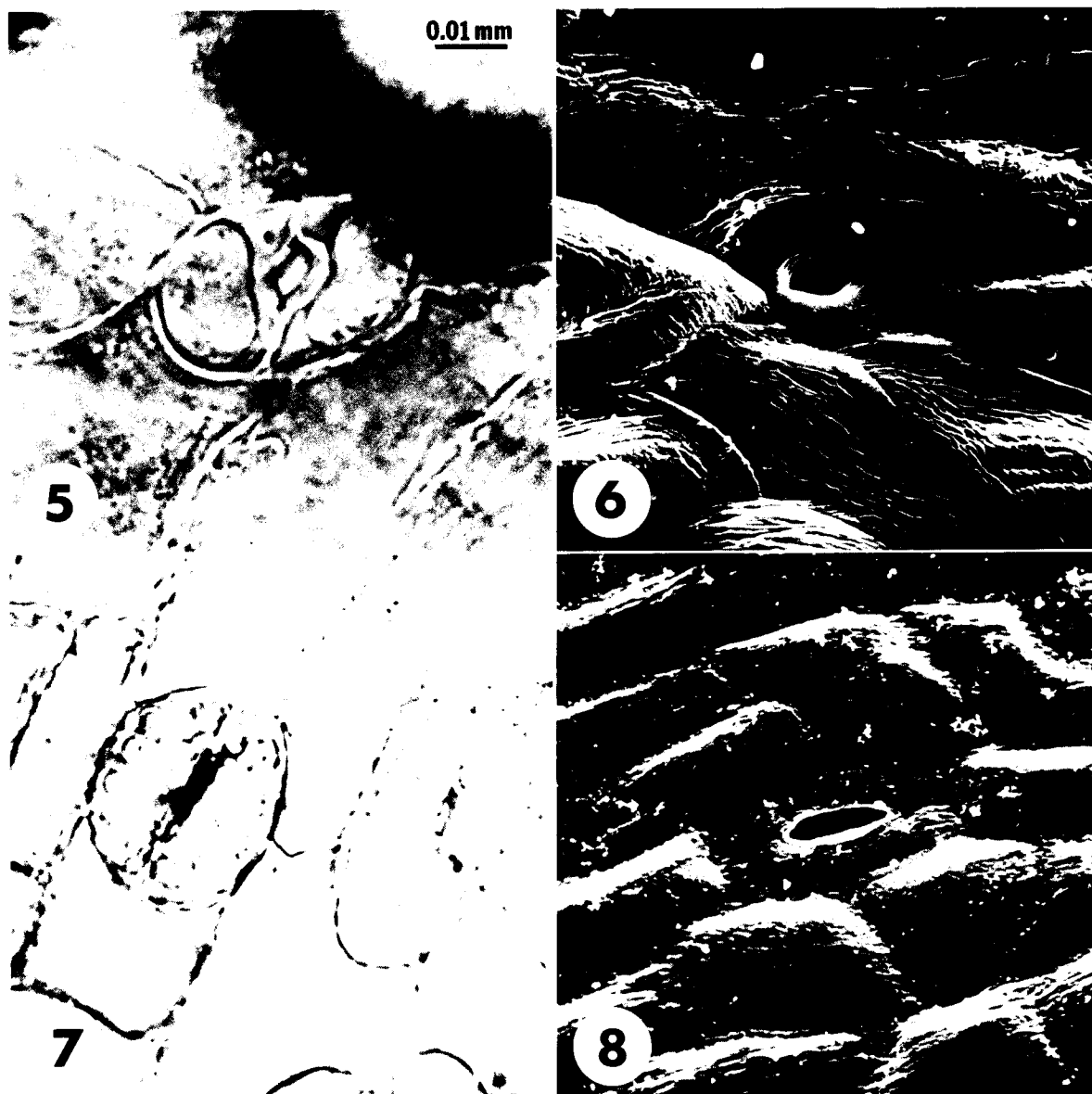


Fig. 5-8. Stomata on fruits of *Eschscholzia*: light micrographs to the left, SEM photographs at right. 5. *E. californica*, inner fruit wall, guard cells plasmolyzed. 6. *E. californica*, inner fruit wall. 7. *E. californica*, outer fruit wall. 8. *E. covillei*, outer fruit wall.

located at the ribs, with an additional smaller bundle in each intercostal region (Fig. 1). Each bundle consists of a small amount of xylem (6-12 conducting elements per bundle in cross section in *E. lemmonii*) toward the interior of the fruit, associated with a similarly small area of phloem. The rib bundles are characterized by conspicuous fiber caps external to the phloem, comprised of 80-150 cells per bundle in cross sections of *E. lemmonii*. The intercostal bundles are also partially surrounded by a fibrous bundle

cap, but these consist of only 5-25 cells per bundle in cross section (Fig. 1). Chlorenchyma is found directly beneath the outer epidermis in the regions between the ribs (Fig. 1).

Like the other species in the genus, the ovule of *E. lemmonii* is anatropous and bitegmic, with the micropyle formed by both integuments. The cells of the outer epidermis of the chlorophyllous outer integument are large and cutinized. In certain locations they expand radially, resulting in ridges of elongate cells which form a reticulum

TABLE 1. Rates of net carbon dioxide uptake by fruits, seeds, leaves and stems ($\text{mg CO}_2/\text{g tissue/hour}$). Results of representative experiments at comparable levels of irradiation^a

	Light ^b		Dark	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Intact fruits	0.932	4.702	-1.176	-5.939
Fruits alone	-0.170	-0.852	-1.155	-5.804
Seeds alone	-0.513	-1.743	-1.605	-5.451
Leaves and stems	5.772	41.712	-1.488	-10.750

^a Air temperature, 24–25 C.

^b Incident quantum flux 108–111 nanoeinsteins/cm²/sec.

over the seed surface. Stomata (Fig. 3, 4) are present on seeds of all 13 species in the genus. They are typically located in the flattened regions, or facets, between the ridges (Clark and Jernstedt, 1978). The remaining 4–5 layers of the outer integument are thin-walled and become flattened as the seed matures. The outer layers of the 3- to 4-layered inner integument become sclerified and add strength to the seed coat. Our observations of the ovules and seeds of *E. lemmonii* are in agreement with earlier studies of *E. californica* (Meunier, 1891; Shaw, 1904; Sachar and Mohan Ram, 1958).

Together the fruits and seeds of *Eschscholzia* form a unit structurally well adapted for photosynthetic activity. A pathway exists for gas exchange from the external environment to the photosynthetic interior. Gases presumably enter via the stomata on the outer epidermis and diffuse through intercellular spaces in the fruit wall. Then the gases enter the lumen of the fruit through the stomata of the cutinized inner epidermis, where they become available to the chlorophyllous tissues of the seed coat. The stomata of the outer fruit wall (Fig. 7, 8) are able to open and close, providing control of transpiration and gas exchange. Stomata of the seed coat (Fig. 3, 4) and inner fruit wall (Fig. 5, 6) are always open, even under plasmolyzing conditions, as demonstrated by immersion of stomata in glycerine.

Light reaches the photosynthetic regions of the fruit after anthesis and especially after abscission of the other floral parts. The seeds are illuminated by light passing through the costal regions of the fruit (as seen in Fig. 2). Because of the small number of chloroplasts present, the fibrous bundle caps and the associated tissues in these regions may act as windows (Fig. 1).

Preliminary results show that total photosynthesis by the capsules and seeds of *E. californica* is low, but more than balances respiratory CO₂ losses (Table 1). Photosynthesis by the capsules or seeds separately does not quite balance res-

piration. This is not surprising, in view of the likelihood of wound respiration and resulting depression of net photosynthesis in both fruits and seeds following removal of the seeds. Because of this, we are at present unable to determine the separate contributions of fruits and seeds to the total photosynthesis of the two. The photosynthetic rate of the leaves and stems together is approximately six times that of the intact fruits on a fresh weight basis (Table 1) at comparable light intensities. Similar results have been reported with various legumes, although photosynthesis did not exceed respiration by as much as it apparently does in *Eschscholzia californica* (Flinn and Pate, 1970; Quebedeaux and Chollet, 1975; Atkins et al., 1977; Olikier, Poljakoff-Mayber, and Mayer, 1978). It has been suggested that in legume fruits and seeds photosynthesis functions mainly in the recapture of CO₂ released by respiration. This may also be true for *Eschscholzia californica*. Legume fruits and seeds lack the extensive gas exchange pathway found in those of *Eschscholzia* (Atkins et al., 1977). Therefore photosynthesis in legume fruits and seeds may be dependent upon, and even limited by, recycled CO₂, whereas that in *Eschscholzia* is not. The extensive gas exchange pathway in *Eschscholzia* fruits and seeds may be one reason that rates of net photosynthesis are higher in *Eschscholzia californica* than in legume fruits and seeds.

The photosynthetic activity of *Eschscholzia* fruits and seeds may play a larger role in the overall economy of the plant than appears to be the case in leguminous species. In the course of normal development in the annual species of *Eschscholzia*, a large proportion of the leaves wither and die at about the time of fruit set. This results in a photosynthetic area consisting largely of fruits, seeds and stems. That photosynthesis by capsules and seeds seems to more than balance respiratory CO₂ losses indicates that these structures do not constitute a major carbon sink for the plant during daylight hours. The extent to which photosynthesis exceeds respiration suggests that under favorable conditions carbon assimilation by fruits and seeds may balance a significant portion of night-time respiratory CO₂ losses. Further studies of carbon transfer between vegetative and reproductive parts are needed.

The rates of photosynthesis of *Eschscholzia californica* fruits and seeds are not great relative to those of leaves and stems (Table 1). Yet, the annual species of *Eschscholzia* inhabit arid and semi-arid regions, and the slight advantage provided by seed and fruit photosynthesis could in unusually dry years be crucial for the plants' reproductive success.

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