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MORPHOLOGY OF GLANDULAR HAIRS OF CANNABIS SATIVA FROM SCANNING ELECTRON MICROSCOPY[1]

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ABSTRACT

Three distinct types of glandular hairs of increasing morphological complexity which occur on flowering tops of Cannabis *sativa* L. (marihuana) are described from scanning electron microscopy. These gland types-termed bulbous, capitate-sessile, and capitate-stalked, described from pistillate plants-occur in greatest abundance on the outer surface of bracts ensheathing the ovary. Bulbous and capitate-sessile glands, which arise at an early stage in bract development, are scattered over the bract surface. Mature bulbous glands have a small swollen head on a short stalk, whereas capitate-sessile glands have a large globular head attached directly to the bract surface. Because of their numbers and large size, capitate-sessile glands are the most conspicuous gland type during the early phase of bract development. Capitate-stalked glands, which have a large globular head on a tall, multicellular stalk, differentiate during subsequent bract development. These stalked glands arise first along the bracteal veins and then over the entire bract surface. A voluminous, fluid secretory product accumulates in the glandular head of all three types. These glands are believed to be a primary site of localization of the marihuana hallucinogen, tetrahydrocannabinol.

CANNABIS SATIVA SHOOTS bear numerous types of glandular and nonglandular epidermal appendages described originally in the classic works of Briosi and Tognini (1894, 1897). The nonglandular trichomes, such as cystolith hairs, recently have been widely studied from a forensic viewpoint with light (Asahina et al., 1967; Shimomura et al., 1967; Nakamura, 1969; Nordal, 1970) and scanning electron microscopy (SEM) (Bradford and Devaney, 1970; Devaney and Bradford, 1971; Siegesmund and Hunter, 1971) in an effort to establish a positive microscopic identification of marihuana. The glandular hairs, seemingly not as taxonomically distinct in their morphology as nonglandular hairs, have correspondingly received less attention. Bouquet (1950), Mohan Ram and Nath (1964), and Shimomura et al. (1967) have described various aspects of the morphology of glandular hairs in marihuana from light microscopy. The glands were found to have a typical structure, being stalked or stalkless with the head a flattened disc of few to many cells covered by a secretory product. The secretory product accumulated beneath a membranous sheath derived from the outer surface of the cellular disc. Published descriptions of the morphology of marihuana glands from scanning electron microscopy are nonexistent, although occasionally (Stearn, 1970; Siegesmund and Hunter, 1971) one gland type has been included in photomicrographs used principally for other illustrative purposes.

The present paper, describing the morphology of the glandular hairs of marihuana from scanning electron microscopy, is the result of our initial investigation into the developmental and functional relationships between glandular hairs and biogenesis of the marihuana hallucinogen tetrahydrocannabinol **(THC) .**

MATERIALS AND METHODS—Plants from a Mexican strain of marihuana, started from seed, were raised in a greenhouse for 35 days under long-day conditions of 16 hr-8 hr, light-dark cycles. Low intensity incandescent light was used to extend natural day length. Flowering was induced by subjecting the 35-day-old plants to 10 short days of 8 hr-16 hr, light-dark cycles (Heslop-Harrison, 1956) by covering the plants with a dark cloth. Flower buds were visible to the unaided eye after as few as six or seven short days. Following the short-day induction period plants were returned to long-day conditions. Bracts, which in marihuana ensheath the ovary, were collected at various developmental stages. The freshly excised bracts were prepared for SEM viewing either by (1) slow drying in a calcium sulfate desiccator, (2) freezing in freon 12 cooled by liquid nitrogen, followed by subliming under high vacuum in a Denton DV-502 evaporator, or (3) fixing in glutaraldehyde, dehydrating in ethanol, immersing in amyl acetate, and critical-point drying. Specimens were coated with carbon and gold-palladium or only gold-palladium and

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viewed at 20 kv accelerating voltage with a Cambridge, Ultrascan, or Autoscan, scanning electron microscope.

OBSERVATIONS-Glandular hairs occur on both pistillate and staminate plants, although in the latter they are of limited abundance and type. Glands on staminate plants are not considered at this time; rather, all observations are limited to pistillate plants. Pistillate plants bear glandular hairs for the most part scattered over the entire shoot surface, with the greatest concentration occurring in flowering tops, especially on bracts.

Three morphologically distinct types of glandular hairs which we refer to as bulbous, capitate-sessile, and capitate-stalked occur on the outer surface of the bracts. The inner surface of bracts, although possessing occasional nonglandular trichomes, is devoid of glands. Bulbous glandular hairs are the smallest and least complex structurally. In early bract development on l-mm-long bracts they appear as small, 10–15 μm in diam spheres scattered over the surface. They frequently arise slightly beneath or become closely appressed to the well-developed, densely aggregated, capitate-sessile glands (Fig. 1, B at arrows). As a result of continuing expansion of the bract, bulbous glands become separated from the other glands. Full-sized bulbous glands as seen laterally (Fig. 2, top center; Fig. 5) are approximately 25-30 μm high with a swollen head 20 μm in diam terminating a short stalk which tapers toward its base. One gland seen from above (Fig. 2, right lower center) bears a circular depression which may be indicative of a rupture in the gland surface, exposing the internal secretory product.

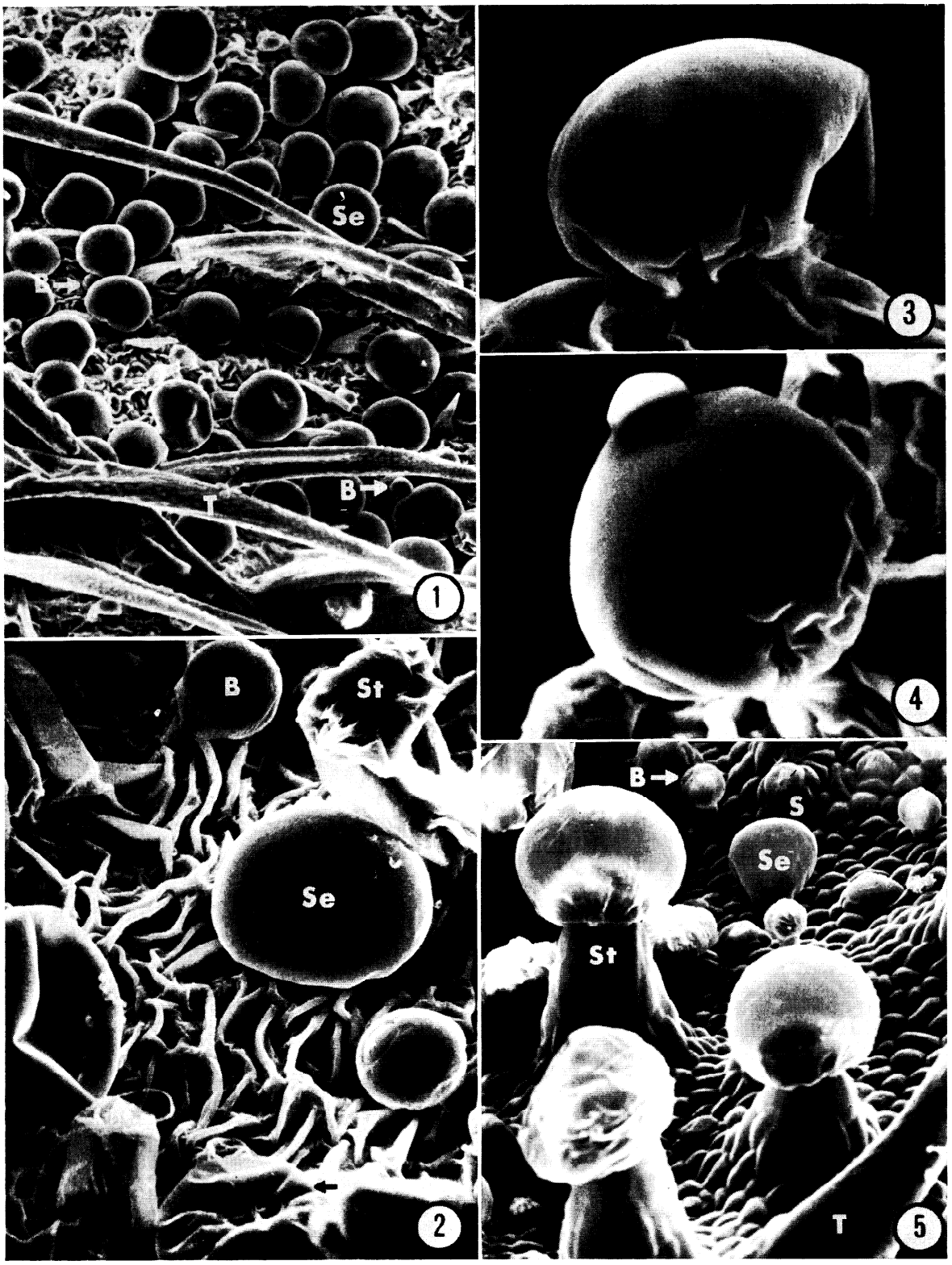
Capitate-sessile glandular hairs are the most conspicuous type during early stages of bract development. Young bracts of barely discernible pistillate flowers already possess well-developed capitate-sessile glands in large numbers (Fig. 1) . These glands, seen in detail in Fig. 3, 4, are stalkless, consisting of a large globose head, 50–70 μm in diam, circular to slightly oblong in outline, and attached directly to the bract surface. They contain an abundant, fluid secretory product which is evident from the flexible nature of the glandular head. This is apparent, for example, in collapsed gland heads (Fig. 2, lower left) or in the regularly occurring vertical folds which radiate from the base of heads (Fig. 3). Mature capitate-sessile glands often bear small, hemispherical protrusions on the head, which may represent extrusion of the secretory product beneath a thin or weak region in the gland surface (Fig. 4).

Capitate-stalked glandular hairs, largest and most structurally complex of the gland types, are the last to mature on developing bracts. They arise among well-developed bulbous and capitate-sessile glands, occurring first along the prominent longitudinal veins of the bract and later scattered over the bract surface. They become the most conspicuous feature of older bracts. Capitate-stalked glands have a 50–70 μm, large globose head positioned on a multicellular, 150-200 μm high stalk (Fig. 5). The globular head contains an abundant secretory product, evidenced again by the flexible nature of its surface, as in cases where the head has completely collapsed upon the underlying cellular disc (Fig. 2, upper right). Capitate-stalked glands, like capitate-sessile glands, often bear small hemispherical protrusions on the head. We have observed similar protrusions on these glands with light microscopy from fresh mounts of bracts. Of special interest in the stalked glands is the juncture of head and stalk. There is an abrupt tapering of the stalk tip, forming a deep circular groove around the base of the head. This juncture is a point of weakness associated with abscission of the gland head. It is common especially in roughly handled samples to find numerous headless stalks. In one such instance, a head still fully turgid fell upside down at the base of its stalk, affording a view of the abscission region (Fig. 2, bottom center at arrow). Here the 4-celled nature of the abscission region is evident from the x-shaped pattern formed by the common radial walls of the basal cells of the head. The presence of regularly occurring, equally spaced, vertical folds radiating from the base of the head remains evident despite inversioa of the head. The number of folds corresponds closely to the number of cells which compose the cellular disc of these glands. The regular occurrence of the folds, their spacing, and number suggest that they may represent regions of incomplete separation of the membranous sheath from the underlying disc cells and that they are not an artifact of preparation.

DISCUSSION—Marihuana glands of pistillate plants are of three types as described for developing bracts; however, they vary in their distribution on the shoot surface. Bulbous and capitate-sessile glands occur on all parts of vegetative and flowering shoots except for the hypocotyl and cotyledons. In contrast, capitate-stalked glands are restricted to flowering regions.

The developmental interrelationships of the three gland types remain to be clarified. Presently, we consider the marihuana glands to be of three distinct types, each with a special developmental pathway, and not just one type arrested at different stages in a common pathway. This is suggested by the fact that all three gland types attain a state of functional maturity as evidenced by the accumulation of the secretory product.

No one method of preparation of SEM specimens is certain to be free of artifact. The chem-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ical alterations and physical forces associated with fixation and dehydration can cause structural abnormalities. Thus, interpretation of real structure can best be achieved through comparison of specimens prepared by a variety of methods. For example, the small hemispherical protruberances found on capitate-sessile and capitate-stalked glands were observed on fresh specimens prepared by chemical desiccation and freezing-subliming methods. Such protuberances were not observed on specimens prepared by fixation followed by critical-point drying. However, the presence of the protruberances on fresh mounts viewed at the light microscope level suggests that there are weak regions in the membranous sheath which protrude outwardly. In our material, various degrees of structural artifact resulted from cell collapse following chemical desiccation or freezing-subliming of fresh material. This is evident in the structure of epidermal cells and gland stalks in Fig. 2 where collapse has left the common walls of adjacent cells in high relief. The absence of such extreme collapse in gland heads on the same specimen suggests a high degree of structural rigidity. The best overall representation of gland structure is achieved by fixation and critical-point drying (Fig. 5). The translucent nature of the gland heads of this micrograph may be due to the extraction of the fluid content of the head.

The lack of developmental and histochemical investigations of marihuana glands is surprising, for these glands appear to be a major site of localization of the hallucinogen THC. The absence of basic information in this area is in contrast to the plethora of biochemical studies on marihuana components (Mechoulam, 1970). Evidence supporting the localization of THC in glands, although limited, is derived from both indirect and direct sources. Indirectly, a correlation exists between the abundance of glands and the amount of THC in plant parts. Bracts have a greater concentration of glands than any other structure on pistillate plants. Fetterman et al. (197 1) demonstrated by gas-liquid chromatography (GLC) that bracts contain a greater amount of THC by weight than any other plant part. Conversely, roots and seeds, plant parts which lack glands, were found to contain small or negligible amounts of THC. Other indirect evidence is obtained from studies of hashish, one of the more potent drug preparations of marihuana. Samples of hashish which are traditionally prepared by mechanically scraping or pounding mature flower tops show the principal structural components to be gland heads, gland stalks, and nonglandular hairs (Shimomura et al., 1967; Nordal, 1970). Our light and scanning electron microscopic observations of a marketed sample of hashish of reputed Lebanon origin confirms the presence of glands as a major component. The only direct evidence for the occurrence of THC in marihuana glands is provided by Fujita et al. (1967). They demonstrated by GLC that THC was present in isolated capitate-sessile and capitate-stalked gland types. Interestingly, the THC was found to be located in the cellular disc within the head and not in the secretory product.

The functional significance of the marihuana glands, although variously described as secretory or protective, may be clarified as their role in the biogenesis, transport, and accumulation of the marihuana cannabinoids, specifically THC, is studied. Nevertheless, the localization of THC in glands of marihuana certainly has been an effective species dispersal mechanism through its attraction to both early and modern man as a medicament and hallucinogen.

LITERATURE CITED

ASAHINA, H., M. ONO, K. TAKAHASHI, AND Y. ONO. 1967. Identification of *Cannabis* resin. Bull. Nat. Inst. Hyg. Sci. Tokyo 85: 123-125.

BOUQUET, R. J. 1950. Cannabis. Bull. Narcotics 2: 14-30.

BRADFORD, L. W., AND J. DEVANEY. 1970. Scanning electron microscopy applications in criminalistics. J. Forensic Sci. 15: 110-119.

BRIOSI, G., AND F. TOGNINI. 1894. Intorno alla anatomia della canapa (*Cannabis sativa* L.). Parte prima: Organi sessuali. Atti 1st. Bot. Pavia, Ser. 2. 3: 91-209.

———, AND ——— 1897. Intorno alla anatomia della canapa *(Cannabis sativa* L.). Parte seconda: Organi vegetativi. Atti 1st. Bot. Pavia, Ser. 2. 4: 155-3 29.

DEVANEY, J. R., AND L. W. BRADFORD. 1971. Applications of scanning electron microscopy to forensic science at Jet Propulsion Laboratory, 1969-1970, pt. 2, p. 561-568. *In* 0. Johari and I. Corvin [ed.], Scanning electron microscope symposium, Scanning electron microscopy proceedings. IIT Research Institute, Chicago.

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Fig. l-5. Glandular hairs on bracts of pistillate plants of *Cannabis*. *1.* Portion of young bract with numerous capitate-sessile glands, young bulbous glands, and non-glandular trichomes. × 255. 2. Portion of old bract with full sized bulbous, capitate-sessile, and capitate-stalked glands. Note complete collapse of head of stalked gland in upper right and abscised head from gland stalk in lower center (arrow). × 700. 3. Capitate-sessile gland. × 1100. 4. Capitate-sessile gland with protrusion on head. × 1140. 5. Portion of old bract bearing full sized bulbous, capitate-sessile, and capitate-stalked glands. Note several stalked stomates. × 390. Specimens were prepared by freezing and subliming (Fig. 1, 2), chemical desiccation (Fig. 3, 4), or critical-point drying (Fig. 5). *B*, bulbous gland; *S*, stalked stomate; Se, capitate-sessile gland; *St*, capitate-stalked gland; *T*, nonglandular trichome.

FETTERMAN, P. S., D. S. KEITH, C. W. WALLER, 0. GUERRERO, N. J. DOORENBOS, AND M. W. QUIMBY. **197 1.** Mississippi-grown *Cannabis sativa* L. **:** Preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part. J. Pharm. Sci. 60: 1246–1249.

FUJITA, M., S. HIROKO, **E. KURIYAMA, M. SHIGEHIRO, AND M. AKASU.** *1967.* Studies on *Cannabis*. II. Examination of the narcotic and its related components in hemps, crude drugs, and plant organs by gas-liquid chromatography and thin-layer chromatography. Annu. Rep. Tokyo Coll. Pharm. 17: 238-242.

HESLOP-HARRISON, J. 1956. Auxin and sexuality in *Cannabis sativa.* Physiol. Plant. 9: 588-597.

MECHOULAM, R. 1970. Marihuana chemistry. Science 168: 1159-1166.

MOHAN RAM, H. Y., AND R. NATH. 1964. The morphology and embryology of *Cannabis sativa* Linn. Phytomorphology 14: 414-429.

NARAMURA, G. R. 1969. Forensic aspects of cystolith hairs of *Cannabis* and other plants. J. Ass. Offic. Anal. Chem. 52: 5-16.

NORDAL, A. 1970. Microscopic detection of *Cannabis* in the pure state and in semi-cornbusted residues, p. 61-68. *In C.* R. B. Joyce and S. H. Curry [ed.], The botany and chemistry of *Cannabis*. Churchill, London.

SHIMOMURA, H., M. SHIGEHIRO, E. KURIYAMA, AND M. FUGITA. 1967. Studies on *Cannabis*. I. Microscopical characters of their internal morphology and spodogram. Annu. Rep. Tokyo Coll. Pharm. 17: 232-237.

SIEGESMUND, K. A., AND G. M. HUNTER. 1971. Scanning electron microscopy of selected crime laboratory specimens, pt. 2, p. 577-584. *In 0.* Johari and I. Corvin [ed.], Scanning electron microscope symposium, Scanning electron microscopy proceedings. IIT Research Institute, Chicago.

STEARN, W. T. 1970. The *Cannabis* plant: botanical characteristics, p. l-10. *In C.* R. B. Joyce and S. H. Curry [ed.], The botany and chemistry of *Cannabis*. Churchill, London.