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STRUCTURE, DEVELOPMENT AND COMPOSITION OF GLANDULAR TRICHOMES OF *CANNABIS SATIVA* L.

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ABSTRACT

The glandular secretory system of Cannabis sativa L. *is composed of bulbous, capitate-sessile, and capitate-stalked glands which are distinguishable from each other by morphogenesis and physiology. Bulbous and capitate-sessile forms occur on vegetative and floral axes whereas the highly evolved capitate-stalked form is present only on floral-related organs. In studies of cloned plants, gland initiation occurred on leaves and pistillate bracts throughout organ ontongeny. Gland density and time of appearance varied between both clones and organs, indicating that control of development is independent for each trichome type. Cannabinoid synthesis also occurred throughout organ ontongeny but with a decreasing rate in leaves as compared to an increasing rate in bracts. In individual glands, cannabinoid content decreased during maturation. Capitate-stalked glands contained higher cannabinoid levels than the sessile form although the glands maintained the profile characteristic of the clone. Analyses of glands and tissues indicated cannabinoids may occur in cells other than glands. Capitate glands develop a disc of secretory cells, and secretions accumulate in a cavity beneath a sheath derived from separation of the cuticularized outer wall surface of the disc cells. Presumed secretions, including cannabinoids, occur at the surface of plastids and appear to migrate to the cell surface adjoining the secretory cavity. Materials appear to be compartmentalized into spheres of variable size in the cavity. Cell fractionation studies are in progress to define the cannabinoid sythesizing activities within the dynamic glandular system of* Cannabis.

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1. INTRODUCTION

The epidermal glandular secretory system of *Cannabis* is an external type consisting of capitate glandular trichomes which secrete a resinous product. First descriptions of this system were made during the 19th century (Martius 1855; Unger 1866; Flückiger and Hanbury 1878; Tschirch 1889) and most extensively by Briosi and Tognini (1884). More recently, other studies utilizing both light and electron microscopy have focused on the glands for their potential utilization in *Cannabis* systematics and forensics, and for their association with cannabinoids (Bouquet 1950; Mohan Ram and Nath 1964; Shimomura 1967; Fairbairn 1972; De Pasquale 1974; Ledbetter and Krikorian 1975; Dayanandan and Kaufman 1976). Other studies have been concerned primarily with the cannabinoids, terpenophenolic compounds present only in *Cannabis*, that accumulate in the glandular trichomes (Fujita et al. 1967; Fairbairn 1972; Malingré et al. 1975; André and Vercruysse 1976). Several investigations in our laboratory have been directed specifically to studies of individual glandular trichomes (Hammond and Mahlberg 1973, 1977, 1978; Turner, Hemphill and Mahlberg 1978) as well as populations of trichomes on different organs (Turner et al. 1977, 1980). Our studies have emphasized morphogenetical and ultrastructural features of trichome development, and the analyses of cannabinoid contents of the various glandular trichomes. In addition, we have examined the interrelationships of the gland populations and the cannabinoid composition on developing plant organs. In this report we wish to bring together a number of salient features that we have found to be associated with trichome morphogenesis and cannabinoid production during the development of the glands that compose the secretory system in *Cannabis*. The objectives of our extensive studies are to determine the morphogenetic factors that control trichome development and cannabinoid synthesis, and to determine the pattern of evolution of the complex trichome system present on the different strains of *Cannabis*.

2. MATERIALS AND METHODS

Cannabis plants were specific strains (Hammond and Mahlberg 1973) or clones (Turner et al. 1977) derived and cultured as previously described. Scanning electron microscopy (SEM) was done with an ETEC Autoscan (Hammond and Mahlberg 1977; Turner et al. 1977), and SEM also was utilized for gland quantitation (Turner et al. 1977). Transmission electron microscopy was done on a Hitachi HU-11C, and samples were prepared as described previously (Hammond and Mahlberg 1978). Cannabinoid content was determined by gas-liquid chromatography (GLC) on a Hewlett-

Packard 5710A chromatograph equipped with a Hewlett-Packard 3380A integrator as described previously (Turner et al. 1977).

3. RESULTS AND DISCUSSION

3.1 *Gland description*

Three types of glandular trichomes are present on the epidermis of the outer surface of bracts from the pistillate plant of *Cannabis* as illustrated in Figure 1. The three types of glands include bulbous, capitate-sessile, and capitate-stalked (Fig. 2). Nonglandular trichomes also are present on the plant in abundance (Figs. 1,2), but will not be discussed here in detail. When mature, each of the three glandular trichome types consists of two distinct components. A secretory head comprises the top portion that is supported for each gland type by an auxiliary portion consisting of a layer of stipe cells subjacent to the head and a layer of base cells associated with the epidermis.

Bulbous glands are the smallest of the three glandular types. They are approximately 25-30 μm high with a secretory head about 20 μm in diameter (Fig. 3). The secretory head may contain one to four secretory cells in a single layer. The auxiliary portion consists of a one or two-celled base layer adjacent to the epidermis and supporting a one or two-celled stipe layer. Capitate-sessile glands have a large globose head approximately 40-60 μm in diameter (Fig. 4). Although the glands appear to be positioned directly on the epidermal surface, the secretory gland head is supported on a short axis consisting of base cell and stipe cell layers. Secretory cells within the gland head are arranged in a single discoidal layer of 8 to 13 cells bounded on the top with a membranous sheath under which accumulate the glandular secretory products. Capitate-stalked glands, also supported by an auxiliary portion, have gland heads similar in size and structure to the capitate-sessile gland heads, although they are frequently as large as 100 μm in diameter (Fig. 5). Capitate-stalked glands are distinguishable from other glands by the presence of a stalk which is derived secondarily from epidermal and subepidermal tissues rather than from immediate derivatives of the gland initial.

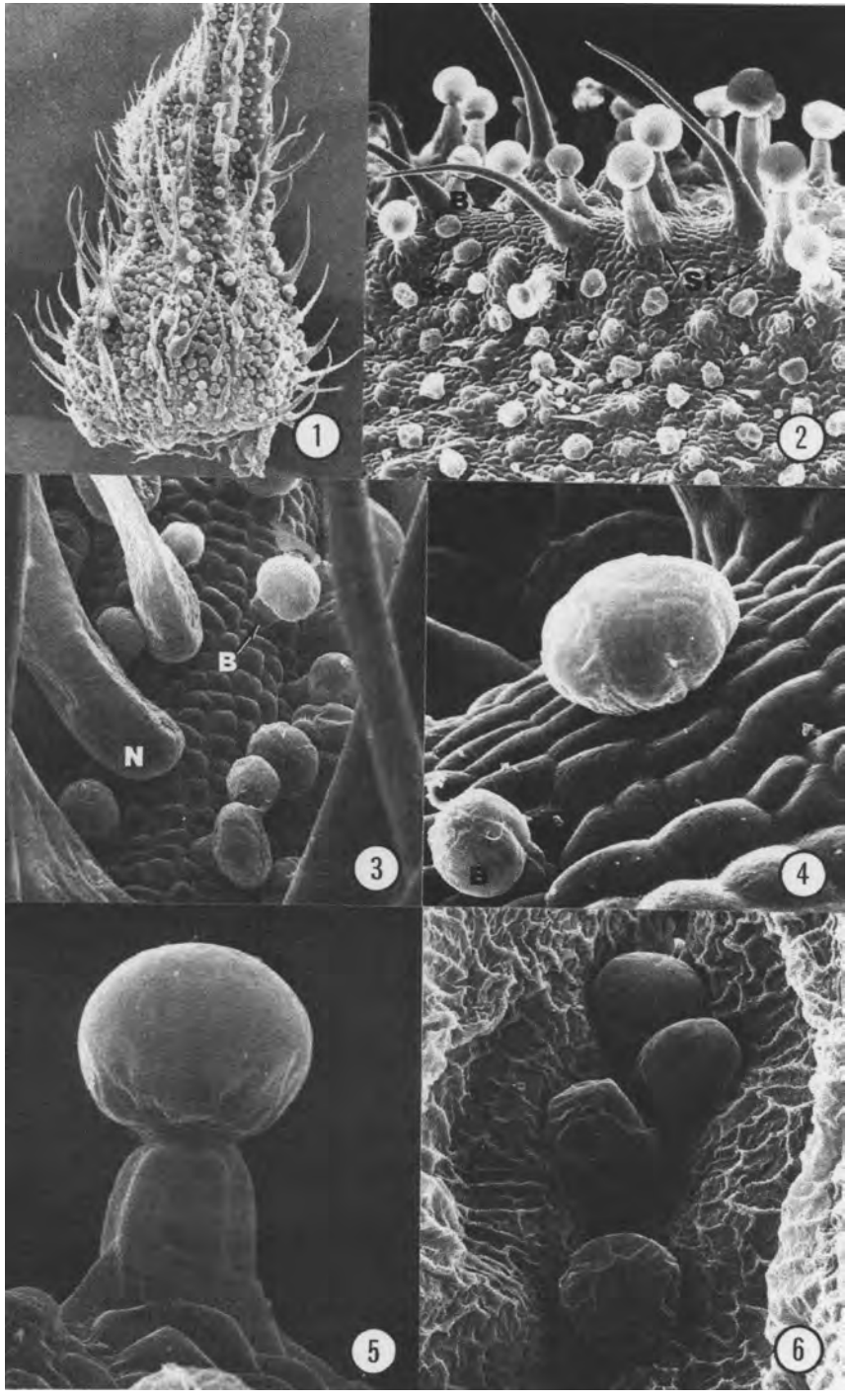
Bulbous and capitate-sessile glands are present on most aerial epidermal surfaces of both pistillate and staminate plants. Capitate-stalked glands are found only on flowering regions of pistillate plants, specifically on bracts and small leaves adjacent to bracts. A capitate gland also develops selectively along the clefts, but not sutures, between anther sacs of the staminate plants, and is interpreted to represent a fourth type of glandular trichome which we identify as the antherial capitate-sessile gland. This gland, although it is composed of a secretory

Key to labeling

B - bulbous gland BC - base cell CW - cell wall
D - dictyosome ER - endoplasmic reticulum
F - fibrillar material L - lipid body
M - mitochondrion N - nonglandular trichome
Nu - nucleus P - secretory product Pl - plastid
S - secretory cell SC - secretory cavity
Se - capitate-sessile gland SP - stipe cell
St - capitate-stalked gland V - vacuole

Figures 1-6. Morphology

Fig. 1. Young 3 mm long pistillate bract with abundant glandular and nonglandular trichomes. x 35.
Fig. 2. Mature bract with nonglandular trichomes and three glandular trichome types. x87.
Fig. 3. Bulbous gland. x 540.
Fig. 4. Capitate-sessile gland. x 810.
Fig. 5. Capitate-stalked gland. x 810
Fig. 6. Antherial capitate-sessile glands. x 205.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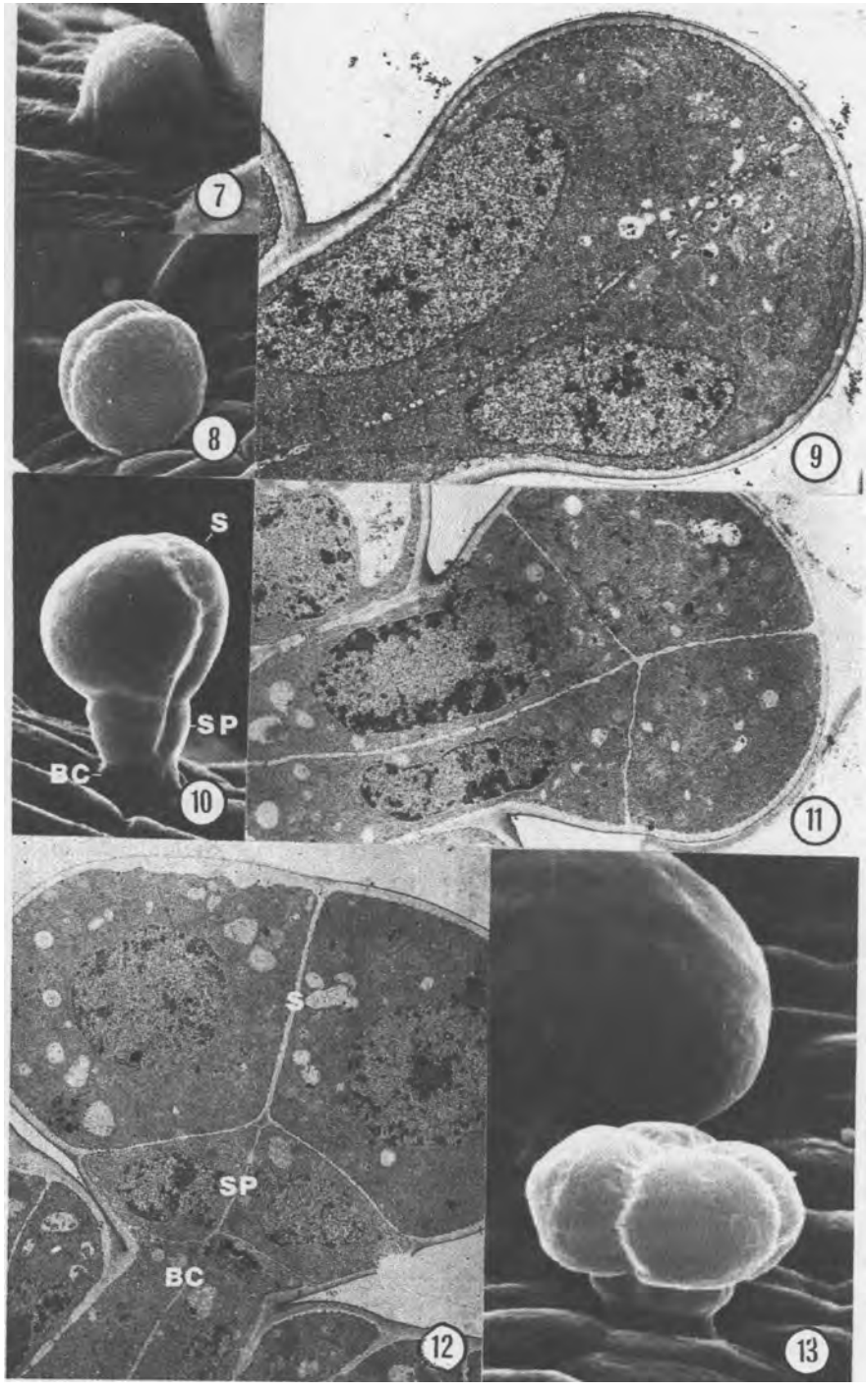
Figures 7-13. Early capitate-stalked gland development.

Fig. 7. Gland initial. x 2,250.
Fig. 8. Two-celled gland stage. x 2,045.
Fig. 9. TEM of two-celled gland stage. x 9,000
Fig. 10. Young gland following periclinal division in lower half of auxiliary portion producing basal and stipe cell layers. x 1,390.
Fig. 11. TEM of first periclinal division in gland creating upper secretory portion and lower auxiliary portion. x 4,100.
Fig. 12. TEM of periclinal division in lower half of auxiliary portion producing basal and stipe cell layers. x 3,900.
Fig. 13. Formation of flattened, four-celled gland head. x 1.905.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head on a short auxiliary portion, is sometimes found to be at least twice as large as any of the capitate gland types. It often develops into an oblong shape with its elongated axis parallel with the cleft of the anther. Further studies are in progress to elucidate more fully the morphogenesis and secretory content of the antherial capitate-sessile gland. Both capitate-stalked and capitate-sessile glands have been shown to contain cannabinoids. Bulbous glands also are observed to produce a secretory product, but as yet there is no direct evidence for the presence of cannabinoids in these glands. The small size of the bulbous gland makes it very difficult to collect samples from the head for chromatographic analyses.

3.2 *Development of individual glands*

At very early stages in development of individual glands, it is difficult to distinguish between the three gland types by morphological features. As the small bulbous gland matures, it can be identified by the presence of a small secretory cavity (Fig. 3). Mature bulbous glands typically consist of a two-celled base and two-celled stipe layers supporting a two to four-celled secretory head. However, we have observed base and stipe tiers each composed of only one cell. Capitate-sessile and capitate-stalked glands initially follow a similar development sequence during enlargement of the head. The head of the capitate-sessile gland appears in close proximity to the epidermis as it matures to the secretory phase (Fig. 4), whereas the head of the capitate-stalked gland becomes elevated above the dermal surface during this phase (Fig. 5).

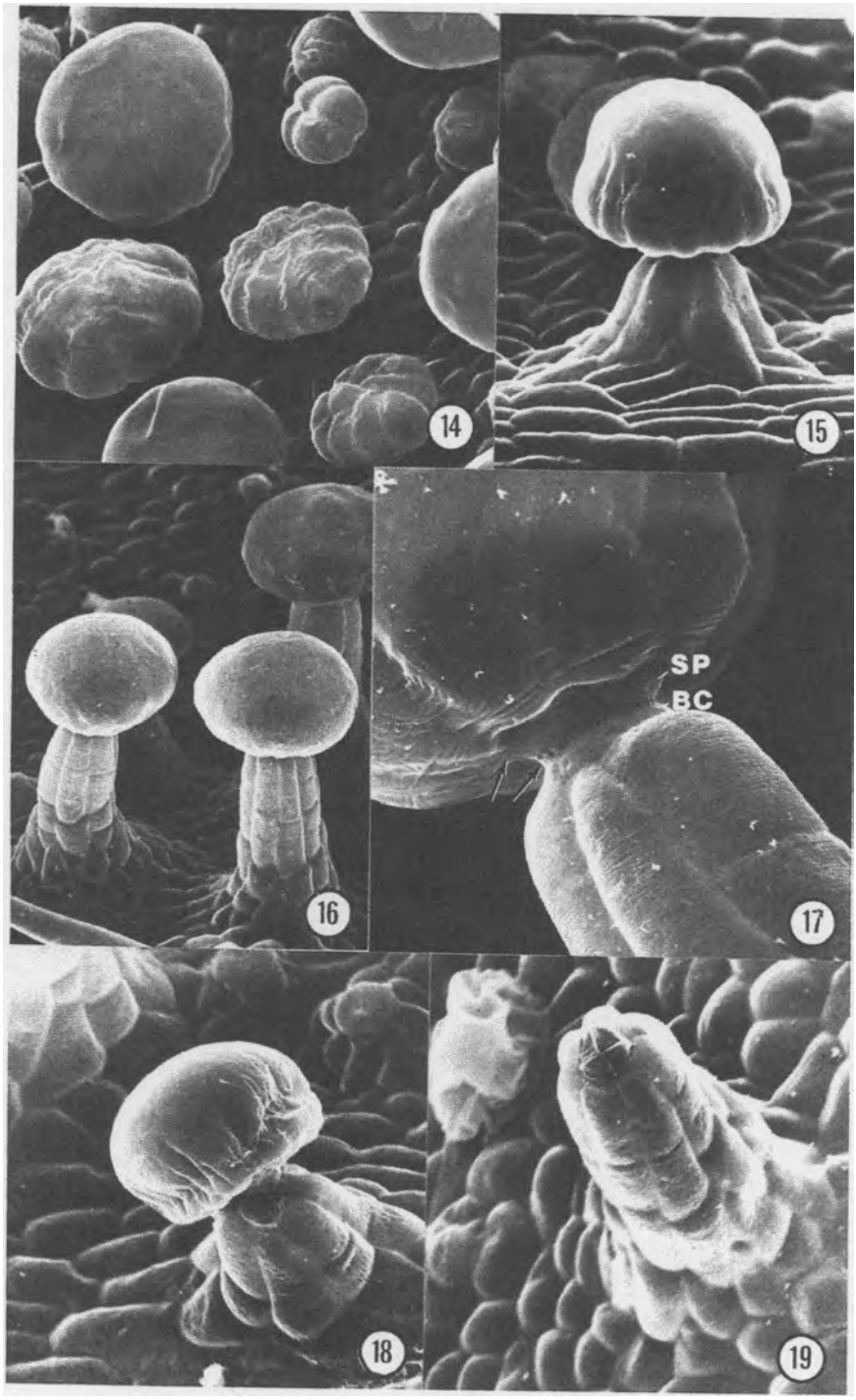
The early stages in the initiaion of capitate-stalked glands were studied on areas of the bract where numerous such glands were known to be undergoing development. Gland initiation is first evident when a dermal initial enlarges to form a protrusion on the epidermis (Fig. 7). At a very early stage in development of this initial an anticlinal division bisects the cell in the longitudinal plane (Figs. 8,9). Large nuclei are conspicuous in each of the derived cells. A periclinal division occurs to form a lower pair of auxiliary cells and an upper or distal pair of secretory cells (Figs. 10,11). Subsequently, a second periclinal division below the first divides the auxiliary portion of the young gland into base and stipe layers (Figs. 10,12). The base tier remains a two-celled layer, while the stipe tier and the secretory tier undergo additional anticlinal divisions. The auxiliary portion is complete with the formation of a two-celled base layer and a four-celled stipe layer. The four-cells of the secretory head appear to flatten into a disc by radial enlargement and the cells divide anticlinally to produce a secretory disc of

approximately 8 to 13 cells (Fig. 13). At this stage in development, the cells no longer divide but continue to increase in size (Fig. 14). During the final stages of disc enlargement, secretory activity is initiated in the disc cells and products from this activity are deposited in a space formed above the disc of secretory cells. As the head approaches its full size it becomes elevated on a multicellular stalk (Fig. 15). The stalk is formed from surrounding epidermal as well as subepidermal cells which elongate vertically to raise the gland proper--base, stipe and secretory cells--well above the surface of the bract (Figs. 15, 16). Epidermal cells in the upper portions of the stalk may undergo transverse division during their elongation. Mature capitate-stalked glands possess a dehiscence mechanism that aids in detaching the gland head from the stalk (Figs. 17-19). Abscission regions develop in both the base and stipe tiers (Fig. 17) which allows the head to separate from the stalk (Fig. 18) leaving either the base cells or stipe cells in relief (Fig. 19).

Based on morphology, development, and distribution on the plant, we consider the *Cannabis* glandular system to consist of at least four distinct and independent gland types. Although they apparently have a similar developmental sequence during their early formative phase of growth, the mature structures are quite distinguishable from each other. Thus, these glands do not represent a single morphological type arrested at various stages in development. Bulbous glands are readily identified by their small size and accumulation of secretory products in the few-celled gland head. Capitate-sessile and capitate-stalked glands differ from each other in their distribution on the plant and in the production of the stalk. Capitate-sessile glands are ubiquitous on the aerial epidermis, whereas capitate-stalked glands are observed only on bracts or adjacent leaves on the pistillate plant. Development of the stalk, although derived from cells other than the gland initial, is dependent upon some factor related to the gland proper, because stalks do not form in the absence of a gland head. The gland in some way exerts control on surrounding cells to trigger and maintain the growth of the stalk. Since capitate-stalked glands are restricted to flowering regions of pistillate plants, the physiology of this region must in some way interact with this gland type to control morphogenesis. Similarly, the selective localization of the antherial capitate-sessile gland on the reproductive structure of the staminate plant suggests that it represents a distinctive gland type in the complex trichome system of *Cannabis*. These variations in glandular types, and the presence of several types on a particular organ such as the bract, indicate that each type reflects subtle differences in the evolution of glandular trichomes in this genus.

Figures 14-19. Capitate-stalked gland development.

Fig. 14. Stages of increasing numbers of secretory disc cells and of increasing size. x 775.
Fig. 15. Elevation of gland on multicellular stalk. x 725.
Fig. 16. Mature capitate-stalked glands. x 255.
Fig. 17. Two abscission layers (arrows) in auxiliary portion of gland at juncture of gland and multicellular stalk. x 1,510.
Fig. 18. Gland head abscission. x 300
Fig. 19. Mature stalked gland after abscission of gland head at stipe cell layer. x 600.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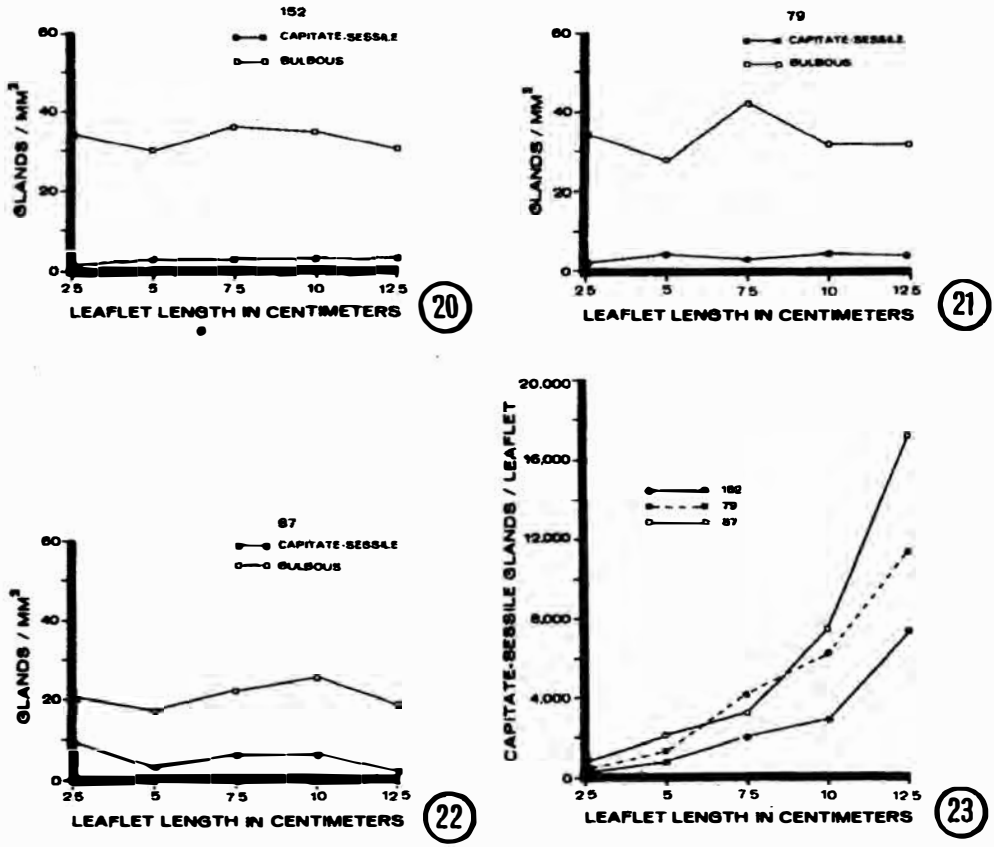


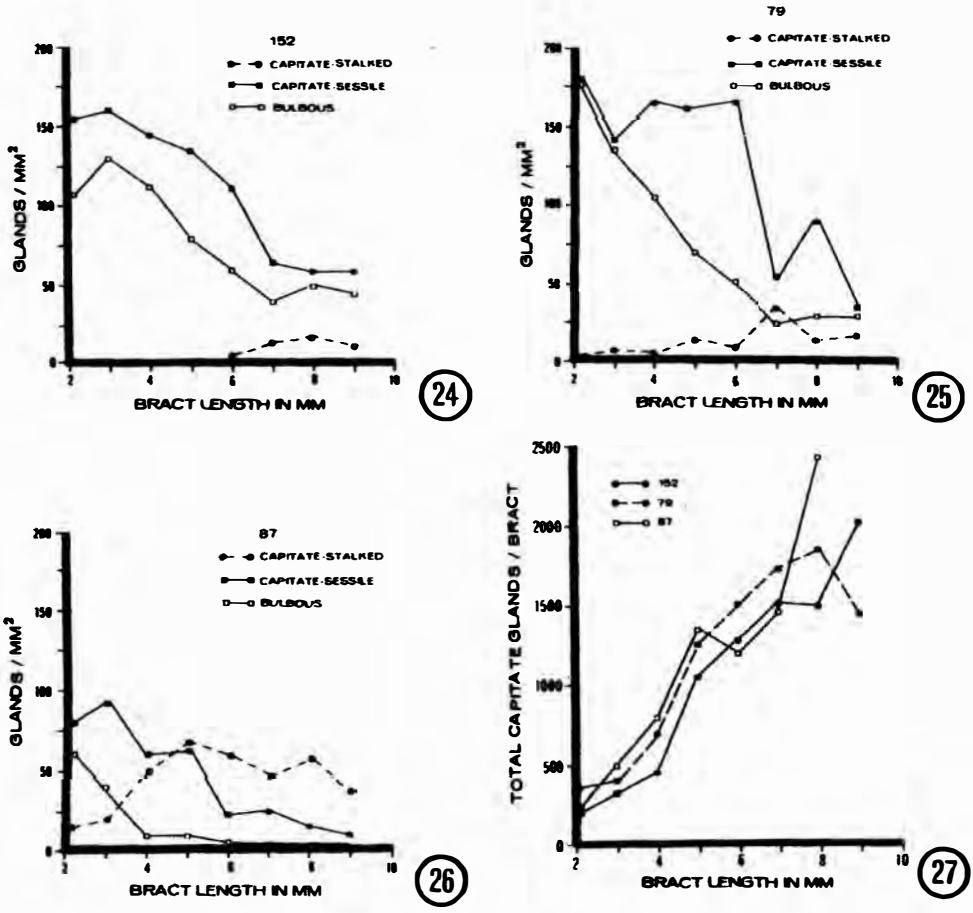
Figures 20-23. Gland populations on developing plant organs.

Fig. 20-Fig. 22. Glands per sq mm on leaves of clones 152, 79 and 87.
Fig. 23. Total capitate-sessile glands on leaves.

3.3 *Gland populations on developing plant organs*

Differences in patterns of gland initiation during organ development also indicated that these gland types were distinct and independent of each other. In addition, the trend of the gland population on an organ appears to depend on whether the organ is located on flowering or vegetative regions of the plant. These studies were performed on clones of *Cannabis*, each derived from a single parent plant, so as to maintain genetically uniform experimental material and to grow the plant over a multiple year period. A comparison of the gland types on the three clones, representing drug, non-drug and fiber type plants, indicated that the developing leaves and bracts of each clone possessed distinctly different populations of glands. On vegetative leaves the gland density remained relatively constant throughout leaf ontogeny (Figs. 20-22). No capitate-stalked glands were observed to develop on these leaves. Bulbous glands on all three clones were present in greater numbers than were capitate-sessile glands. Gland density remained constant on enlarging leaves and indicated that new glands were being initiated from dermal cells throughout leaf development. This is readily seen upon examining the total gland number per leaf (Fig. 23). On bracts the density of capitate-sessile and bulbous glands decreased while the density of capitate-stalked glands increased during bract ontogeny. (Figs. 24-26). Note that initiation of capitate-stalked glands was continual throughout bract ontogeny. The densities of capitate-sessile and bulbous glands decreased during bract ontogeny, although the rate of decrease for these glands was slower than the rate of increase of the surface area of the bract. Thus, new glands of each type were initiated throughout bract development. This conclusion is evident upon examination of the data illustrating the total number of glands per bract (Fig. 27).

The capitate-stalked gland was of particular interest because the time of appearance of this gland during bract development varied between clones. On the drug clone 152, capitate-stalked glands did not appear until midway through bract development (Fig. 24). This was also found to be the case for a Mexican drug strain. However, on the non-drug clone 79 (Fig. 25) and fiber clone 87 (Fig. 26), capitate-stalked glands were present on the youngest bract stages observed although in higher numbers on clone 87 than on clone 79. This temporal difference for gland initiation between clones indicates the existence of a subtle genetic mechanism which controls the initiation of this gland type. While the environment also may influence the timing of gland formation, additional studies are necessary to determine the interrelationships of genetic and environmental factors on gland initiation.



Figures 24-27. Gland populations on developing plant organs.

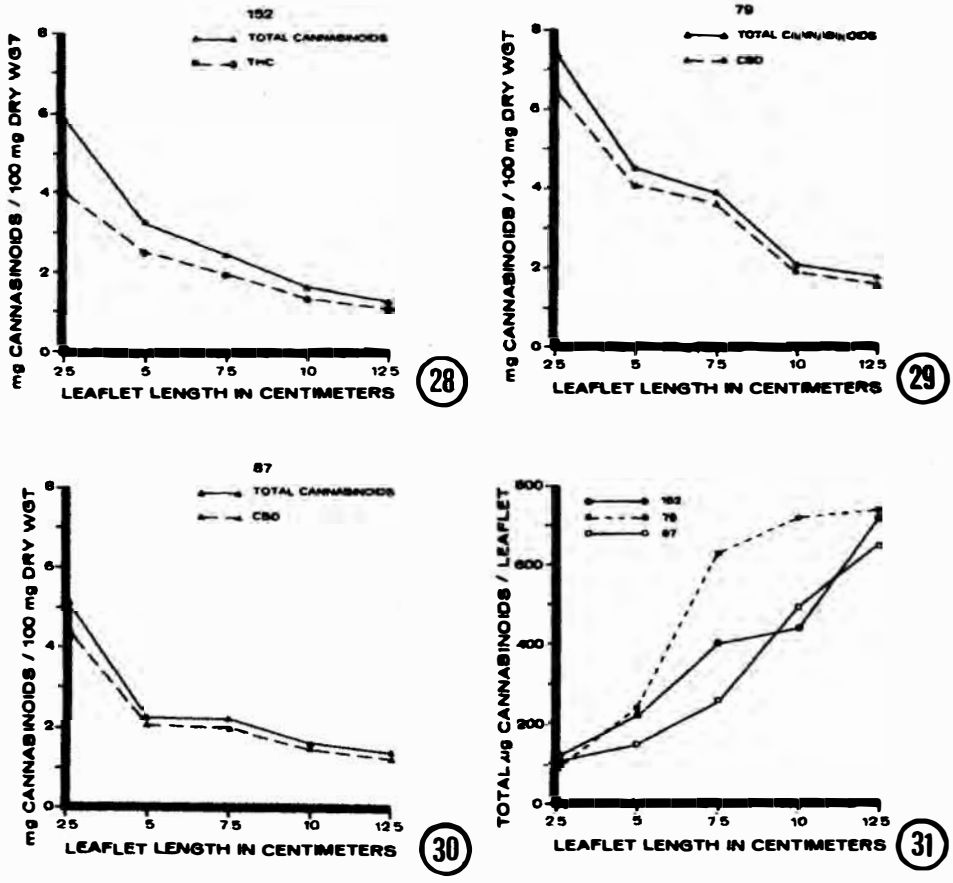
Fig. 24-Fig. 26. Glands per sq mm on pistillate bracts of clone 152, 79 and 87.
Fig. 27. Total capitate glands on bract.

The different patterns of the gland populations on leaves as compared to bracts may reflect the possible interaction of the physiology of the flowering region on gland type and on gland initiation. Although glands are continually initiated throughout development on both organs, the stable gland population on leaves contrasts with the progressively changing density for each gland type on the bracts. These changes in gland densities on developing bracts may be reflective of a progressive evolutionary phenomenon for the glandular system and may be related to a changing functional role of the bract during its development. Young bracts, covered with an abundance of capitate-sessile glands (Fig. 1), may protect floral organs from dessication. On old bracts, which surround developing seeds, the stratified system of bulbous, capitate-sessile and capitate-stalked glands (Fig. 2) may function to protect the maturing seed against herbivory as well as against dessication. The protective role of capitate-stalked glands on the bract is supported further by the trends in the population of nonglandular trichomes. These nonglandular trichomes are silicified, stiff hairs presumably functioning to protect the plant surface (Figs. 1,2). On clone 152, for example, no capitate-stalked glands are present on young bracts (Fig. 24) whereas the density of nonglandular trichomes is high. As the bract develops, capitate-stalked glands appear and increase in density, while the density of nonglandular trichomes progressively decreases during bract ontogeny.

3.4 *Cannabinoids in developing plant organs*

A major aspect of the glandular system of *Cannabis* is the presence of cannabinoids among the secretory products. In our initial studies (Turner et al. 1977), we started with the simple hypothesis that if specific glands were associated with cannabinoids, a correlation should exist between gland density and cannabinoid concentration of a plant organ. The results showed the system to be more complex and dynamic than originally expected. Cannabinoid content within the glands varied with gland age and, as already discussed, gland populations varied with the plant organ and developmental stage of the organ. Therefore, parallel to studying gland populations during organ development, cannabinoid content was also studied.

Cannabinoid content in leaves, based on dry weight, decreased during leaf ontogeny in the three clones studied (Figs. 28-30). The identity of the major cannabinoid component, whether Δ^9 -tetrahydrocannabionol or cannabidiol, was unrelated to the cannabinoid concentration in developing leaves. All three clones showed the same decreasing trend for cannabinoids, but quantitative differences in cannabinoid concentration existed between the clones. Although the cannabinoid concentration expressed as dry weight decreased during organ development, the



Figures 28-31. Cannabinoid content of developing plant organs.

Fig. 28-Fig. 30. Cannabinoid content, based on dry weight, in leaves of clones 152, 79 and 87.
Fig. 31. Total cannabinoids in leaves.

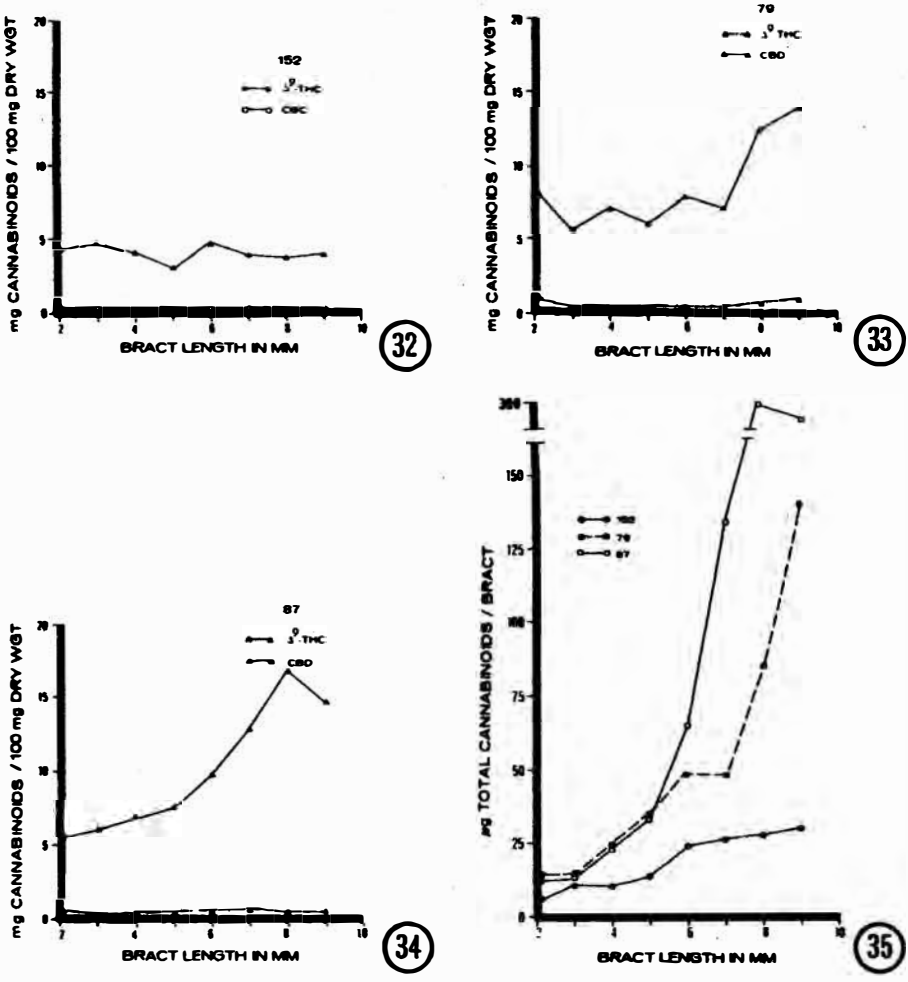
progressive rate of leaf expansion still resulted in an increase in the total cannabinoid content in a leaf as it matured (Fig. 31). All three clones showed very much the same pattern, although clone 79 had a higher total cannabinoid content at mid-stages in organ development when compared to the other clones. Thus, there is continued synthesis of cannabinoids during those stages of leaf development examined in this study.

On bracts, the trends in the cannabinoid concentrations, based on dry weight of tissues during the ontogeny of this organ, varied between the clones (Figs. 32-34). The drug clone, 152, was found to have a relatively constant level of cannabinoids throughout bract development (Fig. 32). However, non-drug clone 79 (Fig. 33) and fiber clone 87 (Fig. 34), both containing cannabidiol as the major cannabinoid, each showed an increase in cannabinoid concentration as the bract matured. In clone 87 the cannabinoid concentration began to increase at an earlier developmental stage of the bract than it did in clone 79. It was apparent from these studies that cannabinoid synthesis was occurring throughout bract development. This was readily evident when the three clones were compared for total cannabinoids per bract (Fig. 35).

Thus, on both leaves and bracts, it was apparent that cannabinoid synthesis occurred throughout organ development. Since gland initiation also was found to occur throughout organ ontogeny, it was possible to determine whether a positive correlation existed between total cannabinoids and total gland number for an organ. Calculations based upon the total cannabinoid content distributed among the gland populations on the leaf and bract, showed that there were differences in the cannabinoid concentration in glands between these organs. For bracts, the estimated content matched relatively well with actual analyses indicating that the gland population on bracts is capable of accomodating most of the cannabinoids present in a bract. In contrast, on leaves the estimated cannabinoid content of an individual gland was much higher than that found in actual analyses indicating that the other leaf cells in addition to the glands may be producing or accumulating cannabinoids.

3.5 *Cannabinoids in individual glands*

Looking specifically at the analyses of individual glands for cannabinoids, several factors become apparent. An initial finding was that cannabinoid content varied with gland age. Among a population of capitate-stalked glands in the secretory phase, it was possible to recognize three stages of maturation. In the initial mature stage, the gland possessed a head with a clear liquid content. The second stage, referred to as aged, had a head that appeared yellow with a sticky, dense content. The final senescent stage was represented by a gland with a red, dried head.



Figures 32-35. Cannabinoid content of developing plant organs.

Fig. 32-Fig. 34. Cannabinoid content, based on dry weight, in bracts of clones 152, 79 and 87.
Fig. 35. Total cannabinoids in bracts.

TABLE 1. Cannabinoid content of capitate stalked gland.

Clone	Gland age	ng Cannabinoid/gland		
		CBD	Δ^9-THC	CBN
152 (drug)	Mature	-	57	-
	Aged	-	35	21
	Senescent	-	9	1
87 (fiber)	Mature	229	-	-
	Aged	113	-	-
	Senescent	29	-	-

TABLE 2. Principal cannabinoid present in glandular trichomes of clone 152.

Gland type	Plant organ	ng Δ^9-tetrahydrocannabinol/gland		
		October	December	March
Capitate-stalked	Bract			
	Vein	55.05	60.25	43.66
	Nonvein	20.51	31.45	16.29
Capitate-sessile	Leaf			
	Vein	ncd[a]	ncd	ncd
	Nonvein	ncd	ncd	ncd (2.93[b])

[a]ncd, no cannabinoids detected
[b]100 gland sample

TABLE 3. Principal cannabinoid present in glandular trichomes of Turkish strain.

Gland type	Plant organ	ng cannabidiol/gland	
		October	December
Capitate-stalked	Bract		
	Vein	56.64	193.25
	Nonvein	102.97	145.95
Capitate-sessile	Leaf		
	Vein	8.31	ncd[a]
	Nonvein	13.42	11.72 (11.15)[b]

[a]ncd, no cannabinoids detected
[b]100 gland sample

Cannabinoid contents decreased greatly as the gland head aged, although at each stage the cannabinoid character of the clone was maintained (Table 1). Thus, cannabinoid contents in individual glands appear to be related to the age of the gland and to the specific strain of plant.

Analyses of individual glands were expanded to include other morphological parameters for these clones as well as for several strains. Mature capitate-stalked glands from vein and nonvein areas of the bract, and capitate-sessile glands from vein and nonvein areas of the leaf, were analyzed for their cannabinoid composition. On clone 152, capitate-stalked glands from vein areas of the bract had higher levels of cannabinoids than did capitate-stalked glands from nonvein areas (Table 2). Capitate-sessile glands on leaves of 152, whether from vein or nonvein areas, contained little or no cannabinoids. A sample of 100 glands from the leaf did show the presence of cannabinoids suggesting that low levels of cannabinoids were present in capitate-sessile glands of 152. Comparable analyses of glands on clone 87 showed very similar results. On bracts and leaves of a Turkish strain, high in cannabidiol, glands from vein and nonvein areas varied but without any specific trends (Table 3). Cannabinoid levels in the Turkish strain were considerably higher than levels found in clones 152 or 87. Capitate-sessile glands generally were found to contain cannabinoids. Analyses of a Mexican strain high in Δ^9-tetrahydrocannabinol, were found to be quite similar to the analyses of the Turkish strain even though the glands differed for the principal type of cannabinoid present in the plant.

The conspicuous differences in cannabinoid concentration between the capitate-stalked and capitate-sessile glands is a character that aids to distinguish between these two gland types. However, it is possible that the differences in cannabinoid content between these gland types, as found in these studies, may be related to their location either on bracts or leaves. The larger size of the capitate-stalked gland head compared to the head of the capitate-sessile gland also may be a factor in their differing cannabinoid content. Yet other factors must be involved because the ratio for size and volume of these two gland types does not correlate with the ratio of cannabinoid levels. It is interesting to note that capitate-stalked glands have the highest cannabinoid concentration. The development of a stalk and the increased concentration of cannabinoids, therefore, indicate the evolutionary trends for the glandular system of *Cannabis*.

3.6 *Gland Ultrastructure*

While gland morphology as well as the cannabinoid content of glands have been studied in some detail by a number of investigators, little has been done to relate gland ultrastructure to the production of cannabinoids. It is well known that the formation

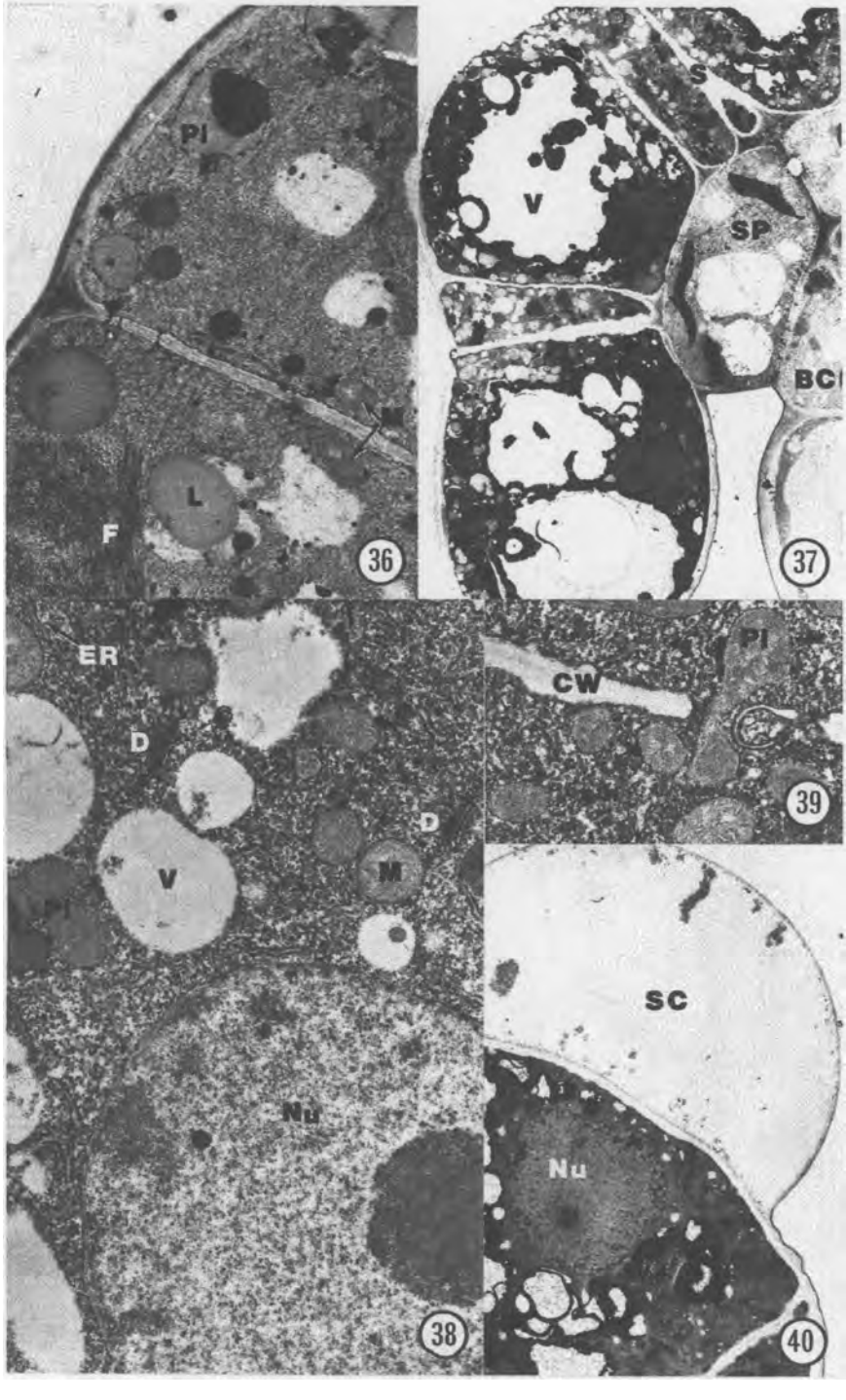
of secretory products in glands of *Cannabis* results in the formation of a distended cuticular sheath area in which accumulate the secretory products derived from the disc cells. We have examined the ontogeny of the gland and development of the secretory cavity in an effort to determine the possible cytoplasmic components involved in cannabinoid synthesis and the process by which the secretory sac is formed during gland development.

In the pre-secretory stage, the head of the young capitate-stalked gland undergoes radial enlargement to initiate formation of the disc cells. The protoplasm of the immature disc cells contain few endoplasmic reticulum (ER) elements, dictyosomes or plastids (Figs. 9,11,12). The plastids in these cells contain a poorly developed thylakoid system compared to chloroplasts in adjacent cells. As the disc of the gland develops to the 4-celled stage, ephemeral lipid bodies and fibrillar material become evident (Fig. 36). Plastids at this stage frequently accumulate an electron dense material within the stroma and at scattered locations within the plastid envelope (Fig. 36).

As the disc cells progress from the 4-celled to the 8-13 celled stage, they develop large central vacuoles that appear to be derived from ER. Prior to secretory activity, the large vacuoles accumulate an electron dense material that typically occurs along the inner surface of the vacuole membrane (Fig. 37). Also prior to the onset of the secretion, the cytoplasm of the disc cells becomes highly electron dense. Plastids, mitochondria, tubular and branched ER are abundant at this stage in development, but dictyosomes as well as dictyosome-derived secretory vesicles are few in number (Fig. 38). A distinctive feature of the glands

Figures 36-40. Gland ultrastructure.

Fig. 36. Four-celled disc stage, with fibrillar material and lipid bodies. x 10,300.
Fig. 37. Secretory disc with large vacuoles that have accumulated electron dense material. x 1,200.
Fig. 38. Secretory cells just prior to secretion. x 18,230.
Fig. 39. Cytoplasmic connection between cells of secretory disc. x 25,750.
Fig. 40. Formation of secretory cavity. x 3,300.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in *Cannabis* at this stage in development is the formation of a symplast within the disc of secretory cells. Large, intercellular cytoplasmic connections develop between secretory disc cells (Figs. 37,39). These intercellular connections, which permit free exchange of cytoplasm between cells of the disc, were never observed between secretory cells and the underlying auxiliary cells or between the auxiliary cells.

Secretory activity of the gland begins with synthesis of secretory product and its storage in a secretory cavity above the disc cells (Figs. 15,40). As secretory activity commences, there are corresponding developmental changes occurring in the plastids. While increasing greatly in number, the plastids also form complex paracrystalline membranous inclusions (Fig. 41). At maturity, the paracrystalline body occupies nearly all the open stroma area, the plastids cease dividing and remain spherical in shape with a diameter of 1.4-1.6 μm (Figs. 42, 43). Plastids containing the paracrystalline bodies were only observed in secretory cells and may be the potential source of the main secretory product. Material interpreted as secretory product appears at the surface of the plastids and then migrates through the cytoplasm to accumulate along the cell surface adjoining the secretory cavity

Figures 41-45. Gland ultrastructure.

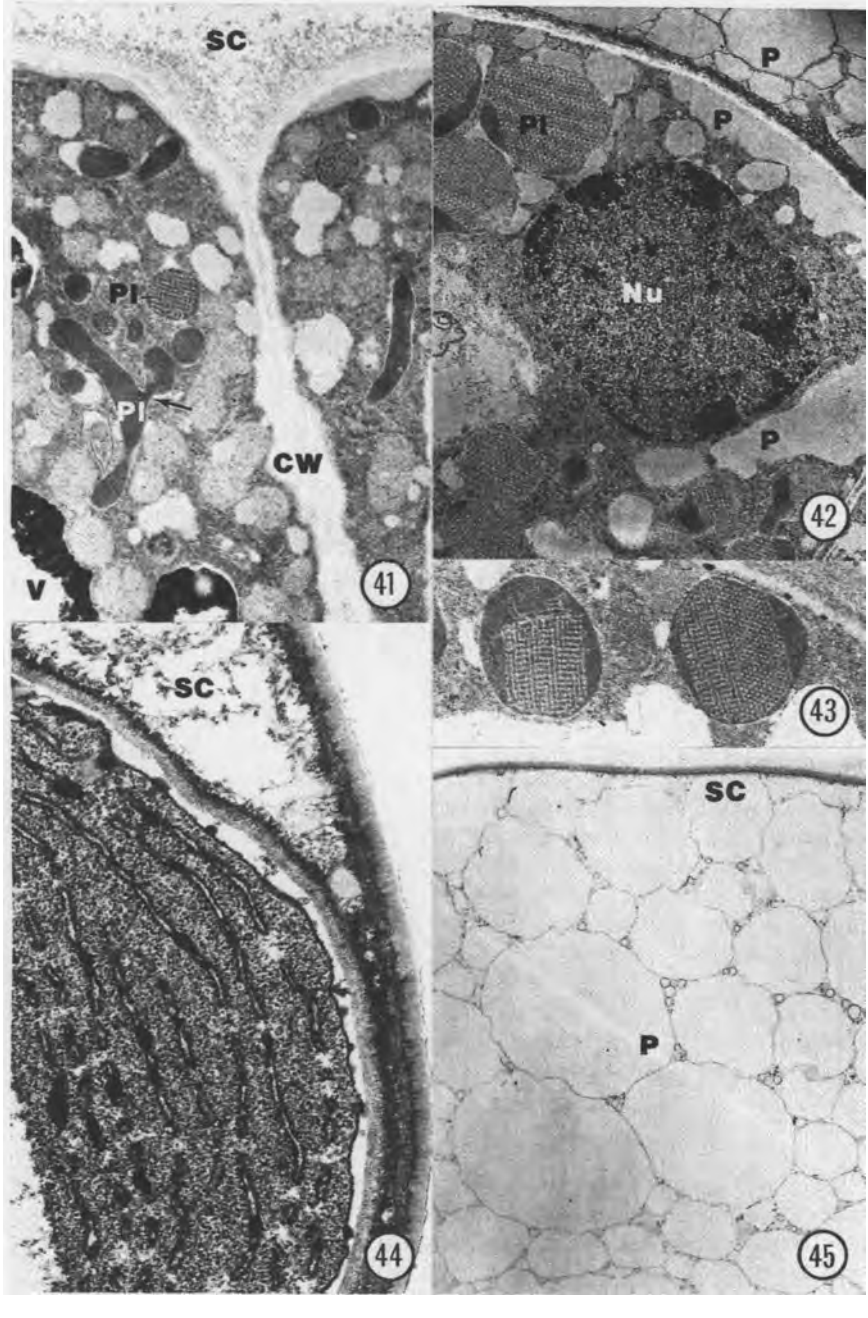
Fig. 41. Early secretory stage. Plastids increase in number by pinching (arrow) and begin to organize paracrystalline inclusions. x 11,000.

Fig. 42. Late stage in secretory activity. Plastids with secretion product at their surface, stages of migration and accumulation of secretory product along cell surface adjoining secretory cavity. x 12,500.

Fig. 43. Plastids with growing and well-developed paracrystalline bodies. x 13,300.

Fig. 44. Formation of secretory cavity by separation in middle layer of three-layered cell wall. x 32,100.

Fig. 45. Secretory product within the secretory cavity. x 4,900.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(Fig. 42). The secretory cavity is formed when the outer wall layers of the secretory disc separate from inner layers allowing the outer sheath to stretch and swell (Fig. 44). Histochemical staining of the sheath to demonstrate the presence of polysaccharide, has indicated that the outer sheath membrane appears to be comprised of both cuticle and a portion of the primary cell wall (Hammond and Mahlberg 1978). Within the secretory cavity, the secretory product can be observed to be organized into spherical bodies that seem to be delimited by a membrane-like structure (Fig. 45). No obvious features, such as pores, were observed in the wall of the disc cell that would facilitate movement of the secretory product into the secretory cavity. Since this was the case, it also was assumed that the organization of the secretory product into spherical bodies occurred in the secretory cavity rather than in the cytoplasm. Studies are in progress, using a micromanipulator, to analyse the contents of the secretory cavity not only for cannabinoids but other secretory products including proteins, lipids, or polysaccharides which may be related to the cannabinoid components.

3.7 *Cell fractionation*

Because the secretory product of *Cannabis* is chemically complex and contains various mono- and sesquiterpene essential oils in addition to the terpenophenolic cannabinoids, the identification of a particular product at a specific organellar site can be difficult. In addition, the lipophilic products may be extracted during typical processing procedures employed in electron microscopy. To solve some of these problems and provide another approach for determining the site of cannabinoid synthesis within the cell, we have begun fractionation studies. Our initial efforts have been directed toward preparing a fraction of viable plastids and analyzing it for the presence of cannabinoids. The preliminary results thus far obtained have indicated that no cannabinoids are present in the fraction containing viable plastids. Additional studies of the plastid fraction as well as other fractions isolated from the leaf and bract are necessary. However, it does appear that fractionation procedures may make it possible to correlate cannabinoid synthesis with a particular cytoplasmic or organellar fraction.

4. CONCLUSIONS

In conclusion, our research to this point has revealed numerous facets of the complex and dynamic glandular system of *Cannabis sativa*. Based on morphological and physiological differences, we recognize that several distinct gland types have evolved in

Cannabis. Although they may share similar ontogenetic origins, for reasons yet unknown and as a result of factors not yet know, their structure and functions differ. Also, gland populations differ as a result of their location on the plant. While initiated throughout organ development, the number of each gland type present is correlated with vegetative or flowering regions of the plant. Production of cannabinoids occurs throughout organ development but differs qualitatively and quantitatively based on a number of factors. The strain on which the glands are located dictates the qualitative aspect of the cannabinoid profile. However, gland type, gland age and location of the gland on the plant and on the organ help determine the quantitative aspects of the cannabinoid profile. Research so far has provided insight into a number of the complexities of the cannabinoid producing glandular system. Further studies should elucidate more of the controls governing gland and cannabinoid formation.

Having some information on gland and cannabinoid production and factors involved in their control, the site of cannabinoid synthesis within the gland is also of interest. Ultrastructural studies have provided an indication of plastid involvement in cannabinoid synthesis, and cell fractionalization studies are in progress to investigate this further. Once the site of synthesis is known, it should be possible to determine more fully the biosynthetic process of cannabinoid formation in the plant and perhaps be able to manipulate it.

Cannabis sativa provides a desirable system for studying plant trichomes, secondary product formation and interrelationships of the two. Distinct gland types are present and their presence is apparently related to physiological aspects of the plant. This allows an investigation of factors involved in gland initiation. In addition, genetic strains exist with apparent differences in gland populations. Secondary products produced by these glands also appear to be regulated genetically by the plant strain and environmentally by the physiological condition of the plant. *Cannabis sativa* provides a dynamic and complex secretory glandular system with variables present that allow definitive studies into the biosynthesis and control of that system.

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