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TRICHOMES AND CANNABINOID CONTENT OF DEVELOPING LEAVES AND BRACTS OF CANNABIS SATIVA L. (CANNABACEAE)[1]

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ABSTRACT

Trichome density and type and cannabinoid content of leaves and bracts were quantitated during organ ontogeny for three clones of *Cannabis sativa* L. Trichome initiation and development were found to occur throughout leaf and bract ontogeny. On leaves, bulbous glands were more abundant than capitate-sessile glands for all clones, although differences in density for each gland type were evident between clones. On pistillate bracts, capitate-sessile glands were more abundant than the bulbous form on all clones, and both types decreased in relative density during bract ontogeny for each clone. The capitate-stalked gland, present on bracts but absent from vegetative leaves, increased in density during bract ontogeny. The capitate-stalked gland appeared to be initiated later than bulbous or capitate-sessile glands during bract development and on one clone it was first found midway in bract ontogeny. Nonglandular trichomes decreased in density during organ ontogeny, but the densities differed between leaves and bracts and also between clones. Specific regulatory mechanisms appear to exist to control the development of each trichome type independently. In addition, control of trichome density seems to be related to the plant organ and clone on which the gland type is located. Cannabinoid synthesis occurs throughout organ development and is selectively regulated in each organ. Typically, cannabinoid synthesis occurred at an increasing rate during bract development, whereas in developing leaves synthesis occurred at a decreasing rate. Cannabinoid content on a dry weight basis was generally greater for bracts than leaves. Analyses of leaves indicate that other tissues in addition to glands may contain cannabinoids, while for bracts the gland population can accommodate the cannabinoid content for this organ. The functional significance of trichomes and cannabinoids in relation to evolution is discussed.

OUR INVESTIGATIONS indicate that the epidermal glandular trichomes present on *Cannabis* are components of a complex and dynamic secretory system. Three types of glandular trichomes, as well as a nonglandular trichome, are recognizable on the shoot. Vegetative leaves possess bulbous and capitate-sessile glands. Pistillate bracts and specialized leaves in association with the inflorescence possess bulbous and capitate-sessile forms as well as the more highly evolved capitate-stalked gland (Hammond and Mahlberg, 1973, 1977, 1978). Both capitate-sessile and capitate-stalked glands have been implicated as reservoirs for cannabinoids (Fujita et al., 1967; Fairbairn, 1972; DePasquale, 1974; Malingré et al., 1975; André and Vercruysse, 1976). However, few definitive qualitative or quantitative data on the cannabinoid content of capitate-sessile and capitate-stalked glands on plants of the same or various strains were presented in these studies. Our initial comparative studies (Turner, Hemphill, and Mahlberg, 1977) on gland density and cannabinoid concentration of various plant organs on several clones have shown that there is no simple relationship between glands and cannabinoid content. A subsequent analytical study of individual capitate-sessile and capitate-stalked glands (Turner, Hemphill, and Mahlberg, 1978) reported conspicuously higher levels of cannabinoids in capitate-stalked glands than in capitate-sessile glands. In addition, the cannabinoid content of an individual gland type varied with position on the plant organ and with gland age, although the gland retained the cannabinoid profile characteristic of the clone. Thus, data from these studies indicated the existence of a complex relationship between glands on an organ and the biosynthesis of component cannabinoids. In a related study (Hemphill, Turner, and Mahlberg, 1980), it was shown that leaves and bracts of various ages from a particular clone or strain differed quantitatively in their cannabinoid content. This suggested that the quantitative differences for these compounds were controlled directly or indirectly by factors associated with the ontogeny of a particular organ.

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In the current study, we examine the relationship between the cannabinoid composition and gland populations during leaf and bract ontogeny in each of three clones. Our understanding of the involvement of the glandular system in the overall formation and localization of cannabinoids within the plant, as well as individual cells, remains unclear. This study, therefore, will provide a morphogenetical basis for interpreting data on cannabinoid content of plant organs and their associated glands. It also will contribute to the objective of identifying the protoplasmic site of cannabinoid synthesis in the cell by identifying cells potentially involved in cannabinoid biosynthesis.

MATERIALS AND METHODS—*Clones*—Pistillate plants of three *Cannabis* strains (79, 87, 152) were selected and cloned (Turner et al., 1977). Strain 152 is a drug strain high in Δ^9-tetrahydrocannabinol (Δ^9-THC). Strain 79 is a non-drug, non-fiber strain having cannabidiol (CBD) as the major cannabinoid, and strain 87 is a fiber strain high in CBD. Clones were grown under ambient greenhouse conditions.

Plant parts sampled—Maturing leaves and bracts of each clone were collected for analysis. Leaves from 2.5 cm (very young) to 12.5 cm (mature) in length were sampled at length intervals of 2.5 cm. Leaf lengths were based on the center leaflet of the compound leaves, and only center leaflets were collected for analysis. Bracts from 2 mm (very young) to 9 mm (mature) in length were sampled at length intervals of 1 mm. A minimum of three leaves or bracts of each length were processed for scanning electron microscopy (SEM), while a minimum of 100 mg dry weight (DW) of leaves or bracts of each length was processed for analysis by gas-liquid chromatography (GLC). GLC data were prepared from duplicate samples of the very small organs and triplicate samples of organs of larger size. Leaflet samples were collected from vegetative plants at 3 pm on one day both in mid-July and again in early September for analysis. Data presented in the figures are from the September collection. Bract samples were collected at 3 pm on three consecutive days, one clone each day, during late October.

Gas-liquid chromatography and scanning electron microscopy—Plant parts to be analyzed were processed as described previously (Turner et al., 1977). Samples prepared for SEM were examined with an ETEC Autoscan. Analyses by GLC were performed on a Hewlett-Packard 5710A chromatograph equipped with a 3380A H-P integrator. An analysis of variance (F-ratio) was calculated for each cannabinoid for each size of leaf and bract, and between the lengths of the organs for each clone, as well as between the July and September collections for leaf samples.

Gland quantitation—Gland number per unit area on leaflets and bracts was determined by counting glands directly on the SEM screen (Turner et al., 1977). On leaflets, counts were made at the midpoint of the blade, from the midrib to the margin. Multiple counts (16 fields, totaling 1 sq mm) of both the adaxial and abaxial vein as well as nonvein areas were made, and the results were averaged to provide a mean for the sample. An analysis of variance (F-ratio) was calculated for each gland type at each leaf length, and among the five lengths of the leaf, as well as between the July and September collections for each leaf length. In the current experiments, the abaxial nonvein areas on young leaves (2.5–7.5 cm) were too densely covered with nonglandular trichomes to obtain an accurate gland count. Thus, for comparative reasons all values for the leaflet samples were averaged without including counts from the abaxial nonvein areas. The data for each leaf-length sample were calculated as glands per sq mm and also as total glands per leaflet. For the estimated cannabinoid content of individual glands, glands counted on abaxial nonvein areas of older leaves (10–12.5 cm) were included in the calculations.

On bracts, one-half of the total surface area, from tip to base, of each bract was quantitated. Measurements of surface area encompassed a minimum of 2 sq mm (32 fields) for the 2 mm long bracts and a maximum of 42 sq mm (672 fields) for the 9 mm long bracts. Data for each sample from each length of bract represent the mean, and were calculated as glands per sq mm, as well as total number of glands per bract. An analysis of variance (F-ratio) was calculated for each gland type at each bract length, as well as among all samples from the eight lengths of bracts examined from each clone.

RESULTS—Pubescence on leaves and bracts of *Cannabis* clones examined in this study included a nonglandular trichome and three types of secretory glandular trichomes, identified as bulbous, capitate-sessile and capitate-stalked glands. Leaves possessed bulbous and capitate-sessile glands, but lacked the more specialized capitate-stalked form. Bracts possessed all three types of glandular trichomes. Both leaves and bracts possessed nonglandular trichomes.

Gland quantitation on leaves—The number

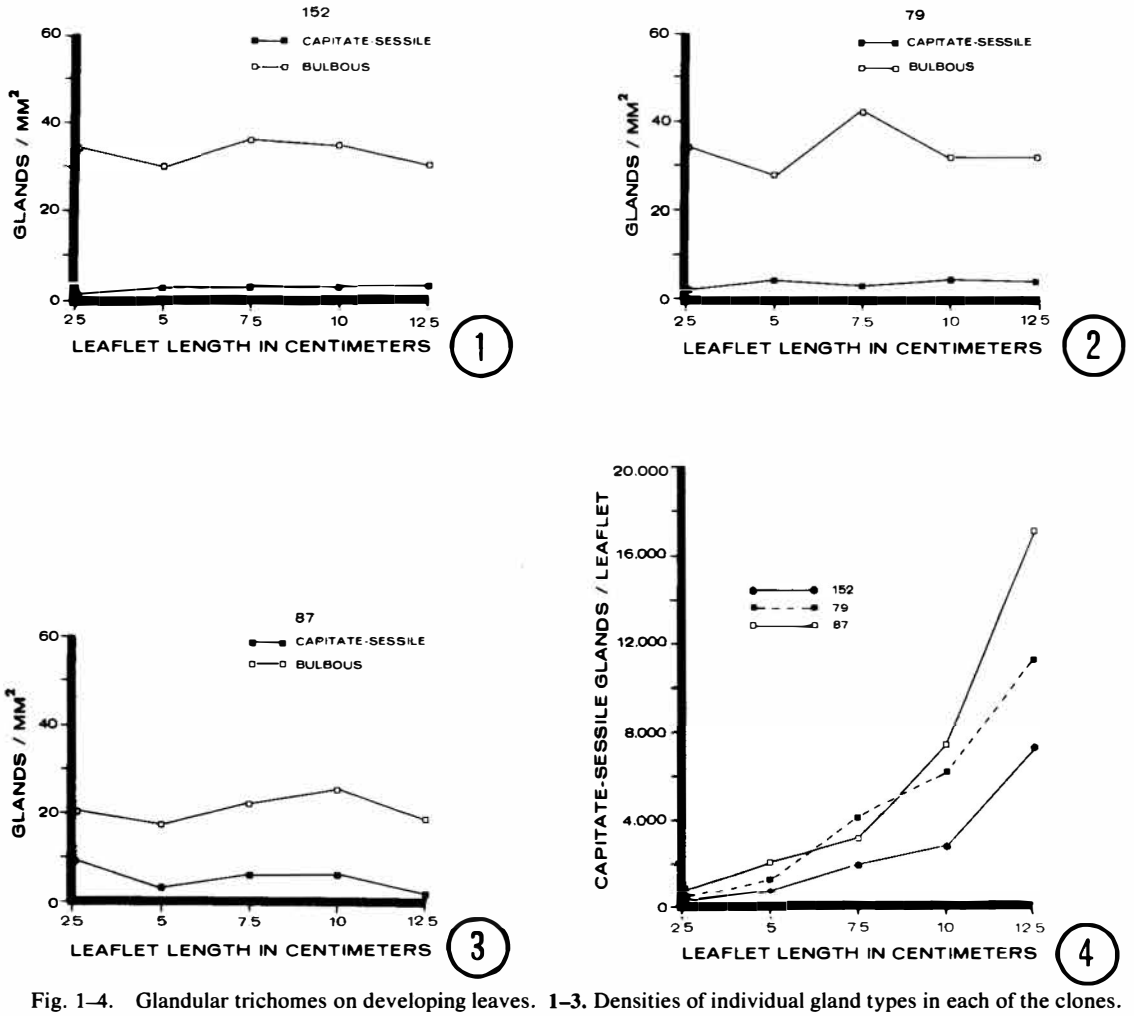
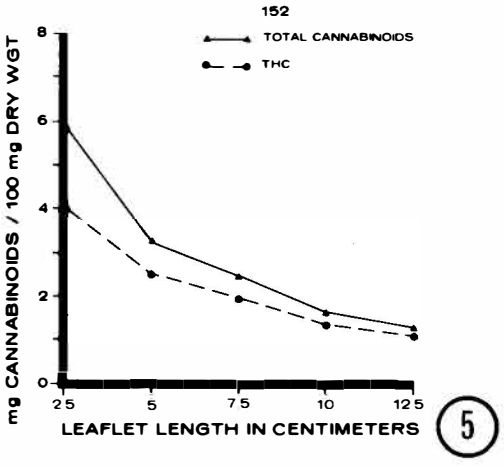


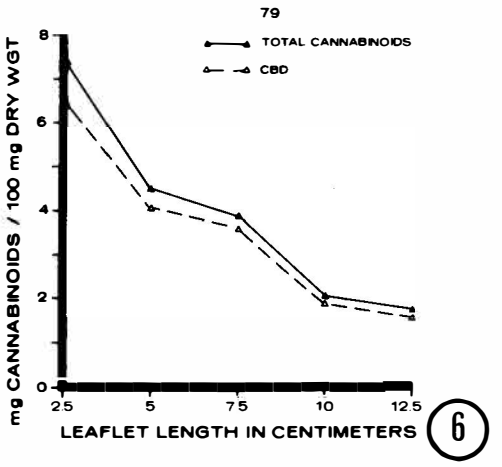
Fig. 1–4. Glandular trichomes on developing leaves. **1–3.** Densities of individual gland types in each of the clones. **4.** A comparison among the clones for total capitate-sessile glands per leaflet.

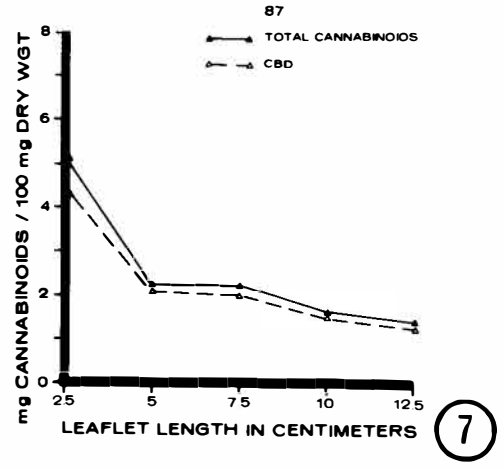
of each gland type per sq mm was determined for each of the three clones at intervals during leaf ontogeny (Fig. 1–3). On leaflets, capitate-sessile and bulbous glands maintained a relatively constant density throughout leaf ontogeny (Fig. 1–3) and no capitate-stalked glands were observed. Clone 87 averaged a greater number of capitate-sessile glands per sq mm than did either clone 79 or 152; the latter two clones possessed a similar number of capitate-sessile glands per unit area. Clones 79 and 152 also possessed similar numbers of bulbous glands per sq mm, and significantly more than clone 87 (Fig. 1–3). The *F*-ratios calculated among the five leaf lengths were not significant for either capitate-sessile or bulbous glands on any of the three clones. However, at each leaf length the *F*-ratios for each gland type were significant on all three clones. The *F*-ratios between samples of each leaf length from two samplings, July and September, were not found to be significant.

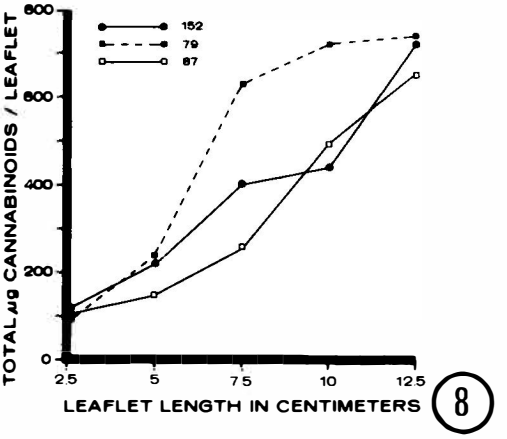
The total number of capitate-sessile glands per leaflet also was determined for these clones during leaf development (Fig. 4). On each clone, an increase in total gland number was seen as the leaf developed, although the clones differed in the specific numbers of capitate-sessile glands found on a leaf (Fig. 4). Clone 152 had fewer glands at each developmental stage than did clones 79 and 87 (Fig. 4). Clones 79 and 87 had similar numbers of glands except on mature leaflets (12.5 cm) where clone 87 had a higher number of capitate-sessile glands (Fig. 4).

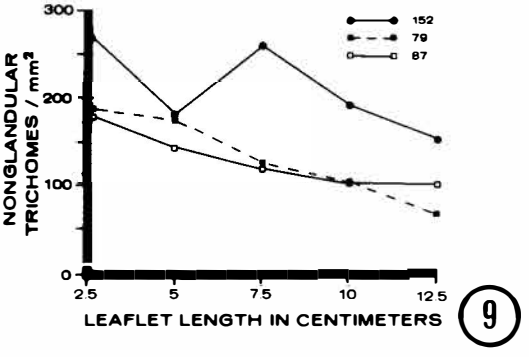
Cannabinoid content of leaves—Cannabinoid concentration, on a dry weight basis, decreased in each of the clones throughout leaf ontogeny (Fig. 5–7). Although both clones 79 and 87 characteristically had high levels of











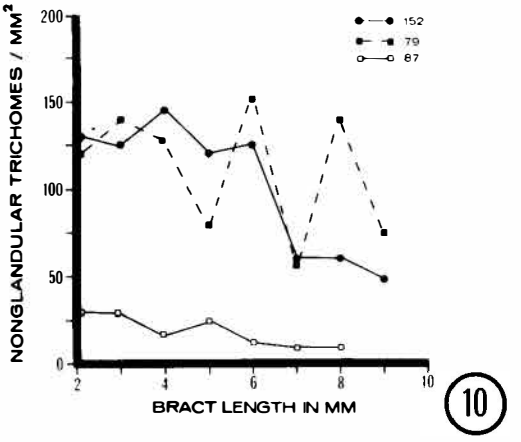


Fig. 5–10. Cannabinoid concentration and nonglandular trichomes on developing leaves and bracts. **5–7.** Concentrations of cannabinoids during leaf ontogeny in each of the clones. **8.** A comparison among the clones for total cannabinoid content per leaflet. **9.** Nonglandular trichome density compared among the clones during leaf ontogeny. **10.** Nonglandular trichome density compared among the clones during bract ontogeny.

CBD, clone 79 had higher levels of CBD at most stages of leaflet development than clone 87 (Fig. 6, 7). Clone 152, a Δ^9-THC strain, had levels of cannabinoids (Fig. 5) comparable to cannabinoid levels found for clone 87 (Fig. 7). Each clone contained concentrations of its characteristic cannabinoid at levels similar to the total cannabinoid concentration detected (Fig. 5–7). The *F*-ratios for both CBD and Δ^9-THC among the five leaf lengths were found to be significant for all three clones. However, the *F*-ratios for each of the cannabinoids at each leaf length were not found to be significant for any of the three clones. The *F*-ratios between samples of each leaf length from the two sampling periods, July and September, were not found to be significant.

Expressed on a per leaflet basis, total cannabinoids increased during leaf ontogeny in each of the clones (Fig. 8). The three clones were found to have comparable levels of cannabinoids at the youngest stage of leaflet development and again to have comparable although higher levels of cannabinoids in the most mature leaflets (Fig. 8). However, at intermediate stages, clones 152 and 87 had lower quantities of cannabinoids than clone 79 (Fig. 8). The increase in total cannabinoids per leaflet, in general, paralleled the increase in total capitate-sessile glands per leaflet throughout leaf ontogeny (Fig. 4, 8).

Nonglandular trichomes quantitation—On leaves, the density of nonglandular trichomes decreased slightly and at a similar rate on each clone as the leaflets enlarged (Fig. 9). Clones 79 and 87 were found to have comparable densities of trichomes during leaf ontogeny. However, clone 152 had approximately 25% more trichomes per unit area throughout leaflet development than clone 79 or 87 (Fig. 9). The *F*-ratios calculated among the five leaf lengths, and for each leaf length, were found to be significant on all three clones. However, *F*-ratios between the July and September collections for each of the clones were not found to be significant.

On bracts, the nonglandular trichomes on clone 152 were found to maintain a uniform density until the bract was 6 mm in length, whereupon the density progressively decreased to a smaller number of nonglandular trichomes per unit area on mature bracts (Fig. 10). The trend for clone 79 was less clear, although a slight overall decrease in trichome density during bract development appeared to exist (Fig. 10). The density of trichomes on clone 87 was lower than that found for clones 152 or 79, and a slight decrease in trichome density was observed as the bracts matured (Fig. 10). The *F*-ratios for each bract length were found to be significant for most of the samples from clones 79 and 152, while most of the length samples on clone 87 were not found to be significant. The *F*-ratios between the bract lengths for each of the clones were found to be significant, however.

Gland quantitation on bracts—On bracts, capitate-stalked glands were present along with bulbous and capitate-sessile forms on all three clones. A comparison of gland densities during bract ontogeny indicated that similar trends occurred among the clones for each gland type (Fig. 11–14). The density of capitate-stalked glands increased as the bract developed. However, the increase occurred at a different developmental stage for each clone. Capitate-stalked glands were absent from young bracts of clone 152 and appeared only after the bracts had attained a length of 6 mm (Fig. 11). In contrast, for clones 79 and 87, capitate-stalked glands were present on the youngest bracts sampled, with clone 87 possessing a greater number of these glands than clone 79 at this early developmental stage (Fig. 12, 13). On clone 87, the density of this gland type increased during the early developmental stage, whereas for clone 79 gland density increased during later stages of bract development. There also were numerical differences among the clones, when the densities of capitate-stalked glands were compared. Both clones 152 and 79 had relatively low gland densities (Fig. 11, 12), while clone 87 was found to have the highest density of capitate-stalked glands (Fig. 13). The *F*-ratios were not significant for most of the bract lengths on each of the clones. However, the 7 mm bract length for clone 79 and the 4 mm bract length for clone 87 had significant *F*-ratios for capitate-stalked gland counts. Analyses of variance among the eight bract lengths showed significant *F*-ratios for each of three clones.

The density of capitate-sessile glands on bracts of both clones 152 and 87 decreased as the bracts increased in length from 3 mm to 6 mm (Fig. 11, 13). The number of these glands per unit area then remained relatively constant as the bracts matured to 9 mm in length. In contrast, the density of capitate-sessile glands on clone 79 remained relatively constant until the bracts had developed to about 6 mm in length, whereupon gland density decreased with further bract development (Fig. 12). Quantitatively, clones 152 and 79 had similar densities of capitate-sessile glands, while clone 87 had a lower gland density (Fig. 11–13). The *F*-ratios were not significant for most of the bract-length samples, although for clone 79 the

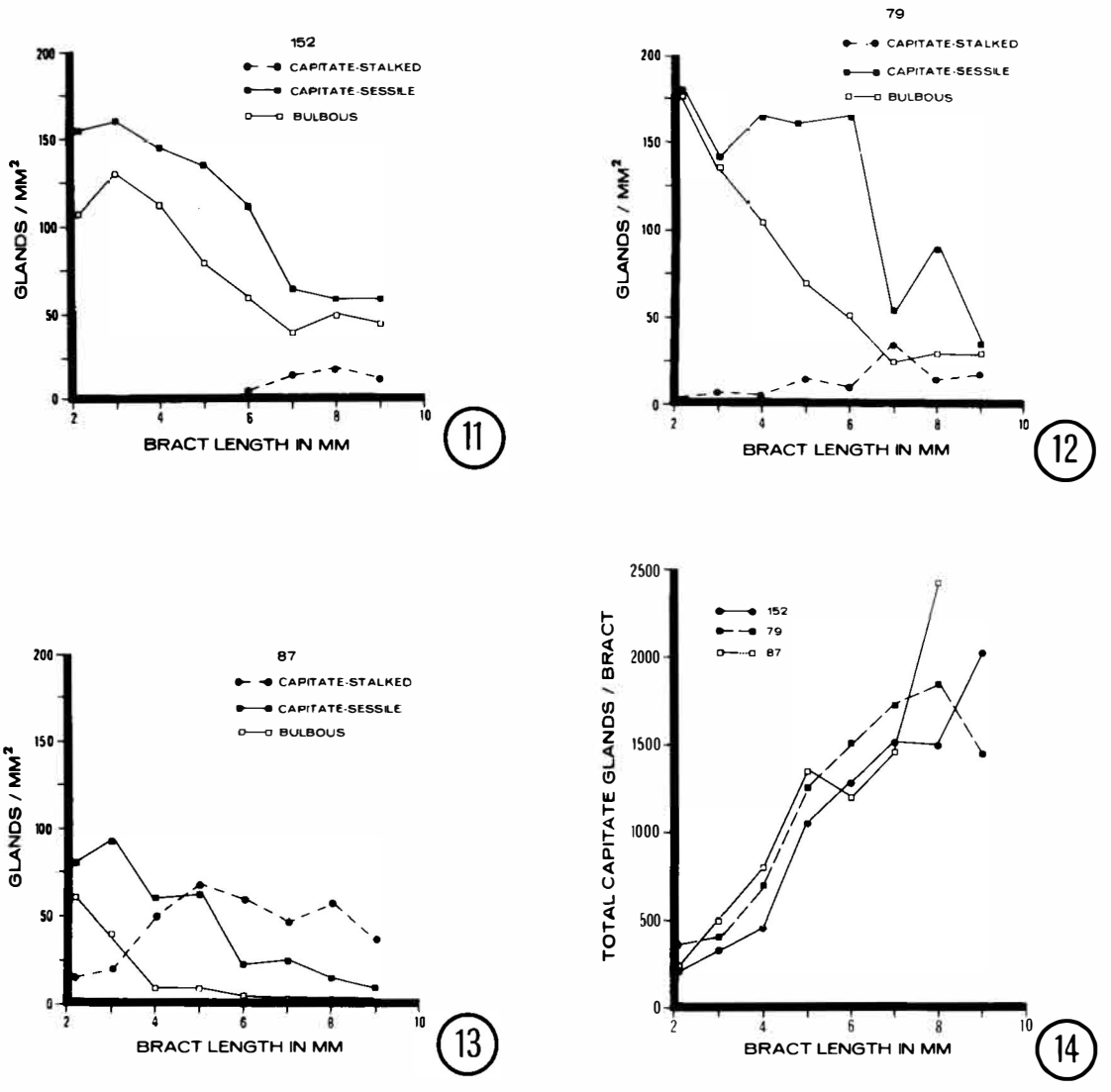
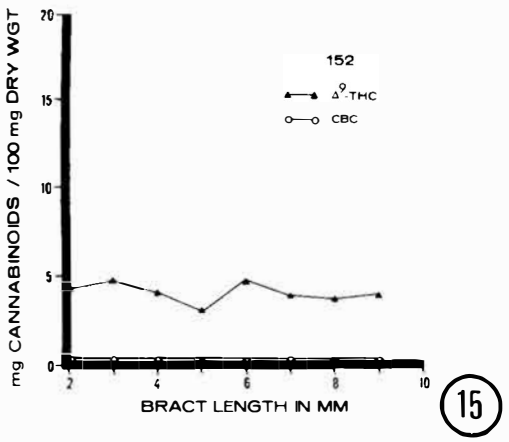


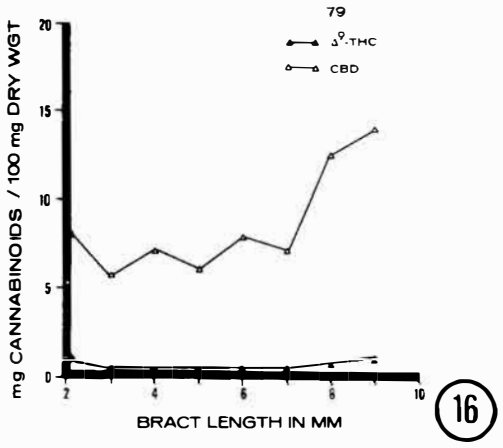
Fig. 11–14. Glandular trichomes on developing bracts. **11–13.** Densities of individual gland types in each of the clones. **14.** A comparison among the clones for total capitate glands per bract.

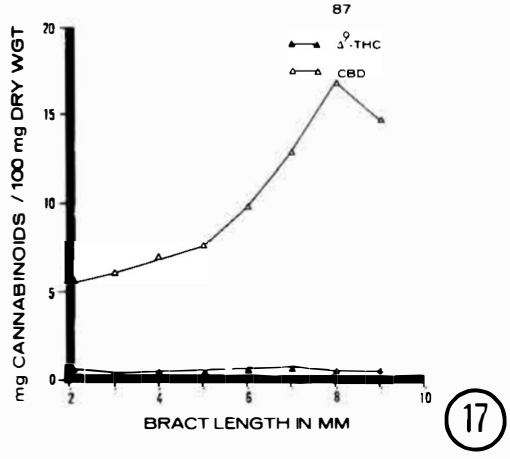
7 mm and 9 mm lengths were significant along with the 5 mm and 7 mm bract lengths from clone 87. The *F*-ratios among the eight bract lengths were significant for each clone.

The density of bulbous glands decreased on each of the clones during early to middle stages of bract ontogeny and then remained at a relatively constant number per sq mm as the bracts approached maturity (Fig. 11–13). However, while the number of bulbous glands per sq mm on clones 152 and 79 decreased gradually until the bracts were about 7 mm in length (Fig. 11, 12), the density of this gland type on clone 87 decreased only during the earliest stages of bract development (Fig. 13). Also, clone 87 was found to have a lower density of bulbous glands than clones 152 and 79 (Fig. 11–13). The *F*-ratios were not significant for most of the samples, although the 6 mm length on clone 152, the 9 mm length on clone 79, and the 4 mm length on clone 87 were significant. The *F*-ratios among the bract length samples for all clones were significant.

In addition to monitoring gland density during bract ontogeny, the total number of both types of capitate glands per bract was compared among the clones (Fig. 14). All three clones were found to have an increase in gland number as the bract developed to maturity. When compared, the clones showed little difference in the number of capitate glands present throughout bract development until the







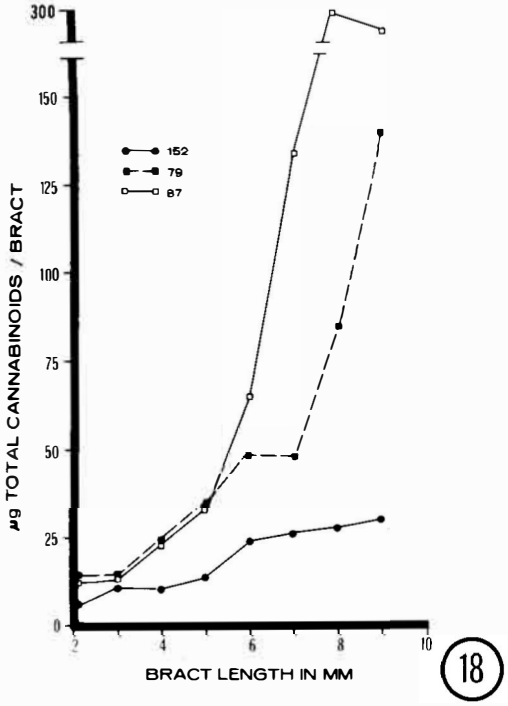


Fig. 15–18. Cannabinoid concentration during bract ontogeny. **15–17.** Concentrations of cannabinoids in each of the clones. **18.** A comparison among the clones for total cannabinoid content per bract.

most mature stages at which time quantitative differences became apparent (Fig. 14).

Cannabinoid content of bracts—Cannabinoid concentration of developing bracts varied considerably for each of the clones (Fig. 15–17). Clone 152, a drug strain which is characteristically high in Δ^9-THC, was found to have relatively similar concentrations of cannabinoids at each stage of bract development (Fig. 15). The major cannabinoid detected was Δ^9-THC, averaging approximately 4–5 mg/100 mg DW, whereas cannabichromene (CBC) was detected in very small amounts. Clone 79, a non-drug strain, accumulated characteristically high levels of CBD, although a small quantity of Δ^9-THC was present as well. Clone 79 was found to have relatively similar concentrations of cannabinoids at immature stages of bract development, but as the bract matured the cannabinoids increased in concentration (Fig. 16). Concentrations of CBD in clone 79 averaged 6–7 mg/100 mg DW or higher. In Clone 87, a fiber strain characteristically high in CBD, the

cannabinoid concentration increased at each stage of bract development except for the most mature stage (Fig. 17). As in clone 79, high levels of CBD were detected along with small concentrations of Δ^9-THC. Quantities of cannabinoids detected in clone 87 were comparable with the levels found in clone 79, whereas clone 152 contained lower concentrations of these compounds (Fig. 15–17). The *F*-ratios for each of the cannabinoids at each bract length were not significant in any clone. However, the *F*-ratios for CBD and Δ^9-THC among the eight bract lengths were significant for clones 79 and 87, whereas the *F*-ratios were not significant for either CBC or Δ^9-THC among the bract lengths on clone 152.

Total cannabinoid content per bract also was compared among the clones at each stage of bract development (Fig. 18). Differences were apparent, with clone 152 having the lowest total cannabinoid content, particularly at the more mature stages. Clones 79 and 87 had similar amounts of total cannabinoids per bract present at young developmental stages, but clone 87 had considerably higher amounts of total cannabinoids per bract at the more mature stages (Fig. 18). A comparison of total cannabinoid content per bract with total capitate glands per bract showed an increase in both parameters throughout bract development for each of the clones (Fig. 14, 18).

DISCUSSION—The dynamic character of the trichome system on *Cannabis* is evident from these analyses of trichomes and cannabinoids for leaves and bracts during organ ontogeny. Evidence shows that new trichomes are initiated and that cannabinoids are synthesized continually throughout all stages of leaf and bract development. However, there are several aspects of the interrelationship between the trichome population and cannabinoid composition during organ morphogenesis which emphasize the complexities of this dermal secretory system.

A comparison of gland densities among the clones revealed a difference for the density of individual gland types present on each clone. On both leaves and bracts, for example, bulbous glands were present at a lower density on clone 87 than on either clone 152 or 79. The densities of capitate-sessile and capitate-stalked glands also varied between the clones. These data indicated that the number of each gland type present on a specific organ may be a genetical characteristic for a clone. For each bract length, most of the *F*-ratios were not significant, indicating a uniformity of the gland population on the several individual bracts within each sample. The significant *F*-ratios found for the individual gland types for each leaf-length sample reflected the nonuniform distribution of glands on the leaf surfaces as previously reported (Turner et al., 1977).

In addition to differences in gland densities among the clones, differences in density also were found between leaves and bracts. On leaves, both bulbous and capitate-sessile glands maintained a relatively constant density throughout leaf development on each of the clones. The observed trends were supported by *F*-ratios from the analyses of variance of the glandular trichome populations. On bracts, we observed that bulbous and capitate-sessile glands decreased, while capitate-stalked glands increased in density on all clones during organ ontogeny. Among bract samples the *F*-ratios for each gland type on each clone were significant, which indicated that the observed differences were real. Further, gland densities generally were found to be higher on bracts than on leaves. Physiological differences between the flowering region and vegetative region of the axis may have resulted in evolutionary modification or amplification of the role of glandular trichomes on bracts.

Although the factors regulating gland development and cannabinoid formation are not yet clear, interrelationships between these two parameters appear to exist. A comparison of gland number with cannabinoid content at each stage of bract development made it possible to estimate the cannabinoid content of a single gland on the bract. The estimate (for example, 20 ng for clone 152, and 44 ng for clone 87) compared favorably with results obtained previously for individual glands (Turner et al., 1977, 1978), and indicated that bracteal glands could contain all of the cannabinoids found in this organ, although other bracteal tissues could be involved in the synthesis of cannabinoids. In contrast, comparisons of gland numbers with cannabinoid content of leaflets indicated that the capitate-sessile gland population could contain only a part of the total cannabinoid content of the leaf. The estimated content per individual gland (45 ng for clone 152, 47 ng for clone 79, and 88 ng for clone 87) was higher than the content found previously for individual glands on the leaf (Turner et al., 1978). Other leaf tissues, therefore, may be involved in cannabinoid synthesis or accumulation.

Quantities of individual cannabinoids, like gland density, varied among the clones. Clone 87 had the highest cannabinoid levels on bracts, while clone 79 had the highest cannabinoid levels on leaves. However, the cannabinoid profile characteristic of each clone was maintained throughout organ development,

although the levels of cannabinoids present in a clone varied during organ ontogeny. The cannabinoid profile for a particular clone, therefore, appeared to be genetically stable. Hemphill, Turner, and Mahlberg (1980) have shown for certain strains that different plant parts can vary both quantitatively and qualitatively in cannabinoid contents. It was more typical, however, for strains to possess a characteristic cannabinoid profile, and this feature has been employed to interpret the taxonomic affinities between strains (Small and Cronquist, 1976; Small, Jui, and Lefkowitch, 1976).

The occurrence of capitate-stalked glands, shown to be new structures rather than existing glands stimulated to produce a stalk (Hammond and Mahlberg, 1978), distinguished bracts of the pistillate plant from leaves of the vegetative axis. Physiological variations between vegetative and flowering regions may account for the absence of capitate-stalked glands on vegetative leaves and for their presence on bracts. Variations of the capitate-stalked gland population on bracts among clones indicate the existence of a mechanism that controls the morphogenesis of this gland independently from that for other gland types. Decreases in densities of bulbous and capitate-sessile glands on bracts did not appear to be influenced by the increase in capitate-stalked gland density, because the former can occur in the absence of the capitate-stalked gland as seen during early stages of bract enlargement on clone 152. If there exists an independent mechanism which controls the development of capitate-stalked glands, then the development of bulbous and capitate-sessile glands also might be controlled by individual regulatory mechanisms. The bases of these mechanisms, however, are unknown at the present time.

The variations among clones in time of appearance of capitate-stalked glands on young bracts indicate that the density of capitate-stalked glands relative to bract development may represent a genetical characteristic of all strains. It should also be noted that although Small et al. (1976) have used gland density and the percentage of stalked glands as taxonomic characters, they did not take into consideration changes in gland numbers related to organ ontogeny. The high density of glands on bracts and the emergence of the specialized capitate-stalked gland, with its concomitant increase in cannabinoid content for the bract, does indicate the evolutionary trends for these characters in *Cannabis*. Similar analyses of other strains very probably would identify additional variations for these characters as well, emphasizing the morphogenetical plasticity of this species. Studies of these characters among other strains could reveal the pattern in their progressive evolution and identify the presumptive form that would be analogous to the wild-type plant.

The influence of environmental factors on gland density, or on the development of a particular gland type on an organ, is unknown at present and additional studies in this area are desirable. However, data derived from leaf samples collected both in July and September, and from *F*-ratio determinations of the samples, indicate no significant differences between the two groups of samples at each length. Further, the occurrence of a uniform gland density on the leaf for each gland type indicates the existence of precise internal regulatory balance and control mechanisms. It would appear that gland development is dependent upon genetical factors which influence gland density and initiation on a clone during leaf development independent of various environmental changes.

Cannabinoid concentrations also showed different patterns between leaves and bracts. In each clone there was a progressive decrease in concentration in leaves during their development, whereas there was a trend toward increased concentrations in bracts during their development. These trends were independent of the characteristic type of cannabinoid, whether Δ^9-THC or CBD, present in the clone. For example, cannabinoid levels in leaves of clones 152 and 87 were similar, although 152 was high in Δ^9-THC and 87 was high in CBD. Differences in cannabinoid patterns between leaves and bracts may be indicative of physiological differences for vegetative as contrasted to flowering organs of the plant. The protoplasmic site of cannabinoid synthesis remains unknown and, therefore, it is unclear whether the cannabinoid profile in an organ reflects compounds synthesized in or transported to the organ. Thus, the differences in cannabinoid concentrations detected in leaves, as contrasted to bracts, may be related to different roles which have evolved for these compounds in the different organs.

Nonglandular trichomes, reported to lack cannabinoids (Malingré et al., 1975), were found to decrease in density during both leaf and bract ontogeny. The rate of decrease in all cases observed was slower than the rate of increase in the surface area of the plant organs indicating that nonglandular, like glandular, trichomes were continually initiated during both leaf and bract development. Leaves typically possessed a greater density of these trichomes than did bracts, and trends of the trichome population differed among the clones

and between leaves and bracts. Thus, their progressive decrease in density on leaves, while glandular trichomes did not decrease in density, indicates that the formation of nonglandular trichomes is controlled by a mechanism independent of that for glandular trichomes.

Variations among the clones in the population of nonglandular trichomes on bracts, which represent specialized leaves related to reproductive organs, may reflect a progressive change in functionality of this specialized leaf. The nonglandular trichomes, which are silicified and rigid (Dayanandan and Kaufman, 1976), may have developed phylogenetically as a defense mechanism against herbivory on leaves. In general, leaves have a higher density of nonglandular trichomes and a lower density of glandular forms than do bracts. On bracts, however, the trend is toward a decreased density of nonglandular trichomes and an increased density of glandular trichomes. These data indicate that the assumed defense role of nonglandular trichomes against herbivory or desiccation have been replaced on bracts by a different function involving the present development of a complex stratified trichome system of both glandular and nonglandular trichomes. The evolution on the bract of the capitate-stalked gland with a high cannabinoid content and positioned well above the epidermal surface may now serve a function related to reproduction or survival possibly through enhanced seed dispersal (Hammond and Mahlberg, 1973).

Cannabis, because of the presence of diverse morphological and biochemical characters in the numerous varietal forms, represents an excellent plant for morphogenetical studies to determine the genetical or environmental factors that control the initiation of the different trichome types and the synthesis of the various cannabinoids in organs of this plant. Such studies also will contribute data to our current research which is directed to determining the subcellular site of cannabinoid synthesis in the cell.

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