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QUANTITATIVE DETERMINATION OF CANNABINOIDS IN INDIVIDUAL GLANDULAR TRICHOMES OF CANNABIS SATIVA L. (CANNABACEAE)[1]

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ABSTRACT

Cannabinoid levels of individual mature glandular trichomes from two clones and two strains of *Cannabis sativa* L., which included both drug and fiber phenotypes, were investigated by gas-liquid chromatographic analyses. Capitate-stalked glands were selectively harvested from vein and nonvein areas of pistillate bracts while capitate-sessile glands were harvested from these areas of leaves. The qualitative cannabinoid profile characteristic of the strain or clone was maintained in the individual capitate-stalked glands while the quantitative cannabinoid profiles varied with each strain or clone and between vein and nonvein areas as well. Capitate-sessile glands were found to contain conspicuously lower levels of cannabinoids than capitate-stalked glands. This study emphasizes that glands of *Cannabis* represent a dynamic system within the cannabinoid synthesizing activities of this plant.

GLANDULAR trichomes are prominent features on the shoot system of *Cannabis sativa* L. Several studies using histochemical and analytical procedures support an interpretation that cannabinoids, terpenophenolic compounds present only in *Cannabis,* accumulate in glandular trichomes (Fujita et al., 1967; Fairbairn, 1972; Malingré et al., 1975; André and Vercruysse, 1967; Turner, Hemphill, and Mahlberg, 1977). Of the numerous cannabinoid compounds known, the major ones include cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and Δ^9-tetrahydrocannabinol (Δ^9-THC) (Mechoulam, 1973; Report, United Nations, 1976). These compounds may vary qualitatively and quantitatively in analyzed plant material derived from the different strains of this species (Small and Beckstead, 1973). Commonly, those strains which contain a high ratio of Δ^9-THC to CBD are referred to as drug type plants, whereas strains which possess a high ratio of CBD to Δ^9-THC are considered non-drug or fiber strains (Small and Cronquist, 1976).

The diversity of the glandular trichomes as well as their development and distribution (Hammond and Mahlberg, 1973, 1977, 1978) on different strains of *Cannabis* provides an excellent tissue system in which to study the production and accumulation of specialized products, such as cannabinoids. In a recent study we reported that the cannabinoid content of capitate-stalked glands from floral bracts varied with gland age and between clones, although the glands retained the cannabinoid profile characteristic of the clone (Turner et al., 1977). However, the results suggested that the cannabinoid content might not be equal in quantity in all glands throughout the glandular system of the plant. The purpose of this investigation, therefore, was to analyze mature glands from specific areas on the plant for variability in cannabinoid content. Cannabinoid profiles of capitate-sessile as well as capitate-stalked glands from both drug and fiber strains were compared. These studies are directed toward an interpretation of the function of the glandular trichome in cannabinoid synthesis.

MATERIALS AND METHODS—Plants used in this study were derived from two sources. Strains 152 and 87 were cloned plant material (Turner et al., 1977) while the Mexican and Turkish strains were grown from seed. Seeds of the Mexican strain were obtained from a police seizure (Hammond and Mahlberg, 1973). Seeds of the Turkish strain [TU-A(2):71] were obtained from C. E. Turner, University of Mississippi. The Mexican strain and clone 152 are high Δ^9-tetrahydrocannabinol (Δ^9-THC) producing strains (drug phenotype) while the Turkish strain and clone 87 are high cannabidiol (CBD) producing strains (fiber phenotype). All four strains were grown under ambient greenhouse conditions.

Individual glands were sampled from both vein and nonvein areas of several 9-mm pistillate bracts and several 7.5-cm vegetative leaves from several flowering pistillate plants of each strain and clone in October and December, 1977, and March, 1978. A tungsten needle was used to remove the gland heads and place them directly into chloroform. The needle was rinsed in chloroform after collecting each gland head to avoid any potential carry over of materials throughout

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TABLE 1. *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.

the sampling procedure. Only intact mature (Turner et al., 1977) gland heads were taken for the analyses. Samples consisted of 20 gland heads and were analyzed for cannabinoid content by gas-liquid chromatography as previously described (Turner et al., 1977). Some samples consisting of 100 gland heads were also collected. Collections made in October and December were single samples of 20 glands each while the March samples were collected in triplicate. All glands from any one strain or clone were collected within hours of each other, while all strains or clones were sampled within a few days of each other for each collection period.

RESULTS—Cannabinoid content was analyzed in capitate-stalked glands collected from vein and nonvein regions of bracts at several times during the year (Tables 1–4). Although the tables present the principal cannabinoid present in the glands, infrequently small amounts of a second cannabinoid reflective of the qualitative profile of the strain or clone (Turner et al., 1977) were detected (unpublished). On clone 152, capitate-stalked glands harvested from veins contained a higher cannabinoid content (Δ^9-THC) than glands from nonvein areas at each collection time (Table 1). Clone 87 was found to have the reverse relationship; glands from nonvein areas contained a higher CBD (the principal cannabinoid) content than glands from vein areas (Table 2). The cannabinoid levels were relatively similar throughout the experimental period in each of the two clones, although there was a slight decrease at the March collection period. Triplicate collections of the March samples were similar indicating uniformity of the samples. For example, capitate-stalked glands on nonvein areas of bracts of clone 152 contained a mean of 16.29 ng Δ^9-THC with a standard deviation of 4.23.

Similar analyses of capitate-stalked glands from bracts of the Mexican and Turkish strains showed less stability for cannabinoid content. Glands harvested in October from the vein areas of the Mexican strain contained higher Δ^9-THC levels than those from nonvein areas, while in December the quantitative levels were reversed (Table 3). In comparison, glands harvested from the nonvein areas of the Turkish strain possessed higher CBD levels than vein glands collected in October, but a reversed relationship existed for glands taken in the December collection (Table 4). Furthermore, glands from the Turkish strain contained a higher level of the characteristic cannabinoid (CBD) in December as compared to October, while the opposite relationship was evident for the Mexican strain (Tables 3, 4). Since the Mexican and Turkish strains were grown from seeds and were not cloned to provide new plant material throughout the winter, no plants

TABLE 2. *Principal cannabinoid present in glandular trichomes of clone 87*

Gland type	Plant organ	ng cannabidiol/gland		
		October	December	March
Capitate-stalked	Bract			
	Vein	37.10	31.60	18.73
	Nonvein	—[a]	49.26	39.66
Capitate-sessile	Leaf			
	Vein	ncd[b]	ncd	ncd
	Nonvein	ncd	ncd	ncd

[a] sample not available.
[b] ncd, no cannabinoids detected.

TABLE 3. *Principal cannabinoid present in glandular trichomes of Mexican strain*

Gland type	Plant organ	ng Δ^9-tetrahydrocannabinol/gland	
		October	December
Capitate-stalked	Bract		
	Vein	224.00	69.30
	Nonvein	120.20	97.50
Capitate-sessile	Leaf		
	Vein	9.66	ncd[a]
	Nonvein	6.92	6.05

[a] ncd, no cannabinoids detected.

TABLE 4. *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.

were available for a gland collection in March. In general, neither the Turkish nor the Mexican strain revealed any trends, although the cannabinoid content in the capitate-stalked gland did vary with bract area and sampling time during the year.

Capitate-sessile glands also were sampled for their cannabinoid levels. Although capitate-sessile glands are present on bracts, they are at times difficult to distinguish from young capitate-stalked glands. Therefore, for this study capitate-sessile glands were sampled from vegetative leaves where capitate-stalked glands are rarely present; none have been observed in any of our studies (Hammond and Mahlberg, 1973, 1977; Turner et al., 1977).

Capitate-sessile glands of clones 152 and 87, whether from vein or nonvein areas of the leaf, contained little or no cannabinoids (Tables 1, 2). The leaves from which the glands were sampled were found to have typical cannabinoid levels for the clone (unpublished). Since it was possible that cannabinoids were present in low quantities (less than 3 ng per gland), a sample of 100 glands was collected and analyzed (Table 1). The results showed the presence of cannabinoids in leaf glands. This finding suggests that low levels of cannabinoids may also be present in the other samples of capitate-sessile glands from clones 152 and 87. In contrast, capitate-sessile glands from leaves of the Mexican and Turkish strains were found to contain detectable quantities of cannabinoids in the samples of 20 glands each (Tables 3, 4). A 100-gland sample from the Turkish strain showed comparable amounts of cannabinoids for both sample sizes which indicated that the 20-gland sample size was adequate for the analyses (Table 4).

DISCUSSION—We have previously reported the occurrence of variations, related to the gland age and the clone, in the cannabinoid content of capitate-stalked glands from *Cannabis* (Turner et al., 1977). The current study demonstrates further variations in cannabinoid content found in individual glandular trichomes. There are several possible explanations which will assist in interpreting these results. The position of glands on the plant, such as leaf versus bract or vein as contrasted to nonvein area, may influence cannabinoid content. In addition, factors affecting the general physiological state of the whole plant, such as environment (Phillips et al., 1970; Latta and Eaton, 1975) or nutrition (Coffman and Gentner, 1975), possibly may influence cannabinoid content in individual glands as well as in the whole plant. The data also suggest that the time of year may cause cannabinoid levels to vary. Related to the time of the year would also be the age of individual glands (Turner et al., 1977). Although all gland heads sampled were in the mature category, some samples might have contained glands that were physically, if not visibly, approaching the aged category thus lowering the cannabinoid level for the sample. The fluctuations in cannabinoid levels of capitate-stalked glands on the Mexican and Turkish strains were not found on clones 152 or 87 nor were they apparent in capitate-sessile glands. The reasons for this are not clear although the possibilities previously discussed, along with hormonal influences related to flowering, may be combining to influence cannabinoid variation. However, although glands varied in cannabinoid content within each strain and between strains, the individual glands still retained the qualitative profile of the strain or clone.

The conspicuous differences in cannabinoid levels between capitate-sessile and capitate-stalked glands can possibly be explained by considering them as two distinct gland types (Hammond and Mahlberg, 1977) rather than a single group of capitate glands (Dayanandan and Kaufman, 1976). Although both gland types may have similar ontogenetic origins (Hammond and Mahlberg, 1977), it has become evident that those glands which have a secondary developmental phase to produce the stalk also have the correlated presence of higher cannabinoid content.

The evolutionary development of stalk formation may be interrelated with as yet unknown factors that also stimulate cannabinoid formation to a far greater extent in bracteal glands than in glands on leaves. A high level of cannabinoid production, like the presence of a stalk, may represent a derived condition related to the yet unclear functional role of the capitate-stalked gland.

Differences in cannabinoid levels between the two gland types on a strain or clone may also be related in part to differences in gland size. Reports in the literature indicate capitate-sessile glands to be 40–60 μm in diameter (Fairbairn, 1972; Ledbetter and Krikorian, 1975; Hammond and Mahlberg, 1977). In contrast, the diameter of the capitate-stalked gland head was reported as being approximately 70–100 μm (DePasquale, 1974; Ledbetter and Krikorian, 1975; Hammond and Mahlberg, 1977). Our observations from the collection of hundreds of gland heads for analyses confirmed that capitate-sessile glands were consistently smaller in diameter than capitate-stalked glands. Yet, the quantitative differences for cannabinoids in capitate-sessile glands from leaves of the Mexican and Turkish strains as contrasted to clones 152 and 87 indicate that factors in addition to size must influence and control the cannabinoid content in a gland.

These results emphasize that glands of *Cannabis* represent a dynamic system in which cannabinoid content can vary in glands on different organs, on different areas of an organ, with the time of year, and with gland maturation while retaining the qualitative cannabinoid profile for a given strain or clone. It is also evident that some glandular trichomes may contain only low levels of cannabinoids. However, it is not yet fully clear what factors influence cannabinoid formation and accumulation. The role of individual glands in the synthesis of the cannabinoids in the plant as a whole requires further study.

LITERATURE CITED

André, Cl., and A. Vercruysse. 1976. Histochemical study of the stalked glandular hairs of the female *Cannabis* plants, using Fast Blue Salt. Planta Med. 29: 361–366.

Coffman, C., and W. Gentner. 1975. Cannabinoid profile and elemental uptake of *Cannabis sativa* L. as influenced by soil characteristics. Agron. J. 67: 491–497.

Dayanandan, P., and P. Kaufman. 1976. Trichomes of *Cannabis sativa* L. (Cannabaceae). Amer. J. Bot. 63: 578–591.

DePasquale, A. 1974. Ultrastructure of the *Cannabis sativa* glands. Planta Med. 25: 238–248.

Fairbairn, J. 1972. The trichomes and glands of *Cannabis sativa* L. Bull. Narc. 23: 29–33.

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.

Hammond, C., and P. Mahlberg. 1973. Morphology of glandular hairs of *Cannabis sativa* from scanning electron microscopy. Amer. J. Bot. 60: 524–528.

———, and ———. 1977. Morphogenesis of capitate glandular hairs of *Cannabis sativa* (Cannabaceae). Amer. J. Bot. 64: 1023–1031.

———, and ———. 1978. Ultrastructural development of capitate glandular hairs of *Cannabis sativa* L. (Cannabaceae). Amer. J. Bot. 65: 140–151.

Latta, R., and B. Eaton. 1975. Seasonal fluctuations in cannabinoid content of Kansas marijuana. Econ. Bot. 29: 153–163.

Ledbetter, M., and A. Krikorian. 1975. Trichomes of *Cannabis sativa* as viewed with scanning electron microscopy. Phytomorphology 25: 166–176.

Malingré, T., H. Hendricks, S. Batterman, R. Bos, and J. Visser. 1975. The essential oil of *Cannabis sativa*. Planta Med. 28: 56–61.

Mechoulam, R. 1973. Marijuana. Academic Press, New York.

Phillips, R., R. Turk, J. Manno, N. Jain, D. Grim, and R. Forney. 1970. Seasonal variation in cannabinolic content of Indiana marijuana. J. Forensic Sci. 15: 191–200.

Report of a Working Group. 1976. The botany and chemotaxonomy of *Cannabis*. United Nations Document MNAK/15/CE 77-1387: 1–31.

Small, E., and H. Beckstead. 1973. Common cannabinoid phenotypes in 350 stocks of *Cannabis*. Lloydia 36: 144–165.

———, and A. Cronquist. 1976. A practical and natural taxonomy for *Cannabis*. Taxon 25: 405–435.

Turner, J., J. Hemphill, and P. Mahlberg. 1977. Gland distribution and cannabinoid content in clones of *Cannabis sativa* L. Amer. J. Bot. 64: 687–693.