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CANNABINOID COMPOSITION IN SEEDLINGS COMPARED TO ADULT PLANTS OF *CANNABIS SATIVA*

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ABSTRACT.—Cannabinoid ratios in *Cannabis sativa* were compared in developing seedlings and adult plants of different ages. Analyses were performed on primary and secondary leaves from seedling plants and 4- and 7.5-cm leaves from adult plants. In seedlings of a drug strain, maximum cannabinoid levels were obtained in primary leaves at 120–122 h. Cannabichromene (CBC) was the dominant cannabinoid of young seedlings. The dominant cannabinoid of older plants, Δ^9-tetrahydrocannabinol (THC), was present in the least amount in all seedlings through age 120–122 h. By age 144–146 h, when other cannabinoid amounts were declining, THC was emerging as the major cannabinoid. This was attributed to increased levels of THC in secondary leaves in older seedlings. The cannabinoid profile of young seedlings differs significantly from that of adult plants, and the transition to the adult profile begins with the formation of secondary leaves.

Biosynthesis of cannabinoids in *Cannabis sativa* L. is not well understood. Although it is well-known that cannabinoids occur in glandular trichomes (1–4), reports of their presence in other plant tissues are contradictory (5,6). Factors that control biosynthesis and distribution of cannabinoids within the plant are unknown. As part of our ongoing studies of cannabinoid localization and biosynthesis, we have developed a methodology for examining the sequence of appearance of detectable cannabinoids in developing seedlings (7). We have shown that differences in cannabinoid contents occur during seedling development. In this report we compare cannabinoid quantities in adult plants with those in seedlings to determine the changes that occur as the plant matures.

EXPERIMENTAL

SEED GERMINATION AND SEEDLING HARVEST.—*Cannabis* seeds, obtained from parent stock grown under greenhouse conditions, were from a drug [Δ^9-tetrahydrocannabinol (THC)] strain of Mexican origin used in previous studies (8). Seeds were germinated and harvested using the seedling assay system previously described (7). Seedlings were grouped into categories based upon their age. Each sample contained 20–24 seedlings and weighed ca. 200 mg (dry wt).

MATURE PLANTS AND SEED PRODUCTION.—Mature plants were grown under greenhouse conditions from the same seed supply as that used for seedlings and maintained in the vegetative state under a 20 h/4 h light/dark cycle in the greenhouse and growth chamber (7). Actively growing leaves from both greenhouse-grown (vegetative plants) and controlled-light-grown plants were harvested when their center leaflets reached a length of 4.0 (± 0.5) cm or 7.5 (± 0.5) cm as indicated. All leaflets were used after being separated and randomly distributed (9). The 18–20- and 29-day-old plants were grown at the same time under vegetative greenhouse conditions. Samples of primary leaves were collected randomly from the same plants at day 18–20 and again at day 29. Since primary leaves rarely reached the 7.5 (± 0.5) cm length (2–4), a length of 4.0 (± 0.5) cm was chosen. This was the maximum average length attained by actively growing, healthy primary leaves. Primary leaves have one leaflet only. Leaves from plants with 7.5 (± 0.5) cm center leaflets were at least 40 days old when analyzed. After sufficient vegetative growth, plants were switched to an 8 h/16 h light/dark cycle to induce flowering for "flowering top" analyses and to generate seeds for subsequent experiments. Flowering tops were collected when glandular trichomes on bracts were globose and resinous. Flowering top samples included bracts, small leaves, and stem pieces but no seeds. Each datum point represents triplicate samples; each sample weighed 50–100 mg dry wt. Samples were dried at either 37 or 60° for 15–21 h.

EXTRACTION.—Dried samples were extracted for cannabinoids as previously described (10).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.—Analyses were performed on a Hewlett-

Packard 1084B liquid chromatograph using the procedures of Turner and Mahlberg (10,11), which were modified for seedling samples (7). Cannabinoid standards were obtained from the National Institute on Drug Abuse. Two internal standards (dioctylphthalate and dibenzylphthalate) were used to calibrate the columns. Injection volumes were 20 μl per mature plant sample and 100 μl per seedling or seed sample.

DECARBOXYLATION OF SAMPLES.—Each sample was routinely analyzed twice by hplc. The first analysis was performed after sample preparation as described above with the cannabinoids being primarily in their carboxylated (acidic) forms. Subsequently, each sample was decarboxylated with heat (200° for 10 min) to convert all cannabinoids to the neutral form (12).

RESULTS

All seedlings had well-developed primary leaves, and those seedlings 72–74 h or older often possessed secondary leaves. Whole seedlings were analyzed because virtually all cannabinoids in seedlings are located in the leaves (13). Whole seedling cannabinoid data were compared with leaf samples from older plants (Table 1).

Total cannabinoids in seedlings increase in mean quantities up to age 120–122 h (Table 1). Variation in cannabinoids for a given age is high, as is evident from standard deviation values, although variation tends to decrease with increasing age.

Although mean cannabinoid amounts for THC and total cannabinoids appear to decrease and cannabichromene (CBC) appears to increase from age 120–122 h to age 144–146 h, the means are not significantly different (Student's *t* ratio). The mean amount of cannabigerol (CBG) at age 144–146 h is significantly lower ($p<0.001$, Student's *t* ratio) than the mean at age 120–122 h. Thus, it appears that while THC, CBC, and total cannabinoids are leveling off by 120–122 h, CBG is decreasing. Cannabinoid levels for all cannabinoids in primary leaves decrease from age 120–122 h to 144–146 h to those found at 18 days to 29 days (Table 1).

Quantitative relationships among the three major cannabinoids can be seen by examining their ratios (Table 1). CBC is the dominant cannabinoid in all seedling and primary leaf samples. The dominant cannabinoid of the mature plant, THC (Table 1), is the cannabinoid present in the least amount in all seedling samples through age 120–122 h. However, by age 144–146 h the amount of THC has surpassed that of CBG. Likewise, THC is present in greater amounts than CBG in primary leaves of 18- and 29-day-old plants. Thus, at the same age, 120–122 h to 144–146 h, that cannabinoid levels are peaking or declining, THC is emerging as the major cannabinoid.

All cannabinoids are present in greater amounts in mature plants than in younger plants, and THC is dominant (Table 1). Mean CBG, THC, and total cannabinoid amounts of controlled-light and greenhouse-grown plants are not significantly different (Student's *t* ratio). CBC is significantly greater ($p>0.001$; Student's *t* ratio) in controlled-light plants. Variation among samples is high, as has been routinely observed in *Cannabis*.

Secondary leaves (20- and 29-day-old plants) contain higher levels of cannabinoids than do primary leaves (Table 1). As was observed with primary leaves, there is a decrease in cannabinoids as secondary leaves age. Secondary leaves of 20-day-old plants have THC as the dominant cannabinoid.

CBG means from flowering tops and greenhouse-grown or controlled-light-grown mature vegetative plants are not significantly different (Student's *t* ratio, Table 1). In contrast to the other cannabinoids, CBG did not increase as the plants were induced to flower. The means of THC, CBC, and total cannabinoids from flowering top samples were all significantly higher than their respective means from vegetative plants: THC $p<0.001$, both light conditions; CBC $p<0.001$ and $p<0.01$, controlled-light and greenhouse-grown plants, respectively; total cannabinoids $p<0.01$ and $p<0.001$, controlled-light and greenhouse-grown plants, respectively (Student's *t* ratio). Note

TABLE 1. Mean Cannabinoid Content of *Cannabis* Materials of Different Ages Analyzed by hplc.

Material/Age	Cannabinoid (μg/100 mg dry wt ± standard deviation)				Ratio CBC:THC:CBG[a]	Number of Samples
	CBC[a]	THC[b]	CBG[c]	Total		
Seedlings						
48–50 h	nd[d]	nd	nd	nd	nd	11
52–54 h	0.004 ± 0.009	nd	nd	0.004 ± 0.009	—[e]	12
56–58 h	0.36 ± 0.49	nd	nd	0.36 ± 0.49	—	13
60–62 h	1.1 ± 1.2	0.3 ± 0.6	0.3 ± 0.6	1.7 ± 2.3	4.1:1.1:1	12
66–68 h	10.6 ± 9.1	4.5 ± 3.4	7.0 ± 7.0	22.2 ± 19.1	2.3:1:1.6	13
72–74 h	20.9 ± 13.6	5.4 ± 4.6	14.9 ± 10.1	41.1 ± 27.8	3.9:1:2.8	30
96–98 h	102.3 ± 37.9	21.8 ± 10.0	47.3 ± 20.3	172.9 ± 62.1	4.7:1:2.2	22
120–122 h	191.0 ± 40.0	42.0 ± 13.0	54.6 ± 12.8	288.2 ± 48.7	4.5:1:1.3	24
144–146 h	197.7 ± 41.3	40.6 ± 10.0	30.6 ± 8.4	269.2 ± 54.6	6.5:1.3:1	15
Vegetative, 18–20 days						
Primary leaves	67.1 ± 8.5	24.46 ± 3.7	19.2 ± 7.9	110.8 ± 17.5	3.5:1.3:1	3
Secondary leaves	353.3 ± 12.5	501.00 ± 7.8	110.33 ± 19.9	968.7 ± 32.3	3.2:4.5:1	3
Vegetative, 29 days						
Primary leaves	57.2 ± 9.6	16.9 ± 1.6	6.32 ± 1.8	80.4 ± 9.8	9.0:2.7:1	2
Secondary leaves	213.1 ± 0.3	166.42 ± 5.6	1.67 ± 0.1	381.2 ± 5.4	127.6:99.7:1	3
Adult vegetative						
Controlled lighting	513.1 ± 106	1704.9 ± 407	133.53 ± 139	2354.0 ± 608	3.8:12.8:1	12
Greenhouse	251.8 ± 93	1331.3 ± 968	143.47 ± 57	1731.8 ± 1,094	1.8:9.3:1	12
Flowering						
Greenhouse	127.2 ± 17	3575.3 ± 1,069	101.8 ± 62	3820.6 ± 1,142	1.2:35.1:1	6

[a]Cannabichromene.
[b]Δ^9-Tetrahydrocannabinol.
[c]Cannabigerol.
[d]nd, none detected.
[e]—, no data.

that while THC has been the dominant cannabinoid in all mature plants sampled, its dominance has increased 3–4-fold in flowering top samples.

DISCUSSION

The peaking and subsequent decrease in individual and total cannabinoid contents were evident in both primary and secondary leaves. The pattern was not affected by the different growth environments of controlled lighting or greenhouse conditions. This trend for decreasing levels of cannabinoids also has been observed in the aging leaf and for successively older leaves along a plant axis (4,9). The fate of the cannabinoids is incompletely understood. Because the entire shoot for the seedling and primary leaves was analyzed in this study, the mobilization and transport of cannabinoids within the shoot appear not to occur, indicating that the cannabinoids may be altered during the aging processes in the organ.

In this strain the THC concentration becomes dominant over CBC and CBG only upon development of secondary leaves. Although secondary leaves represent a very small part of the total seedling, they are present on many 72–74 h and older seedlings (13). Since these leaves rather than primary leaves show a predominance of THC they appear to be responsible for the higher level of THC in 144–146 h seedlings. The THC content of primary leaves in seedlings is relatively low when compared with levels in secondary leaves. It can be concluded that the cannabinoid profile of young seedlings differs substantially from that of mature plants and that the transition to the adult profile begins in secondary leaves.

Although total THC and CBG accumulation was not affected by light conditions, CBC content was significantly greater than that of THC and CBG under the lower intensity of controlled light. A similar trend for CBC in relation to other cannabinoids was observed in other plants examined under different light conditions (7, 14, 15) and suggests that its synthesis or accumulation may be influenced by a photomorphogenetic condition. The differential response of CBC and its acid compared to other cannabinoids has been observed in other studies and led to an interpretation that two pathways for cannabinoid formation may exist at different stages in plant development (16).

The increases in THC content between seedling, vegetative, and flowering plants indicate that the synthetic mechanism for cannabinoid formation is developmentally dynamic. While THC is reported here and by others to increase during the transition from vegetative to flowering state (5, 17), we find no increase in CBC or CBG content. The predominance of THC in this strain may contrast with that in different strains in which another cannabinoid is dominant in the adult state (18) and emphasizes further the dynamic character of this biosynthetic process. It remains to be determined whether a similar pattern in sequential appearance of cannabinoids will be detected in other drug strains, and whether specific patterns or ratios occur in nondrug strains. If distinctive patterns do occur in other strains, this phenomenon can provide insight into phylogenetic trends for the biosynthesis of cannabinoids in the numerous strains of *Cannabis*.

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LITERATURE CITED

1. T. Malingre, H. Hendriks, S. Batterman, R. Box, and J. Visser, *Planta Med.*, **28**, 56 (1975).
2. J. Turner, J. Hemphill, and P. Mahlberg, *Am. J. Bot.*, **64**, 687 (1977).
3. J. Turner, J. Hemphill, and P. Mahlberg, *Am. J. Bot.*, **65**, 1103 (1978).
4. J. Turner, J. Hemphill, and P. Mahlberg, *Am. J. Bot.*, **67**, 1397 (1980).

5. P. Fetterman, E. Keith, C. Waller, O. Guerrero, N. Doorenbos, and M. Quimby, *J. Pharm. Sci.*, **60**, 1246 (1971).
6. M. Furr and P. Mahlberg, *J. Nat. Prod.*, **44**, 153 (1981).
7. A. Vogelmann, J. Turner, and P. Mahlberg, *Bot. Gaz.*, **8**, 468 (1987).
8. C. Hammond and P. Mahlberg, *Am. J. Bot.*, **64**, 1023 (1977).
9. J. Hemphill, J. Turner, and P. Mahlberg, *J. Nat. Prod.*, **43**, 112 (1980).
10. J. Turner and P. Mahlberg, *J. Chromatogr.*, **283**, 165 (1984).
11. J. Turner and P. Mahlberg, *J. Chromatogr.*, **253**, 295 (1982).
12. S. Kanter, M. Musumeci, and L. Hollister, *J. Chromatogr.*, **171**, 504 (1979).
13. A. Vogelmann, "Studies on Cannabinoid and Glandular Trichome Occurrence in Seedlings of *Cannabis sativa* L. (Cannabaceae)," Doctoral dissertation, Indiana University (1985).
14. P. Mahlberg and J. Hemphill, *Bot. Gaz.*, **144**, 43 (1983).
15. J. Valle, J. Vieira, J. Aucelio, and I. Valio, *Bull. Narc.*, **30**, 67 (1978).
16. C. Turner and P. Mahlberg, in: "Marihuana 1987." Ed. by G. Chesher, Pergamon Press, New York, in press.
17. C. Turner and M. Elsohly, *J. Heterocycl. Chem.*, **16**, 1667 (1979).
18. E. Small and H. Beckstead, *Lloydia*, **35**, 144 (1973).

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