BOT. GAZ. 148(3):468–474. 1987.

0006-8071/87/4804-0006$02.00

CANNABINOID OCCURRENCE IN SEEDLINGS OF CANNABIS SATIVA L.: QUANTITATION IN SEEDLINGS OF KNOWN AGE AND PRIMARY LEAF LENGTH

ANN F. VOGELMANN,* JOCELYN C. TURNER,† AND PAUL G. MAHLBERG†

*Jet Propulsion Laboratory MS 183-501, California Institute of Technology, Pasadena, California 91109; and †Department of Biology, Indiana University, Bloomington, Indiana 47405

Seedlings of a Δ^9-tetrahydrocannabinol (THC) dominant strain of *Cannabis sativa* were employed to develop a biological system to investigate cannabinoid biosynthesis. High-performance liquid chromatography was used to detect and quantify the cannabinoids. Cannabinoids were first detected in seedlings of light/dark-grown plants at 48–50 h. The first cannabinoid detected was cannabichromene (CBC) at 52–54 h. At 60–62 h both cannabigerol (CBG) and THC were detected along with CBC. A similar sequence appeared in dark-grown seedlings, but CBC became evident only at 56–58 h, and THC and CBG were first detected at 66–68 h. This pattern is significant because previously proposed pathways of cannabinoid synthesis have indicated that CBG is a precursor to CBC, yet CBG is not the first cannabinoid to be detected but occurs later with the appearance of THC. Cannabinoid concentrations were always higher in light-grown than in dark-grown plants of comparable age. Cannabinoid quantities on a dry-weight basis increased with increasing leaf length and/or increasing age.

Introduction

Cannabinoid biosynthesis within *Cannabis sativa* L. is not well understood. Reports on the localization of cannabinoids in specific plant tissues, other than their documented presence in glandular trichomes (MALINGRE et al. 1975; J. C. TURNER et al. 1977, 1978, 1980), are contradictory (FETTERMAN et al. 1971; FURR and MAHLBERG 1981). The site of synthesis within the plant, the pathway sequence, and the factors controlling biosynthesis are unknown. Attempts to investigate cannabinoid biosynthesis in mature plants or specific tissues such as glandular trichomes have been complicated by numerous variables (TURNER et al. 1985).

The purpose of this study was to develop a seedling assay system to determine qualitative and quantitative changes of cannabinoids under defined temporal and morphological parameters during seedling development. Since cannabinoids are known to be absent from the mature embryo (FURR and MAHLBERG 1981; VOGELMANN 1985), the developing seedling can be utilized for examination of the sequence of appearance of detectable cannabinoids in the growing plant.

Material and methods

SEED GERMINATION

Cannabis seeds were from a Δ^9-tetrahydrocannabinol (THC) strain of Mexican origin (HAMMOND and MAHLBERG 1973) and used in previous studies (MAHLBERG and HEMPHILL 1983). Large quantities of seeds have been generated periodically from a small number of pistillate and staminate parents grown under greenhouse conditions in the absence of other *Cannabis* strains.

For each experiment ca. 1,000 seeds were used. Seeds were germinated in the dark in 9-cm glass petri dishes containing 25–30 seeds on 50 mL dry perlite with 24 mL distilled water. At 17–20 h, seeds were examined in the dark with a green photography safelight for germination (defined as the splitting of the seed coat). Any germinated seeds were discarded, and subsequent checks were made every 2 h, thus defining germination within a 2-h interval. Germinated seeds were transferred to covered 16-oz glass jars, each containing 10 seeds on 50 mL dry perlite and 20 mL distilled water. Seedlings were maintained under either 20/4-h light/dark cycles or in darkness in a growth chamber at 25 C (TURNER et al. 1985). Light level was ca. 3,500 lx (ca. 3.6×10^4 erg/cm^2/s).

SEEDLING HARVEST AND EXTRACTION

At a specific age, seedlings were harvested and data recorded for (1) presence of a seed coat enclosing the epicotyl and cotyledons, (2) presence of a nucellar sheath surrounding the epicotyl and cotyledons, (3) relative expansion of the cotyledons, and (4) relative expansion of the primary leaves. Each seedling was classified also for length of its primary leaves and hypocotyl. Dark-grown seedlings were harvested under a green safelight. Harvested seedlings were dried at 60 C for 12–24 h. Seedlings were grouped into categories based on their age and the length of their primary leaves (tables 1, 2). Each sample usually contained 20–24 seedlings and weighed ca. 200 mg dry weight (DW). Three or more samples typically were collected for each seedling category. Samples of ungerminated seeds, containing 25 seeds each (ca. 200 mg DW) were collected in triplicate and dried at 60 C for

Manuscript received November 1986; revised manuscript received April 1987.

Address for correspondence and reprints: PAUL MAHLBERG, Department of Biology, Indiana University, Bloomington, Indiana 47405.

TABLE 1

MEAN CANNABINOID CONTENT OF LIGHT-GROWN *CANNABIS* SEEDLINGS SEPARATED BY AGE AND PRIMARY LEAF LENGTH

CANNABINOID (μg/100 mg DW) AND AGE (h)	PRIMARY LEAF LENGTH (mm)							MEAN	r^2
	2–3	3–4	4–5	5–6	6–7	7–8	>8		
CBG:									
48–50	nd	nd	...	...	...	...	...	nd	nc
52–54	nd	nd	...	...	...	...	...	nd	nc
56–58	nd	nd	nd	...	...	...	...	nd	nc
60–62	nd	.065 ±.16	1.44 ±.41	...	...	...	...	.28 ±.56	.54
66–68	.27	2.48 ±1.30	7.50 ±3.42	20.55 ±4.95	...	...	...	7.02 ±7.25	.71
72–74	2.06 ±2.06	5.95 ±2.82	13.42 ±6.93	23.81 ±7.12	29.24 ±2.91	...	...	14.93 ±10.27	.67
96–98	...	...	30.52 ±7.58	39.85 ±5.54	53.62 ±16.75	64.33 ±11.75	65.47 ±3.84	47.32 ±16.92	.42
120–122	...	...	...	48.82 ±10.57	52.97 ±13.38	61.72 ±13.11	54.11 ±10.51	54.63 ±13.07	.02
144–146	...	...	18.85	32.23 ±5.36	25.73 ±5.78	33.21 ±5.07	33.19 ±9.48	30.62 ±8.66	.29
r^2	.35	.66	.37	.36	.0035	.20	.59	...	
THC:									
56–58	nd	nd	nd	...	...	...	...	nd	nc
60–62	nd	.12 ±.21	1.36 ±1.86	...	...	...	...	.30 ±.57	.47
66–68	nd	2.22 ±.97	5.94 ±2.69	8.98 ±2.39	...	...	...	4.52 ±3.52	.64
72–74	nd	2.03 ±1.89	4.57 ±2.80	7.87 ±3.72	13.87 ±1.84	...	...	5.37 ±4.63	.61
96–98	...	...	11.06 ±3.69	15.50 ±3.65	23.48 ±8.84	33.68 ±3.67	33.07 ±2.07	21.77 ±10.24	.62
120–122	...	...	...	28.05 ±7.32	40.43 ±8.40	43.11 ±9.31	52.33 ±12.48	41.99 ±13.26	.45
144–146	...	...	29.94	33.31 ±.44	35.47 ±8.64	44.33 ±6.52	46.40 ±8.71	40.62 ±10.29	.61
r^2	nc	.27	.72	.77	.46	.22	.036	...	...
CBC:									
48–50	nd	nd	...	...	...	...	...	nd	nc
52–54	nd	.0064 ±.010	...	...	...	...	...	.0038 ±.0096	nc
56–58	nd	.24 .17	1.40 ±.31	...	...	...	...	.36 ±.51	.66
60–62	.017 ±.025	.99 ±.61	3.33 ±.94	...	...	...	...	1.14 ±1.27	.69
66–68	.43	5.09 ±2.01	11.26 ±4.50	27.94 ±6.87	...	...	...	10.62 ±9.46	.71
72–74	1.51 ±1.04	8.93 ±4.91	21.07 ±10.43	31.64 ±12.06	35.18 ±5.89	...	...	20.87 ±13.98	.55
96–98	...	...	60.00 ±11.33	83.62 ±9.38	107.90 ±26.31	130.01 ±32.29	171.07 ±11.38	102.34 ±38.80	.69
120–122	...	...	...	173.17 ±28.64	169.08 ±40.37	213.94 ±34.01	204.17 ±29.68	191.01 ±39.92	.10
144–146	...	...	133.23	157.58 ±16.24	193.57 ±21.48	191.00 ±6.72	227.35 ±42.34	197.75 ±42.79	.62
r^2	.53	.64	.92	.83	.74	.28	.22	...	

NOTE.—nd, none detected; nc, not calculated; r^2, coefficient of determination; ..., no samples of this size or age; ±, SD.

TABLE 2

MEAN CANNABINOID CONTENT OF DARK-GROWN CANNABIS SEEDLINGS SEPARATED BY AGE AND PRIMARY LEAF LENGTH

CANNABINOID (μg/100 mg DW) AND AGE (h)	PRIMARY LEAF LENGTH (mm) 2–3	3–4	4–5	5–6	MEAN	r^2
CBG:						
48–50	nd	nd	...	...	nd	nc
52–54	nd	nd	...	...	nd	nc
56–58	nd	nd	...	...	nd	nc
60–62	nd	nd	nd	...	nd	nc
66–68	nd	.71 ± 1.02	3.78 ± 1.47	...	1.23 ± 1.84	.59
72–74	nd	3.78 ± 2.56	7.45 ± 2.82	16.88 ± 4.30	5.79 ± 5.19	.66
96–98	1.18 ± 1.18	9.08 ± 5.56	19.61 ± 4.87	29.18 ± 2.41	14.99 ± 9.86	.76
120–122	...	9.30 ± 1.55	12.37 ± 1.94	16.92 ± 7.11	13.20 ± 5.55	.03
r^2	nc	.48	.40	.0086	...	
THC:						
48–50	nd	nd	...	...	nd	nc
50–52	nd	nd	...	...	nd	nc
56–58	nd	nd	...	...	nd	nc
60–62	nd	nd	nd	...	nd	nc
66–68	nd	.32 ± .38	1.27 ± .67	...	.45 ± 3.66	.53
72–74	nd	1.65 ± 2.02	4.88 ± 2.30	12.03 ± .21	3.54 ± 3.67	.71
96–98	.47 ± .47	5.30 ± 3.06	12.05 ± 8.29	19.96 ± 5.11	9.38 ± 6.84	.76
120–122	...	7.19 ± .093	20.83 ± 6.50	29.95 ± 10.56	20.84 ± 11.86	.55
r^2	nc	.64	.81	.49		
CBC:						
48–50	nd	nd	...	...	nd	nc
52–54	nd	nd	...	...	nd	nc
56–58	nd	.19 ± .14	...	...	.092 ± .14	nc
60–62	nd	.25 ± .088	.48	...	.18 ± .19	.90
66–68	.24 ± .35	1.75 ± .77	4.44 ± 1.67	...	1.89 ± 1.86	.76
72–74	.73 ± .42	6.91 ± 3.65	19.94 ± 8.85	38.96 ± 5.49	13.73 ± 12.55	.71
96–98	5.18 ± 2.97	21.11 ± 12.51	41.48 ± 10.48	66.89 ± 12.18	33.52 ± 21.89	.72
120–122	...	32.87 ± 3.04	56.23 ± 7.46	132.02 ± 18.33	76.30 ± 44.65	.83
r^2	.66	.71	.79	.85		

NOTE.—nd, none detected; nc, not calculated; r^2, coefficient of determination; ..., no samples of this size or age; ±, SD.

12 h. Dried samples were extracted for cannabinoids as described (TURNER and MAHLBERG 1984).

HIGH-PERFORMANCE LIQUID (HPLC) AND GAS-LIQUID CHROMATOGRAPHY (GLC)

Analyses were performed on a Hewlett-Packard 1084B liquid chromatograph (HPLC) and a Hewlett-Packard 5710A gas chromatograph (GLC) (TURNER and MAHLBERG 1982, 1984). The HPLC was equipped with a variable-wavelength UV detector set at 277 nm. Two different reversed phase columns were used: an Altex C-8 (Ultrasil-octyl, 10 μm; 25 cm × 4.6 mm ID) or a Whatman C-18 (Partisil-10 ODS-2, 10 μm; 25 cm × 4.6 mm ID). Samples were analyzed with a gradient program starting with 25% acetonitrile at time zero and reaching 85% acetonitrile at 35 min (C-8 column) or 25 min (C-18 column). It was necessary to add a cleaning segment to the end of the program using 100% acetonitrile at a flow rate of 0.5 mL/min for ca. 15 min after the cannabinoids and internal standard peaks had been eluted. Cannabinoid standards, courtesy of the National Institute on Drug Abuse, and two internal standards (dioctylphthalate [DOP] and dibenzylphthalate [DBP]) were used to calibrate the columns. Injection volume was 100 μL.

Very young seedling samples contained an oily substance, probably a storage product from cotyledons, that interfered with reproducibility of cannabinoid analyses by GLC. Analyses of cannabinoids by HPLC were unaffected by oily residues in samples (VOGELMANN 1985).

DECARBOXYLATION

Each sample was routinely analyzed twice by HPLC. The first analysis was performed after sample extraction; the cannabinoids were primarily in their carboxylated or acidic forms. Subsequently, each sample was decarboxylated (200 C for 3 min) to convert all cannabinoids to their neutral forms (KANTER et al. 1979), and each sample was reanalyzed. All quantitations were from decarboxylated samples.

STATISTICAL ANALYSIS

Statistical analyses were performed on a DEC System 2060 computer using an SPSS program

package (NIE et al. 1975) at the Academic Computing Center of the University of Vermont, Burlington. For statistical analyses, medians of age and primary leaf length ranges were used.

Results

SEEDLING MORPHOLOGY

Seed germination typically occurred within 17–32 h after wetting. The epicotyl and cotyledons initially remained enclosed by the split seed coat, while the taproot elongated rapidly and often was 8 cm long by 48–50 h. Expansion of the cotyledons and primary leaf typically occurred after 50 h.

The two primary leaves were simple and essentially equal in size. There was a lag time between the expansion of the cotyledons and the subsequent expansion of the primary leaves. Lengths of primary leaves ranged from 2 to 3 mm in 48–50-h seedlings to 12–13 mm in 144–146-h seedlings. Secondary leaves were discernible in 72–74 h and older seedlings when the primary leaves were at least 4–5 mm long. Secondary leaves were rarely longer than 1 mm and were usually simple, rarely tripartite. Subsequent leaves were never observed in seedlings in this study (tables 1, 2).

TEMPORAL OCCURRENCE OF CANNABINOIDS

No cannabinoids were detected in ungerminated seeds or in 48–50-h or younger seedlings grown in either light or total darkness (tables 1, 2). However, cannabinoid residues can occur on the seed coat from contact with gland contents during harvest. The first cannabinoid detected in the seedling was cannabichromene (CBC). Initial detection was in 52–54-h light-grown seedlings with 3–4 mm primary leaves and in 56–58-h dark-grown seedlings with 3–4-mm primary leaves. CBC was the only cannabinoid detected in all 52–54-h and 56–58-h light-grown seedlings and in all 56–58-h and 60–62-h dark-grown seedlings. It was the only cannabinoid in some samples of 60–62-h and 72–74-h light-grown seedlings and 96–98-h dark-grown seedlings (tables 1, 2).

There was no significant difference (Student's *t*-ratio) between the mean amount of CBC in light- and dark-grown samples having CBC as the only detectable cannabinoid. The average CBC amount was 0.48 μg/100 mg DW. Maximum amounts of CBC were 1.71 μg/mg DW in 4–5-mm (56–58 h) light-grown seedlings and 2.21 μg/100 mg DW in 2–3-mm (96–98 h) dark-grown seedlings. Minimum amounts of CBC were 0.018 μg/100 mg DW in 3–4-mm (52–54 h) light-grown and 0.058 μg/100 mg DW in 3–4-mm (56–58 h) dark-grown seedlings. Although mean amounts of CBC (tables 1, 2) were the same in light- and dark-grown seedlings, the appearance of CBC was delayed for 2–6 h in dark-grown seedlings. At comparable ages, the mean quantities of CBC were higher in light-grown than in dark-grown seedlings.

The order of cannabinoid occurrence subsequent to the appearance of CBC is unclear. While the age/size range of seedlings having only CBC is relatively wide, the transition to having all cannabinoids (CBC, THC, and cannabigerol [CBG]) occurs over a much shorter range (tables 1, 2). Of the 259 seedling samples analyzed, only seven had either CBC + CBG or CBC + THC. The earliest occurrence of cannabinoids other than CBC was in 60–62-h light-grown seedlings with 3–4-mm primary leaves where both CBC and THC were detected.

The earliest occurrence in dark-grown seedlings was in 66–68-h plants with 3–4-mm primary leaves where two samples were found with CBC + CBG, and one sample with CBC + THC. Five samples contained CBC + CBG but no THC (three light-grown, two dark-grown), and two samples contained THC + CBC but no CBG (one each light- and dark-grown). By 72–74 h, both light- and dark-grown seedlings with primary leaves 3–4 mm long contained all three cannabinoids.

EFFECT OF AGE AND LEAF LENGTH ON CANNABINOID CONTENT

Seedlings of the same age showed morphological differences, especially for primary leaf length. Consequently, analyses of seedling samples were based on age and primary leaf length, whereas relative expansion of primary leaves was of only limited value.

Cannabinoid contents of seedlings of known age and primary leaf length showed the following trends in light-grown seedlings: cannabinoid concentration increased with increasing primary leaf length for any given age, and with increasing age for any given primary leaf length (table 1). Exceptions occurred at older stages and longer primary leaf lengths. For all leaf length categories, total cannabinoid concentration (CBG + THC + CBC) ceased to increase after seedlings reached 120–122 h and then either leveled off or decreased (table 1).

CBC was the dominant cannabinoid in all light-grown seedlings, although after 120–122 h the quantities of CBC leveled off or decreased. In seedlings up to 120–122 h, THC was present in the lowest quantity. However, by 144–146 h the THC content became equal to or greater than the CBG content. Samples with longer primary leaves showed a greater difference between THC and CBG content.

Cannabinoid data from seedlings grown in total darkness and grouped by primary leaf length (table 2) can be compared with similar data from light-grown seedlings (table 1). While light-grown seedlings often had primary leaves longer than 8 mm, the number of dark-grown seedlings with primary

leaves exceeding 6 mm never occurred in quantities great enough to be analyzed. Trends within categories of dark-grown seedlings were like those described for light-grown seedlings: cannabinoid content increased with increasing age for a given primary leaf length and for a given age with increasing primary leaf length (table 1). In samples of comparable age, cannabinoid quantities were less or, rarely, about the same in those grown in total darkness than in those grown in the light. As in light-grown seedlings, CBC was the dominant cannabinoid in all dark-grown seedlings. However, a leveling off or decrease in CBC quantity was not observed. The relationship of CBG to THC in light-grown seedlings also was apparent in dark-grown seedlings. Young seedlings had higher quantities of CBG, but in seedlings 120–122 h old with 4–5-mm or longer primary leaves, THC was present in higher amounts.

Analysis of variance (ANOVA) revealed no significant differences ($P = .05$) in cannabinoid content between most adjacent age and/or primary leaf length seedling sample categories (tables 1, 2). Correlation analysis was performed to provide a measure of the relationship between cannabinoid content and primary leaf length or age for the various seedling categories. Categories with the highest r^2 values were generally those in which cannabinoid contents were increasing with age or size; those in which cannabinoid contents were leveling off tended to have lower r^2 values (tables 1, 2).

Discussion

The *Cannabis* seedling can be employed as an assay system to investigate cannabinoid biosynthesis. Our demonstration that cannabinoids first become detectable in both light- and dark-grown seedlings at least 50 h after germination makes it possible to monitor progressive changes in cannabinoid composition. This measurable level of control for cannabinoid analyses during ontogeny has been lacking in studies using adult plants (FAIRBAIRN and LIEBMANN 1974; TURNER et al. 1979).

Cannabinoid accumulation was evident in seedlings ca. 2 days old, with the detection of CBC as the first cannabinoid. CBC remained the only cannabinoid detectable for the subsequent 8–12 h, whereupon both CBG and THC became detectable. Other investigators have suggested that CBG was the first formed cannabinoid in a proposed biosynthetic pathway (fig. 1). The CBG was interpreted to be converted into either CBC or cannabidiol (CBD), with the latter being converted into THC (MECHOULAM 1970; C. E. TURNER et al. 1980). Using labeled substrates, SHOYAMA et al. (1975) showed that CBG was a precursor to CBC in mature plants. SHOYAMA et al. (1968) and KUSHIMA et al. (1980) also reported CBC as the first detected cannabinoid in seedlings. DEFAUBERT MAUNDER (1970) stated that THC was the first detectable cannabinoid. However, his "unknowns 1 and 2" in the same samples with THC, based on R_f values and spot colors of his thin-layer chromatographic analysis, were probably CBG and CBC, respectively.

Our data support the interpretation that CBC is the first cannabinoid synthesized in seedlings (SHOYAMA et al. 1968; KUSHIMA et al. 1980; VOGELMANN et al. 1985). Since numerous samples contain greater quantities of CBC than the minimum amount present when additional cannabinoids became detectable, it appears the presence of a minimal amount of CBC is not prerequisite for the synthesis of additional cannabinoids. In no samples were other cannabinoids detected before CBC.

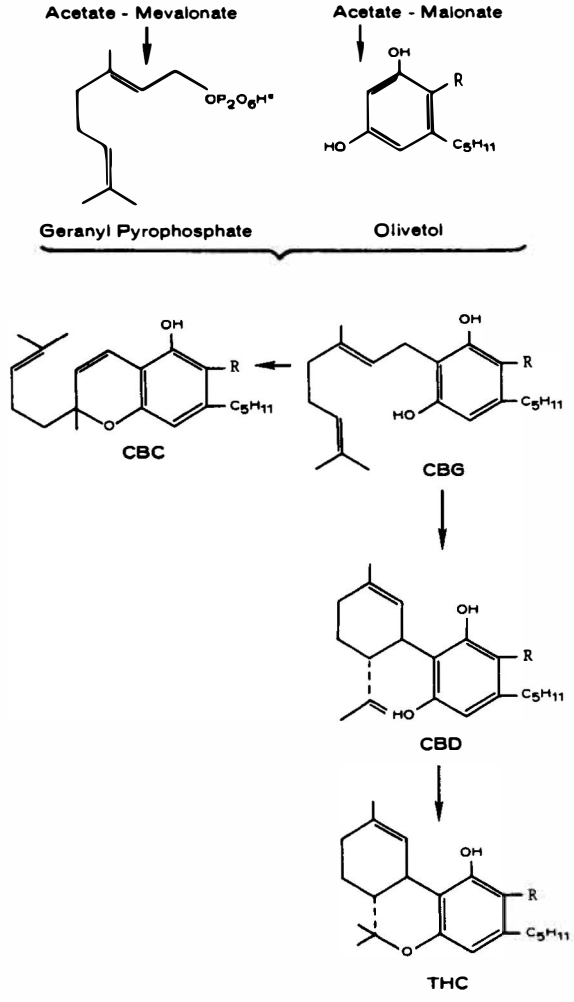

FIG. 1.—Proposed cannabinoid biosynthetic pathway (MECHOULAM 1970; C. E. TURNER et al. 1980). The presumed precursors, geranyl pyrophosphate and olivetol, are interpreted to give rise to *CBG* from which *CBD*, *THC*, and *CBC* are derived. These compounds occur principally as the carboxylated form ($R = COOH$) in the plant and are converted to the neutral form ($R = H$) on heating or drying of plant material.

It is possible that CBG, prior to its detection, was being formed and rapidly and preferentially converted only to CBC to the exclusion of THC. Our studies suggest that CBC is the first cannabinoid synthesized in seedlings, followed later in plant development by the synthesis of other cannabinoids.

Although THC is the dominant cannabinoid in adult plants of this strain, the seedling profile is significantly different. THC is initially present in the lowest quantities, yet equals or surpasses the CBG concentration by the time the seedlings are 6 days old. The changing ratio of THC to CBG is related to the presence of secondary leaves. Seedlings with long primary leaves tend to have large developed secondary leaves. Consequently, the higher THC:CBG content of secondary leaves influences cannabinoid quantities detected in seedlings with longer primary leaves. It is apparent that the cannabinoid profile is not static in nature but instead is developmentally dynamic. This recorded change in the pattern of cannabinoid biosynthesis during ontogeny may reflect evolutionary changes in the cannabinoid profile in *Cannabis* and explain in part the diversity of *Cannabis* strains, each with a different cannabinoid composition in the adult plant.

Primary leaf length was the most important criterion regulating cannabinoid concentration in seedlings of a specific age (VOGELMANN 1985; VOGELMANN et al. 1985). For both light- and dark-grown seedlings, increased quantities of cannabinoids correlated with increased leaf length within each age class. This difference in cannabinoid content may reflect a larger leaf surface with a greater number of differentiated glandular trichomes. Seedling age also was a major factor influencing cannabinoid content. As seedlings aged, cannabinoid quantities increased for seedlings of the same primary leaf length class. Thus, cannabinoid content was influenced by a combination of primary leaf length and seedling age.

A pattern of peaking and leveling off of cannabinoids was found in seedlings of approximately the same age despite differences in leaf sizes and cannabinoid quantities and large variation (SD) between equivalent samples. Physiological and presumably genetic differences among individual seedlings may be responsible for seedlings of the same age having different leaf sizes and amounts of cannabinoids. Light appeared to perform a temporal role in regulating the trends. Quantities of all cannabinoids in light-grown seedlings leveled off and peaked at the same age. Comparable quantities of cannabinoids and similar cannabinoid profile patterns were found in dark-grown seedlings, but they were delayed by the slowed development of these seedlings.

Cannabinoids were analyzed in the neutral form, although it is recognized that cannabinoids occur in vivo predominantly as their carboxylic acids (YAMAUCHI et al. 1967; KIMURA and OKAMOTO 1970; TURNER and MAHLBERG 1982). However, since the cannabinoid acids may be unstable after extraction and are readily converted to their neutral forms on heating or storage, most analyses refer to the neutral forms. It is unknown whether one pathway exists with neutral cannabinoids being formed via decarboxylation, or whether two parallel pathways exist, one for neutral and one for acid forms (MECHOULAM 1970). The seedling assay system can be used to investigate various aspects of cannabinoid biosynthesis and localization and to provide insight into the evolutionary dynamics of the cannabinoid pathway in *Cannabis*.

Acknowledgments

This research was supported with funds from the U.S. Department of Agriculture (53-32U44-234). DEA registration no. PI0043113. We acknowledge the assistance of GARY BADGER with the statistical analyses.

LITERATURE CITED

DEFAUBERT MAUNDER, M. J. 1970. A comparative evaluation of the Δ^9-tetrahydrocannabinol content of *Cannabis* plants. J. Assoc. Public Anal. **8**:42–47.

FAIRBAIRN, J. W., and J. A. LIEBMANN. 1974. The cannabinoid content of *Cannabis sativa* L. grown in England. J. Pharm. Pharmac. **26**:413–419.

FETTERMAN, P. S., E. S. KEITH, C. W. WALLER, O. GUERRERO, N. J. DOORENBOS, and M. W. QUIMBY. 1971. Mississippi-grown *Cannabis sativa* L.: preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinoid content versus age, sex and plant part. J. Pharm. Sci. **60**:1246–1249.

FURR, M., and P. G. MAHLBERG. 1981. Histochemical analyses of laticifers and glandular trichomes in *Cannabis sativa*. J. Nat. Prod. **44**:153–159.

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

KANTER, S. L., M. R. MUSUMECI, and L. E. HOLLISTER. 1979. Quantitative determination of Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid in marihuana by high-pressure liquid chromatography. J. Chromatogr. **171**:504–508.

KIMURA, M., and K. OKAMOTO. 1970. Distribution of tetrahydrocannabinolic acid in fresh wild *Cannabis*. Experientia **26**:819–820.

KUSHIMA, H., Y. SHOYAMA, and I. NISHIOKA. 1980. *Cannabis*. XII. Variations of cannabinoid contents in several strains of *Cannabis sativa* L. with leaf-age, season and sex. Chem. Pharm. Bull. **28**:594–598.

MAHLBERG, P. G., and J. K. HEMPHILL. 1983. Effect of light quality on cannabinoid content of *Cannabis sativa* L. (Cannabaceae). BOT. GAZ. **144**:43–48.

MALINGRE, T., H. HENDRIKS, S. BATTERMAN, R. BOS, and J. VISSER. 1975. The essential oil of *Cannabis sativa*. Planta Med. **28**:56–61.

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

NIE, N., C. HUL, J. JENKINS, K. STEINBRENNER, and D. BENT.

1975. Statistical package for the social sciences. 2d ed. McGraw-Hill, New York.

SHOYAMA, Y., T. FUJITA, T. YAMAUCHI, and I. NISHIOKA. 1968. Cannabichromenic acid, a genuine substance of cannabichromene. Chem. Pharm. Bull. **16**:1157–1158.

SHOYAMA, Y., M. YAGI, and I. NISHIOKA. 1975. Biosynthesis of cannabinoid acids. Phytochemistry **14**:2189–2192.

TURNER, C. E., M. A. ELSOHLY, and E. G. BOEREN. 1980. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J. Nat. Prod. **43**:169–234.

TURNER, C. E., M. A. ELSOHLY, P. C. CHENG, and G. LEVINE. 1979. Constituents of *Cannabis sativa* L. XIV. Intrinsic problems in classifying *Cannabis* based on a single cannabinoid analysis. J. Nat. Prod. **42**:317–319.

TURNER, J. C., J. K. HEMPHILL, and P. G. MAHLBERG. 1977. Gland distribution and cannabinoid content in clones of *Cannabis sativa* L. Am. J. Bot. **64**:687–693.

———. 1978. Quantitative determination of cannabinoids in individual glandular trichomes of *Cannabis sativa* L. (Cannabaceae). Am. J. Bot. **65**:1103–1106.

———. 1980. Trichomes and cannabinoid content of developing leaves and bracts of *Cannabis sativa* L. (Cannabaceae). Am. J. Bot. **67**:1397–1406.

TURNER, J. C., and P. G. MAHLBERG. 1982. Simple high-performance liquid chromatography method for separating acidic and neutral cannabinoids in *Cannabis sativa* L. J. Chromatogr. **253**:295–303.

———. 1984. Effects of sample treatment on chromatographic analysis of cannabinoids in *Cannabis sativa* L. (Cannabaceae). J. Chromatogr. **283**:165–171.

TURNER, J. C., P. G. MAHLBERG, V. S. LANYON, and J. PLESZCZYNSKA. 1985. A temporal study of cannabinoid composition in continual clones of *Cannabis sativa* L. (Cannabaceae). BOT. GAZ. **146**:32–38.

VOGELMANN, A. F. 1985. Studies on cannabinoid and glandular trichome occurrence in seedlings of *Cannabis sativa* L. (Cannabaceae). Ph.D. diss. Indiana University, Bloomington.

VOGELMANN, A. F., J. C. TURNER, and P. G. MAHLBERG. 1985. Sequential appearance of cannabinoids during seedling development in *Cannabis sativa* L. Pages 15–22 *in* D. J. HARVEY, ed. Marihuana '84. IRL Press, Oxford.

YAMAUCHI, T., Y. SHOYAMA, H. ARAMAKI, T. AZUMA, and I. NISHIOKA. 1967. Tetrahydrocannabinolic acid, a genuine substance of tetrahydrocannabinol. Chem. Pharm. Bull. **15**:1075–1076.