

Seasonal Fluctuations in Cannabinoid Content of Kansas Marijuana¹

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Marijuana (Cannabis sativa L.) was sampled at nine progressive growth stages in Riley County, Kansas, and analyzed for four major cannabinoids: cannabidiol (CBD), delta-8-tetrahydrocannabinol (delta-8-THC), delta-9-tetrahydrocannabinol (delta-9-THC), and cannabinol (CBN). Seasonal fluctuation in cannabinoids were related to stage of plant development. Cannabinoids were lowest in seedlings, highest prior to flowering and at an intermediate level thereafter until physiological maturity. Cannabinoids were highest in flowers and progressively lower in leaves, petioles, stems, seeds, and roots. Cannabinoid content of male and female flowers was not significantly different.

Cannabidiol occurred in the highest concentrations (0.01 to 0.94% of dry matter) in all plant parts; delta-9-THC, the next highest (0.0001 to 0.06%) in the study over time. Cannabidiol content of leaf tissue of plants sampled from ten locations at flowering, ranged from 0.12 to 1.7%; delta-9-THC, from 0.01 to 0.49%. Some variation was attributed to environmental factors.

Results indicate transformation of CBD to delta-9-THC to CBN. Environmental stress apparently increased delta-9-THC concentration, and bivalent ions: Mg, Mn, and Fe of leaf tissue could have regulated enzyme systems responsible for cannabinoid synthesis.

INTRODUCTION

Marijuana (*Cannabis sativa* L.), which produces hallucinogenic compounds, has long interested man, and in recent years its use has been associated with drug abuse (1,5). Delta-9-THC, the major hallucinogen in marijuana, was first synthesized *in vitro* in 1964 (9). Despite legal bans, marijuana use has increased sharply in the United States, prompting users to exploit domestic supplies; increased illicit collection in the midwestern states reflects that trend. Increased use encouraged research on the potency and genetic background of midwestern marijuana, but as yet we do not know whether cannabinoid production is controlled genetically or by environmental factors. Fetterman and coworkers (8), who analyzed marijuana from two states and five foreign countries, found a wide range of potency: they noted that growing progeny of those plants under a different environment (in Mississippi) did not alter potency. Similar results were obtained by Ohlsson and co-workers (19), indicating that potency is genetically controlled and that environmental inputs are secondary. Haney et al. (13) found that cannabinoid content of plants grown in Illinois increased with environmental stress (moisture stress, nutrient imbalances, and competition with other plants); the relative potency of those plants, however, was much lower than those of foreign origin (8). Krejci (14), in studies with antibiotic can-

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TABLE I
 MARIJUANA HEIGHT AND STAGE OF DEVELOPMENT AT 11
 SAMPLING DATES (9 PROGRESSIVE STAGES OF DEVELOPMENT)
 IN RILEY COUNTY, KANSAS, 1971.

Time Study		
Sampling date	Plant ht. (cm)	Progressive stages of development
1. 3/17/71	2	Seedling emergence
2. 4/01/71	3	Early seedling growth
3. 4/20/71	12	Advanced seedling growth
4. 5/17/71	75	Rapid vegetative growth
5. 6/02/71	112	Rapid vegetative growth
6. 6/18/71	160	Rapid vegetative growth
7. 7/06/71	186	Preflowering
8. 7/20/71	220	Early flowering
9. 8/03/71	255	Full bloom
10. 8/17/71	255	Seed set
11. 9/09/71	249	Physiological maturity

nabinoids in Czechoslovakia, reported stress responses similar to those of Haney et al. Weber (23) found that inadequate aeration severely stressed marijuana plants. Agrios (2) stated that stress from plant disease organisms could induce biochemical defense reactions in many plant species.

This study was conducted to detect seasonal fluctuations in cannabinoid content of wild marijuana in Kansas and to determine whether environmental inputs affect cannabinoid production.

METHODS AND MATERIALS

Riley County, Kansas, was chosen as the study area because it has abundant marijuana; this county, with an average elevation of 366 m, had 88.35 cm rainfall in 1971, 60% falling from March to September, and a 178-day frost-free growing season.

Plants were sampled from a natural stand throughout the growing season (Time Study); secondly, plants at flowering were sampled from 10 natural stands having different ecological and edaphic characteristics (Location Study).

Time Study. Marijuana plants from a single stand were sampled randomly at 11 dates (Table I) and analyzed for cannabinoids by gas liquid chromatography. We used a randomized complete block design with three replications. Plants emerged 3/8/71 and were sampled at approximately 14-day intervals from March 17 through September 9, 1971. Ten plants from each replication were sampled at all dates except the first four, when as many as 300 plants were required to provide sufficient quantity for analysis. Leaves, stems, roots, petioles, flowers and seeds were separated by hand as those parts developed. Plants were oven dried at 48°C for 24 hours, ground through a 2-mm, stainless-steel screen, and refrigerated at 5°C until analysis.

Location Study. Six plants were sampled randomly at flowering (7/19/71 to 8/6/71) from each of 10 locations with different ecological and edaphic characteristics. Procedures for separating, weighing, measuring, and storing plants were the same as for the time study.

Plant and Soil Nutrient Analysis. Soil samples were collected (0-10 cm depth) for each replication in the time study and for each site in the location study. Soil organic matter was determined according to Graham (11). Total soil nitrogen and nitrate were determined by micro-kjeldahl with steam distillation (6). Soil phosphorus was determined by Bray's weak acid test with 0.025 N HCL in 0.03 N ammonium flouride, 1:10 soil:solution ratio. Potassium was determined by 1 N ammonium acetate with 1:5 soil:solution ratio. All other soil cations were determined by atomic absorption spectroscopy, using the Colorado DTPA method.³ Zn was also examined using a 0.2 N HCL extract. Plant tissue was analyzed by atomic absorption spectroscopy, using the perchloric acid, wet-ashing method (10).

Cannabinoid Extraction. Four cannabinoids, believed to be the major components of the biochemical conversion, were examined: CBD, delta-8-THC, delta-9-THC, and CBN. Plant extraction was basically according to Lerner (15) and Fetterman (8). One gram of dry plant tissue was extracted in 40 ml of chloroform, shaken at 10-minute intervals for one hour, then refrigerated at 5°C. Plant tissue was removed by filtration and the solution evaporated to dryness in vacuo at 40°C. The residue was dissolved in 25 ml of 95% ethanol (in 5× 5-ml aliquots), filtered and evaporated. The remaining residue was dissolved in 1 ml of 95% ethanol containing 1.0 or 0.2 mg of 4-androstene-3, 17-dione, which was the internal standard.

Cannabinoid Analysis. Gas liquid chromatography was used for analysis (Bendix model 2200) and pure standards provided by the National Institute of Mental Health consisted of synthetic cannabidiol, (-)-delta-9-tetrahydrocannabinol, (-)-delta-8-tetrahydrocannabinol, and cannabinol. After extraction, plant samples were centrifuged (5 min. at 400 × G) to precipitate plant tissue not removed by previous filtrations. One microliter samples were injected into the column. Inlet temperature was 255°C. A glass column, 240 cm long and 2 mm-internal diameter, was packed with 2% OV-17 (phenyl methyl silicone on 100/120 mesh Gas Chrom) and operated at an isothermal temperature of 235°. Nitrogen carrier gas was used at a flow rate of 16 ml per minute. A flame ionization detector was used at 260°C. Signal response was recorded on tandem recorders set at different sensitivities to detect high or low concentrations for a single injection. For quantitative determinations, we measured peak height times width at half height. The peak area of each cannabinoid sample was compared with the peak area of the internal standard. Concentration then was determined by comparing the area ratio of internal standard to that of the pure references standards. We calibrated the chromatograph by averaging four one-microliter injections of each pure cannabinoid of known concentration and taking all sample values as percentage of each pure standard. Calibration was conducted before and after analysis to detect column changes.

Statistical Analysis. Data were examined by analysis of variance, simple correlation and multiple regression analysis. A correlation matrix was developed for all variables tested in both studies. Analysis of variance was used to test cannabinoids, plant elements, and plant growth factors in the time study; and to test plant growth factors in the location study. Multiple regression with stepwise deletion was used on 15 variables in the time study.

³Method of analysis outlined and used by the Colorado soil testing laboratory, Fort Collins, Colorado.

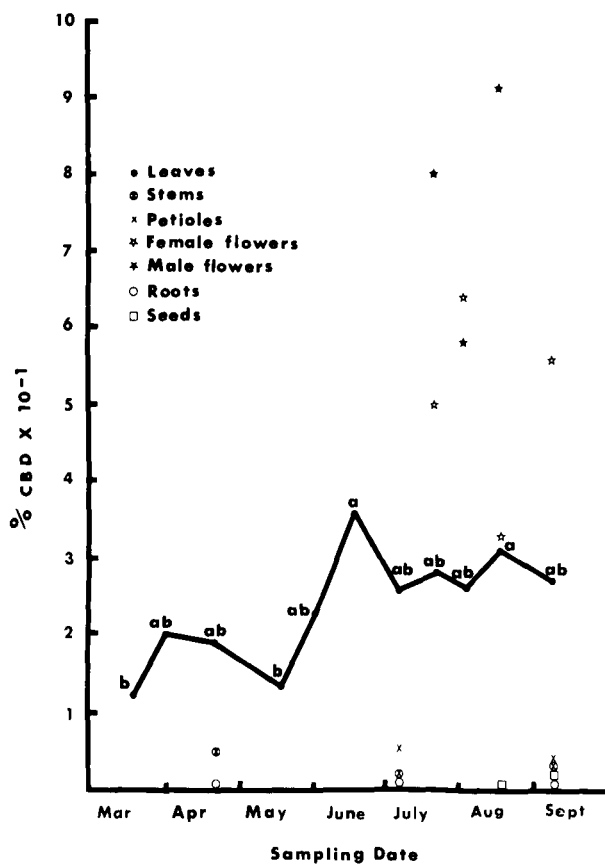


Fig. 1A. Seasonal changes in concentration of cannabidiol in seven indicated components of marijuana plants sampled in Riley County, Kansas, 1971. Data points for leaf tissue followed by the same letter do not differ significantly ($P \leq 0.05$, Duncan's new multiple range test). All data points are an average of 3 replications of 10 plants each.

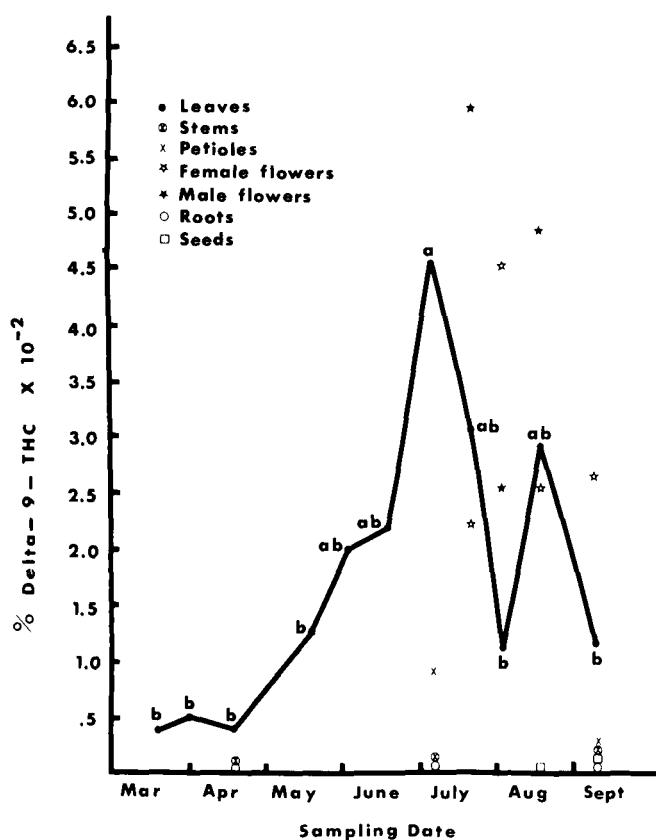


Fig. 1B. Seasonal changes in concentration of delta-9-THC in seven indicated components of marijuana plants sampled in Riley County, Kansas, 1971. Data points for leaf tissue followed by the same letter do not differ significantly ($P \leq 0.05$, Duncan's new multiple range test). All data points are an average of 3 replications of 10 plants each.

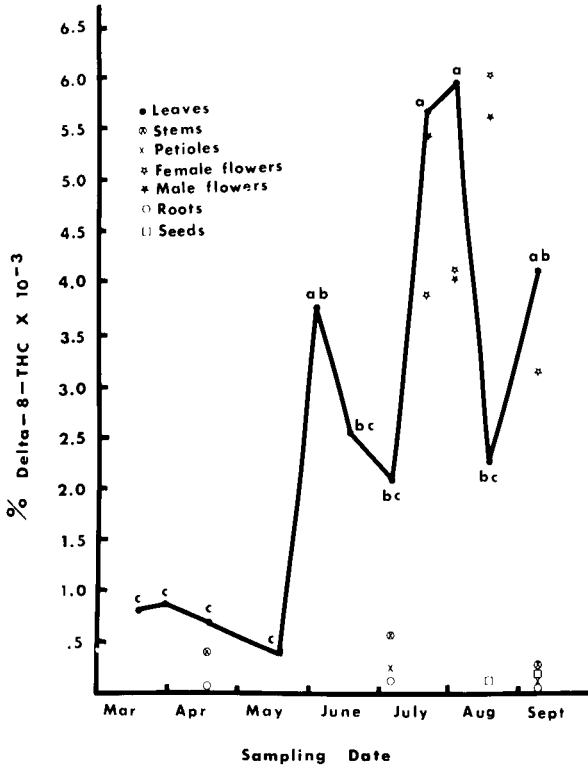


Fig. 1C. Seasonal changes in concentration of delta-8-THC in seven indicated components of marijuana plants sampled in Riley County, Kansas, 1971. Data points for leaf tissue followed by the same letter do not differ significantly ($P \leq 0.05$, Duncan's new multiple range test). All data points are an average of 3 replications of 10 plants each.

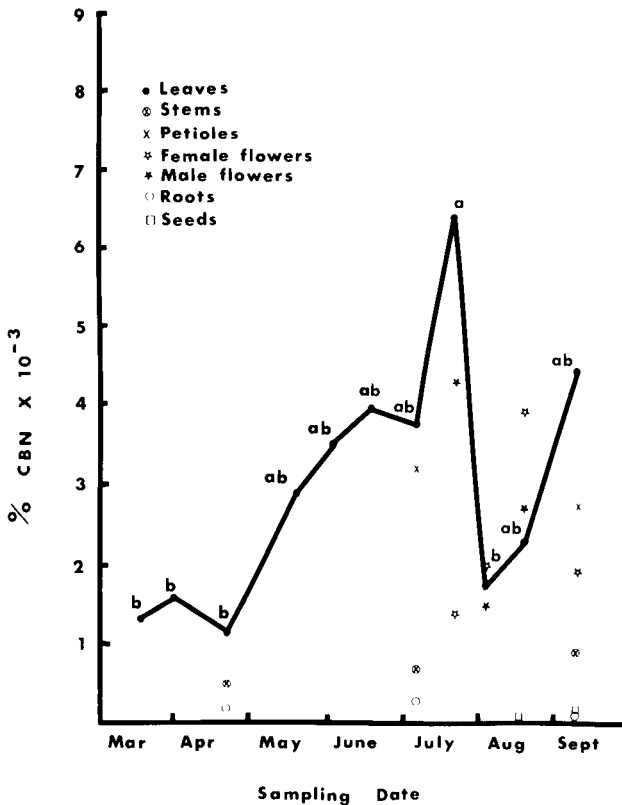


Fig. 1D. Seasonal changes in concentration of cannabiniol in seven indicated components of marijuana plants sampled in Riley County, Kansas, 1971. Data points for leaf tissue followed by the same letter do not differ significantly ($P \leq 0.05$, Duncan's new multiple range test). All data points are an average of 3 replications of 10 plants each.

CBD, CBN, delta-8-THC, and delta-9-THC were found in all plant parts at all growth stages examined (Fig. 1). Limitations on analysis did not permit examination of each plant part on all 11 sampling dates; we performed complete analysis on leaves, flowers, and seeds as those parts evolved in the time study. Roots, stems, and petioles were analyzed at early- and late-sampling dates to detect seasonal changes in cannabinoid concentration during the growing season. Cannabinoid biosynthesis apparently begins with seedling growth and continues until physiological maturity.

In the time study, CBD (ranging from 0.01 to 0.94% of plant dry matter) was 10 to 20 times higher than other cannabinoids in all plant parts (Fig. 1a). Between sampling dates, CBD content was higher and varied more in leaves and flowers than it did in stems, roots, petioles, or seeds. Male and female flowers contained approximately the same concentration of cannabinoid resins. Although seeds were rinsed in chloroform to remove cannabinoids from the exterior seed coat, some contamination from floral bracts may have occurred. CBD content of leaf tissue was lowest (0.12%) on March 17, highest (0.36%) on June 18, then relatively constant at an intermediate level from July to September (Fig. 1a).

The major hallucinogen, delta-9-THC, occurred in all plant parts and ranged from 0.0001 to 0.06% of plant dry matter (Fig. 1b) in the time study. Concentration was highest in flowers, leaves, petioles, stems, seeds, and roots, respectively (Fig. 1b). Plant parts containing the most delta-9-THC also contained the most CBD, but delta-9-THC concentrations were ten times lower than CBD in all plant parts. Delta-9-THC and CBD in leaf tissue exhibited similar seasonal changes, except that delta-9-THC fluctuations came about two weeks later than those of CBD and had the lowest concentration (0.004%) in mid March and the highest (0.046%) in early July.

Delta-8-THC and CBN occurred in approximately the same range of concentration (0.00005 to 0.0064%), which was about a tenth that of delta-9-THC and about one-hundredth that of CBD. Delta-8-THC (Fig. 1c) varied more than did CBN (Fig. 1d), and both varied most late in the growing season. Delta-9-THC, lowest (0.0004%) in mid May, climbed to its highest level (0.006%) in early August. CBN was lowest (0.001%) in late April, highest (0.0065%) in late July which was about two weeks after delta-9-THC had reached its highest concentration.

Several researchers, (7,12,16), proposing the biosynthetic pathway of the major cannabinoids, generally concluded that CBD is the precursor of delta-9-THC, which is converted to the CBN. The fact that CBD, in the time study, was highest in concentration about two weeks prior to the highest concentration of delta-9-THC, followed by the highest concentration of CBN two weeks later, indicates conversion from CBD to THC to CBN. Phillips et al. (21) found similar results with wild marijuana in Indiana. The decreasing concentration we observed, by about 10 fold, of each cannabinoid suggests these conversions are inefficient.

Seasonal fluctuations in cannabinoid content and differences among various plant parts emphasize the need to clearly define samples used for comparisons in future studies. The proportion of plant parts, stage of development, time of collection as well as origin of seed could profoundly affect the interpretation of comparisons. We hope the chemical profile presented here will be of benefit in future studies.

Cannabinoid content of plants was highly variable within sampling dates. Delta-9-THC was consistently higher (three times greater) in the third repli-

TABLE II
FIFTEEN INDEPENDENT VARIABLES EXAMINED BY MULTIPLE
REGRESSION FOR PLANTS SAMPLED ON 11 DATES AND IN NINE
PROGRESSIVE STAGES OF DEVELOPMENT FROM ONE LOCATION
IN RILEY COUNTY, KANSAS, 1971.

Time Study

Stage of plant development
Plant density
Plant height
Root length
Fresh weight
Dry weight
Root weight
Stem weight
Leaf weight
Plant manganese
Plant zinc
Plant iron
Plant copper
Plant magnesium
Plant calcium

cation of the time study, suggesting environmental variables may have caused a significant difference in THC at all sampling dates. All plants in the time study were probably from a homogeneous genetic base, because they were from the same stand and were relatively close together; the species is wind pollinated and all plants flowered at about the same time. Also, the stand had persisted at the same site for at least 10 years. Soil analysis indicated no significant differences in macro- or micro-nutrients among replications. The third replication, however, had a higher stand density, significantly shorter plants, more male plants, and longer exposure to sunlight than other replications; it also produced less biomass. These observations indicate that plant growth proceeded under greater stress than in other replications. Several workers have suggested stress conditions may cause an increase in cannabinoid content (13,14) and stress can induce biochemical defense reactions in some plant species (2).

Data were sufficient to examine 15 independent variables (Table II) in an attempt to elucidate factors responsible for the divergent drug content among replications in the time study. Regression analysis indicated that the combination of plant height, fresh weight, root weight, and leaf weight contributed approximately 50% ($R^2 = 0.497$) to the variance of CBD found in leaf tissue; the effect of these variables was positive and significant ($P < 0.05$). These variables were all growth indicators demonstrating that drug content generally increased as stage of development progressed. Not all growth variables tested were significant, so factors affecting these particular variables may be important in regulating CBD production.

Delta-8-THC was correlated to more variables than were other cannabinoids. Significant ($P < 0.05$) variables were: stage of plant development, plant density, root length, fresh weight, dry weight, stem weight, leaf weight, plant iron and plant copper. These nine variables accounted for 78% ($R^2 = 0.780$) of the variance in delta-8-THC and were positively correlated,

TABLE III

CONCENTRATION OF CANNABIDIOL, DELTA-9-THC, DELTA-8-THC, AND CANNABINOL IN LEAF TISSUE OF MARIJUANA PLANTS AT FLOWERING SAMPLED FROM 10 LOCATIONS IN RILEY COUNTY, KANSAS, 1971. VALUES ARE PERCENTAGES OF DRY WEIGHT.

Location Study				
Location	Cannabidiol	delta-9-THC	delta-8-THC	Cannabinol
1	0.3238	.0123	.0015	.0010
2	0.2958	.0270	.0018	.0040
3	0.5382	.1353	.0053	.0164
4*	0.1226	.0135	.0004	.0029
5	0.9645	.1662	.0085	.0034
6	0.7453	.4900	.0059	.0062
7	1.7148	.0766	.0107	.0066
8	1.0082	.1677	.0097	.0095
9	1.1520	.2327	.0107	.0065
10	0.4641	.1369	.0079	.0066

*Time Study location.

with the exceptions of plant density, plant iron, and plant copper, which were negatively correlated. The negative correlation with plant density was most likely due to plant competition. Early in the growing season there were as many as 643 plants/m²; as growth progressed, competition for sunlight, moisture, and nutrients decreased density to about 127 plants/m² late in the growing season. Negative correlations of delta-8-THC content with plant iron and copper may be because these micronutrients decrease per unit of dry matter as stage of development progresses to maturity. The remaining variables were positive and were growth indicators so factors responsible for their development could, as suggested for CBD, regulate delta-8-THC production.

Root length and plant fresh weight were significantly ($P < 0.05$) and positively correlated with delta-9-THC. These two variables accounted for 27% ($R^2 = 0.274$) of the variance of delta-9-THC. CBN was significantly ($P < 0.05$) and positively affected by plant height and root weight; these variables contributed 23% ($R = 0.228$) of the variance. Root weight or length was correlated with each cannabinoid tested. Nitrogen, phosphorus, and zinc are known to affect root growth. Deficiency of these nutrients can suppress root development, zinc showing the most suppressing effect (20). Nitrogen deficiency generally reduces root branching but stimulates elongation (4). Phosphorus affects root growth more indirectly by reducing top growth; less carbohydrate is photosynthesized, which reduces root growth (18,22). Hence nitrogen, phosphorus, and zinc (along with other factors affecting root growth) could influence cannabinoid biosynthesis.

Marijuana sampled from 10 locations, having a wide range of ecological and edaphic characteristics, illustrates cannabinoid variability in Riley County (Table III). These data place the cannabinoid concentration of time study (Fig. 1) in perspective with cannabinoid content of marijuana found throughout the county (Table III). Location four, the ninth sampling date in the time study, ranked lowest in CBD, ninth in delta-9-THC, lowest in delta-8-THC, and ninth in CBN when compared to the other 9 locations. By random chance, we extensively studied the location where plants were among the lowest in cannabinoid concentration (Fig. 1).

Delta-9-THC ranged from 0.012% to 0.49% and generally increased as locations became less favorable for plant growth, suggesting increased plant stress enhanced delta-9-THC production (Table III). A positive and significant correlation ($r = 0.677$) with associated vegetation and delta-9-THC indicates that competition from associated plants increased delta-9-THC concentration. Plants were sampled at a time in the growing season when moisture was not limiting. Greater difference among locations might have been observed under drought conditions.

Magnesium and iron content in leaf tissue were positively and significantly correlated ($r = 0.662$ and 0.697 respectively) with delta-8-THC in the location study. These elements could serve as cofactors in enzyme(s) responsible for delta-8-THC production. A positive and highly significant correlation ($r = 0.797$) was also found with manganese and CBN. This ion, as the bivalent ions previously mentioned, may serve as a cofactor for enzyme biosynthesis of CBN. However, the level required for micronutrients to function in this capacity may be sufficiently low as to mask their operation. Since micronutrient levels encountered appeared not to be limiting, deficiencies induced in growth chamber studies would be required to further evaluate this possibility.

Researchers generally agree that marijuana falls into two categories: (1) drug types and (2) non-drug types (8,13). Ratios of various cannabinoids proposed to chemically segregate drug types, indicate marijuana in these experiments were of the non-drug type. Marijuana high in CBD and low in THC is characteristic of the non-drug type; the drug type is low in CBD, high in THC. The difference may be due to the efficiency of the drug type to convert CBD to THC. Enzyme systems in the drug type may convert CBD to delta-9-THC, but such systems may not be present (or operative at low efficiency) in non-drug types. Higher drug content in replication three in the time study could indicate that factors in that replication were conducive for the operation of an enzyme system.

Multiple regression analysis indicates that plant growth factors significantly influenced all cannabinoids examined. This suggests that soil fertility and other factors influencing growth and development also influence cannabinoid biosynthesis. Fewer factors were correlated with delta-9-THC, and CBN, indicating conversion from their respective precursor is spontaneous or is both spontaneous and enzyme-catalyzed, depending on the more favorable energy scheme.

CONCLUSIONS

Seasonal changes observed in cannabinoids indicate CBD is transformed to delta-9-THC to CBN. Data suggest that stress may influence cannabinoid production and bivalent ions may regulate enzyme systems responsible for cannabinoid synthesis. Seasonal variability of cannabinoids in plants was observed in Riley County. Potential cannabinoid content of marijuana appears to be genetically controlled, but the level of expression may be regulated by environmental factors regulating plant growth and development. Marijuana growing wild in Kansas is low in potency. Midwestern marijuana, descended from varieties cultivated for fiber and cannabinoid level, apparently has remained unchanged by natural selection.

ACKNOWLEDGMENTS

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Book Reviews (continued from page 152)

Ethnomedizin, Zeitschrift für Interdisziplinäre Forschung: Ethnomedicine, Journal for Interdisciplinary Research. Vol. 1 +, 1971 +. Helmut Buske Verlag, D2000 Hamburg 13. Per volume, DM60 "plus postal and bank expenses."

This journal is designed to lead to "closer relationships between medicine, physics, pharmacology, zoology, botany, and geography, on one side and the anthropological sciences, on the other. Major topics are: primitive medicine and natural history, ethnomedicine, ethnobotany, ethnozoology, ethnopharmacology, early and primitive geography, astronomy, and meteorology." Furthermore, *Ethnomedizin* is concerned also with subjects related to health and environment, such as "anthropogeography, human ecology, geomedicine, sociology of medicine, social psychology and social hygiene, health service in the countries of the Third World, [and] demography." "Its contributions center upon the non-academic healing arts (ethnomedicine or folk medicine) and the sociological and ecological aspects of medicine." *Ethnomedizin* is published irregularly; each volume contains two to four numbers and 450-500 pages. Its contributions are almost all in either German or English; French appears only rarely. Besides articles, it contains book and journal reviews. The most recent issue received by *Economic Botany* is volume 2, number 3/4, 1973.

At first, the coverage of *Ethnomedizin* seems a little confusing, but the possibility exists that one or the other contribution could stimulate serious research in a particular field that might benefit all mankind. Not infrequently, indications from folk medicine lead to discovery of potent drugs used now all over the world, e.g., rauwolfia alkaloids. Scientists looking for problems on the borderline between modern science and folk medicine will be challenged by the

many topics discussed in the various papers. The reading of some of these papers might be only an interesting way to pass the time, but the reading of others might be the spark for a series of worthwhile investigations. Mankind intends to explore other planets of our solar system, but we have not yet explored all the resources of our own earth. *Ethnomedizin* might well be an excellent aid in those aspects of terrestrial exploration it encourages.

G. RITSCHER

Northern Kentucky State College
Highland Heights, Kentucky

Woody Plants of the North Central Plains. H. A. Stephens. xxx + 530 pp. illus. University of Kansas Press, Lawrence, 1973. \$20.00

Doubtless this sizable book comes as a surprise to many who consider the North Central Plains as a place through which to hurry when travelling from the eastern deciduous forest to the forested mountains of the west. Stephens, however, shows it to be an area of prairie but with a larger amount of forest cover that most people fail to observe.

The book includes 255 species of woody or partially woody plants that grow in North Dakota, South Dakota, Nebraska, and Kansas. In the introduction the author clearly and concisely explains, among other ecological aspects, the migration of ligneous plants into the North Central Plains. He devotes 15 pages to keys for identification of the species. Most of the book, 510 pages, contains descriptions and illustrations. For each species, Stephens supplies the scientific name, the synonym, and the common name or names. He gives detailed descriptions of leaves, flowers or strobiles, fruit or cones, twigs, and trunk and statements of habitat and range. He usually follows this with information on economic importance, taxonomic varieties,

Book Reviews (continued on page 170)