



---

Greenhouse Propagation of *Cannabis sativa* L. by Vegetative Cuttings

Author(s): C. B. Coffman and W. A. Gentner

Source: *Economic Botany*, Vol. 33, No. 2 (Apr. - Jun., 1979), pp. 124-127

Published by: Springer on behalf of New York Botanical Garden Press

Stable URL: <http://www.jstor.org/stable/4254038>

Accessed: 23/01/2010 23:51

---

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/action/showPublisher?publisherCode=nybg>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).



Springer and New York Botanical Garden Press are collaborating with JSTOR to digitize, preserve and extend access to *Economic Botany*.

## GREENHOUSE PROPAGATION OF CANNABIS SATIVA L. BY VEGETATIVE CUTTINGS

C. B. COFFMAN AND W. A. GENTNER<sup>1</sup>

Previous work revealed significant variations in cannabinoid profiles of *Cannabis sativa* L. derived from a single seed source (P.I. 378939) and subjected to the same growth environment. Studies were conducted to evaluate the efficacy of propagation of *C. sativa* by vegetative cuttings in order to increase uniformity of cannabinoid concentrations within a given plant population. *C. sativa* was successfully propagated by vegetative cuttings. However, there were both morphological and biochemical differences between seed-derived plants and their vegetative propagules. Delta-<sup>9</sup>-tetrahydrocannabinol concentrations were 4.1 times higher in vegetative propagules than in seed propagules. Vegetative cuttings also generally developed more profuse lateral branch growth; hence, foliage increased relative to their parent plants. Cannabinoid levels within the population of vegetative cuttings remained highly variable.

*Cannabis sativa* L. plants that had been propagated from Afghan seed (P.I. 378939) and subjected to different growth environments exhibited nearly as much biochemical variation within treatments as among treatments (Coffman & Gentner, 1975; 1977). Vegetative propagation methods have been successfully used for development of stability of genetic expression in various horticultural and forest species, as well as cotton (Stassen, 1968; Mansour, 1968) and flax (Dorrell, 1974). Here we describe the effect of vegetative propagation on growth and biochemistry of *C. sativa*.

### MATERIALS AND METHODS

The initial experiment was designed to evaluate the feasibility of vegetatively propagating *C. sativa*. Seeds were sown in soil in 12-cm pots. Pots were placed on greenhouse benches and seedlings were thinned to one plant per pot several weeks after germination. Plants were grown from October to December under ambient photoperiods and watered when needed. Plants were 45 days old and not anthesic when vegetative cuttings were obtained. Terminal cuttings approximately 12 cm long and including the first and second obvious node below the terminal bud were made. Cuttings were made in air from growing, healthy plants. Cuttings were immediately placed in previously washed perlite contained in a mist chamber. The perlite medium was watered several times daily and the mist applicator was activated each half hour for a 15-minute duration. Cuttings developed roots within ten days and were transferred to soil-filled pots, one plant per pot. Potted cuttings were kept in the mist chamber several days before transferal to greenhouse benches. Cuttings grew from February until April under ambient photoperiods. Above-ground portions of the non-anthesic cuttings were harvested, stems were discarded, and leaf tissues were oven-dried and stored before cannabinoid extraction and analyses.

In a second study, seed-derived plants were grown 40 days in the greenhouse during November and December. Cuttings were made and propagated as in the previous study. Forty-nine days after root initiation, the vegetative propagules were anthesic. Leaf tissues were harvested, dried, and stored as in the previous study.

---

<sup>1</sup> Weed Science Laboratory, Agricultural Environmental Quality Institute, USDA, Beltsville, MD 20705.

Submitted for publication February 27, 1978; accepted for publication June 1, 1978.

In both studies, leaf tissues from seed-grown plants were harvested when vegetative cuttings were made. Analytical methods for detection of cannabidiol (CBD) and  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ THC) were those reported by Coffman & Gentner (1975).

#### RESULTS AND DISCUSSION

*Plant morphology.*—Vegetative cuttings in both studies developed into plants dissimilar to their parents in the following ways: petioles and stems displayed red pigmentation (perhaps anthocyanin); leaves were alternate, leaves of parent plants were opposite when cuttings were made; in many instances leaves had only one or an even number (2, 4, 6 . . .) of leaflets; lateral branches developed and were comparable in both height and leafiness to the main stem, parent plants had no lateral branch development; and meristematic growth of the terminal bud produced a loosely plicate pattern of elongation instead of the linear pattern of elongation associated with plants derived from seed. Vegetative propagules of the first study tended to have simple leaves and to lose their lower leaves, whereas plants of the second study had compound leaves (with even or odd numbers of leaflets) and did not display lower leaf abscission. Vegetative propagules obtained in both studies grew from late winter to early spring when daily photoperiods were lengthening. Seed-derived parents grew from late fall to early winter when daily photoperiods were diminishing. It was improbable that this environmental difference *per se* resulted in the observed morphological variations, based on observations since 1972 of seed-derived cannabis plants grown year-around in greenhouses at Beltsville. Morphological expressions herein attributed to vegetative propagules have not been observed on non-stressed, seed-derived plants grown under ambient photoperiod conditions from January through April.

Morphological characteristics of vegetatively propagated plants probably developed in response to stresses created by the propagation method. These stresses were initially manifested by decreased turgidity of the cuttings in the mist chamber. Organization of physiological processes required to initiate root development probably created various biochemical imbalances that were manifested in the plant morphology. Meyer & Anderson (1959, p. 566) described development of morphological characteristics similar to those reported herein, when various plant species were stressed with applications of auxins at high concentrations. Tissue discolorations also have been correlated with moisture and temperature stresses and related starch-sugar transformations (Meyer & Anderson, 1959, p. 389). Thus hormonal imbalances and/or carbohydrate transformations that likely resulted from stresses of the propagation process may have contributed to the unique morphology manifested by the vegetative propagules.

*Cannabinoid Profile.*—Mean CBD concentrations for the first study were 1,369 ppm and 4,083 ppm for seed and vegetative propagules, respectively. The average CRF value for CBD concentrations showed nearly a 4-fold increase in vegetative propagules relative to the seed propagules (Table I). CRF is calculated as follows:

$$\text{CRF (cannabinoid ratio factor)} = \frac{\text{[cannabinoid] in marihuana of vegetative propagules}}{\text{[cannabinoid] in marihuana of seed propagules}}$$

Delta-<sup>9</sup>-THC consistently had a CRF value greater than one for each parent-offspring comparison (Table I). Concentrations of this psychoactive compound ranged from 689 ppm to 6,025 ppm and 3,125 ppm to 17,835 ppm for seed and

TABLE I

CANNABINOID CONCENTRATIONS PER WEIGHT OF DRIED PLANT TISSUE AND CANNABINOID RATIO FACTORS (CRF<sup>a</sup>) FOR LEAF TISSUES FROM SEED-DERIVED *Cannabis* PLANTS AND THEIR VEGETATIVELY-PROPAGATED DESCENDANTS, ALL CONSTITUTING THE FIRST STUDY

Specimen number	Seed		Vegetative		CRF	
	CBD <sup>b</sup>	$\Delta^9$ THC <sup>b</sup>	CBD	$\Delta^9$ THC	CBD	$\Delta^9$ THC
1	1,570	3,291	1,020	10,780	0.6	3.3
2	620	3,690	7,450	6,150	12.0	1.7
3	1,568	1,210	6,970	5,580	4.4	4.6
4	330	2,210	1,265	17,835	3.8	8.1
5	295	6,025	345	14,245	1.2	2.4
6	1,405	1,865	7,131	5,749	5.1	3.1
7	1,980	1,350	4,890	3,125	2.5	2.3
8	2,181	689	5,530	5,110	2.5	7.4
9	1,118	1,198	5,897	5,032	5.3	4.2
10	2,622	1,878	330	7,325	0.1	3.9
$\bar{x}$	1,369	2,341	4,083	8,093	3.9	4.1

<sup>a</sup> CRF (cannabinoid ratio factor) =  $\frac{[\text{cannabinoid}] \text{ in marihuana of vegetative propagules.}}{[\text{cannabinoid}] \text{ in marihuana of seed-derived plants}}$

<sup>b</sup> CBD (cannabidiol);  $\Delta^9$ THC ( $\Delta^9$ -tetrahydrocannabinol); expressed in parts per million (ppm).

vegetative propagules, respectively. Mean concentrations of  $\Delta^9$ THC were nearly four times greater in the vegetative than in the seed propagules.

Similarly, both CBD and  $\Delta^9$ THC concentrations in plants of the second study were higher in the vegetative propagules than in the seed propagules (Table II). CRF values for  $\Delta^9$ THC were greater than one for each parent-offspring comparison. Mean CRF values for CBD and  $\Delta^9$ THC were 4.7 and 2.6, respectively. Thus in both experiments, cannabinoid concentrations were higher in vegetatively-propagated progeny than in their seed-propagated parents.

There were no statistically significant differences between CBD or  $\Delta^9$ THC concentrations either within or between propagation methods for both studies. Differences in cannabinoid concentrations between parents and offspring plants were offset by the variability within the population samples. There were no significant correlations between cannabinoid concentrations of seed and vegetatively

TABLE II

CANNABINOID CONCENTRATIONS PER WEIGHT OF DRIED PLANT TISSUE AND CANNABINOID RATIO FACTORS (CRF<sup>a</sup>) FOR LEAF TISSUES FROM SEED-DERIVED *Cannabis* PLANTS AND THEIR VEGETATIVELY PROPAGATED DESCENDANTS, ALL CONSTITUTING THE SECOND STUDY

Specimen number	Seed		Vegetative		CRF	
	CBD <sup>b</sup>	$\Delta^9$ THC <sup>b</sup>	CBD	$\Delta^9$ THC	CBD	$\Delta^9$ THC
1	374	3,612	1,123	6,774	3.0	1.9
2	290	2,536	3,983	12,815	13.7	5.0
3	2,420	3,769	991	4,898	0.4	1.3
4	1,586	5,322	2,646	11,560	1.7	2.2
$\bar{x}$	1,168	3,810	2,186	9,012	4.7	2.6

<sup>a</sup> CRF (cannabinoid ratio factor) =  $\frac{[\text{cannabinoid}] \text{ in marihuana of vegetative propagules.}}{[\text{cannabinoid}] \text{ in marihuana of seed derived plants}}$

<sup>b</sup> CBD (cannabidiol);  $\Delta^9$ THC ( $\Delta^9$ -tetrahydrocannabinol); expressed as parts per million (ppm).

propagated plants. Mean coefficients of variation (CV) between parent and offspring plants were 78 and 52% for  $\Delta^9$ THC concentrations in experiments 1 and 2, respectively. Mean CV for CBD concentrations were 76 and 72% for the first and second studies, respectively. Photoperiod variations discussed above may have contributed to observed cannabinoid differences. Spectral intensities of radiation received by growing plants also varied as photoperiods varied. Thus the spectral nature and photoperiod differential during plant growth, as well as the photoperiod characteristics at time of plant harvest, may have influenced cannabinoid concentrations of plants used in these studies. Turner et al. (1975) reported variation in cannabinoid concentrations of a Mexican variant as a function of time of day and plant age at sampling.

Another factor that probably contributed to general differences between seed and vegetative propagules was trauma experienced by the cuttings as a result of the vegetative propagation procedure. The physical and physiological violation of the plants subjected to the vegetative propagation procedure probably manifested itself both morphologically and biochemically. Increased cannabinoid concentrations of vegetative propagules relative to seed propagules may have resulted, in part, from stresses of the procedure. Previous studies in which *Cannabis* plants were subjected to various mineral element stresses resulted in increased concentrations of  $\Delta^9$ THC in leaf tissues (Coffman & Gentner, 1975; 1977).

In summation, *C. sativa* was vegetatively propagated and the propagules differed both morphologically and biochemically from their seed-propagated parents. These differences were expressed as increased lateral branch development, various leaf modifications, and increased cannabinoid concentrations. It was speculated that observed responses were due to differences in the growth environment of the seed and vegetative propagules plus stresses that resulted from the vegetative propagation procedure.

#### LITERATURE CITED

- Coffman, C. B. & W. A. Gentner. 1975. Cannabinoid profile and elemental uptake of *Cannabis sativa* L. as influenced by soil characteristics. *Agron. J.* 67: 491.
- & ———. 1977. Responses of greenhouse-grown *Cannabis sativa* L. to nitrogen, phosphorous, and potassium. *Agron. J.* 69.
- Dorrell, D. G. 1974. Vegetative propagation of flax by stem cuttings. *Can. J. Plant Sci.* 54: 197.
- Mansour, A. H. 1968. About the intent offered by propagation by cutting under maximum hygroscopicity in cotton genetics. *Coton et Fibres Tropicales* 23: 459.
- Meyer, B. S. & D. B. Anderson. 1959. *Plant Physiology*. D. van Nostrand Co., Inc., Princeton.
- Stassen, J. P. 1968. Vegetative propagation of cotton. *Farming in S. Africa* 44: 41.
- Turner, C. E., P. S. Fetterman, K. W. Hadley & J. E. Urbanek. 1975. Constituent of *Cannabis sativa* L. X. Cannabinoid profile of a Mexican variant and its possible correlation to pharmacological activity. *Acta Pharm. Jugoslav.* 25: 7.