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UV-B RADIATION EFFECTS ON PHOTOSYNTHESIS, GROWTH AND CANNABINOID PRODUCTION OF TWO *Cannabis sativa* CHEMOTYPES

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Abstract—The effects of UV-B radiation on photosynthesis, growth and cannabinoid production of two greenhouse-grown *C. sativa* chemotypes (drug and fiber) were assessed. Terminal meristems of vegetative and reproductive tissues were irradiated for 40 days at a daily dose of 0, 6.7 or 13.4 kJ m^{-2} biologically effective UV-B radiation. Infrared gas analysis was used to measure the physiological response of mature leaves, whereas gas–liquid chromatography was used to determine the concentration of cannabinoids in leaf and floral tissue.

There were no significant physiological or morphological differences among UV-B treatments in either drug- or fiber-type plants. The concentration of Δ^9-tetrahydrocannabinol (Δ^9-THC), but not of other cannabinoids, in both leaf and floral tissues increased with UV-B dose in drug-type plants. None of the cannabinoids in fiber-type plants were affected by UV-B radiation.

The increased levels of Δ^9-THC in leaves after irradiation may account for the physiological and morphological tolerance to UV-B radiation in the drug-type plants. However, fiber plants showed no comparable change in the level of cannabidiol (a cannabinoid with UV-B absorptive characteristics similar to Δ^9 THC). Thus the contribution of cannabinoids as selective UV-B filters in *C. sativa* is equivocal.

INTRODUCTION

Cannabis sativa L. contains a number of secondary metabolites (cannabinoids) that are species specific. Although the biosynthetic pathway of the major cannabinoids has been established (Mechoulam, 1970; Shoyama *et al.*, 1984), the regulation of cannabinoid production remains unsolved. Some researchers contend that cannabinoid production is genetically controlled and uninfluenced by environmental factors (Doorenbos *et al.*, 1971; Fetterman *et al.*, 1971). Other studies indicate that although the primary control appears to be genetic, cannabinoid production can be modified by an environmental component (Haney and Kutscheid, 1973; Latta and Eaton, 1975; Turner *et al.*, 1982).

Although the mechanism is unknown, a relationship exists between cannabinoid content and the altitude at which *C. sativa* is grown. Mobark *et al.* (1978) suggested that the high-altitude environment was responsible for an increased production of propyl cannabinoids in plants grown at 1300 m. The average total cannabinoid content of wild, mature (flowering) Indian *C. sativa* from elevations between 250 m and 1000 m was 2.43% (by dry wt); between 1000 m and 2000 m was 3.01%; and above 2000 m was 1.39% (Turner *et al.*, 1979). The cannabinoid content in four out of five of these mature Indian *C. sativa* variants decreased when grown at sea level in Mississippi, USA.

One likely factor which may be of significance to cannabinoid production in both high-altitude and tropical environments is ultraviolet radiation. Measurements made in Utah showed that the biologically effective ultraviolet-B radiation flux ($UV\text{-}B_{BE}$ 280–320 nm, weighted with a generalized plant response function, Caldwell, 1971) at 3350 m is 32% greater than at 1500 m (Caldwell *et al.*, 1980). Latitudinal variations of solar UV-B radiation are also considerable. Numerous studies have shown the deleterious effects of UV radiation on many unrelated plant species (for reviews see Caldwell, 1971; Klein, 1978; Teramura, 1983). Tolerance to UV-B radiation in some plants has been attributed to their ability to produce secondary metabolites, such as flavonoids, which absorb and prevent actinic UV-B radiation from penetrating plant tissues (Caldwell *et al.*, 1983; Flint *et al.*, 1985). Cannabinoids in fresh plant tissue are in the form of acids (Doorenbos *et al.*, 1971) that strongly absorb UV-B radiation, making these compounds likely candidates as solar screens.

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Abbreviations: *A*, CO_2 assimilation; CA, cellulose acetate; C_a, ambient CO_2; CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiolic acid; CBG, cannabigerol; CBN, cannabinol; CBNA, cannabinolic acid; DW, dry weight; *E*, transpirational water loss; g_s, leaf conductance to water vapor; PPFD, photosynthetic photon flux density between 400–700 nm; R_d, dark respiration; Δ^8-THC, Δ^8-tetrahydrocannabinol; Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^8-THCA, Δ^8-tetrahydrocannabinolic acid; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid; UV-B, radiation between 280–320 nm; $UV\text{-}B_{BE}$, biologically effective UV-B; VPD, vapor pressure deficit.

Pate (1983) reported that *C. sativa* populations originating from high UV-B environments contained little or no cannabidiol (CBD) but high levels of Δ^9-tetrahydrocannabinol (Δ^9-THC), while the opposite was true for populations from low UV-B environments, and proposed that the two distinct *C. sativa* chemotypes (drug and fiber) evolved as a result of selective pressures brought about by UV-B radiation.

Fairbairn and Liebmann (1974) reported that the Δ^9-THC content of leaf tissue from UV irradiated greenhouse-grown drug-type *C. sativa* was 23% greater than non-irradiated greenhouse-grown plants. However, neither the spectral distribution nor the daily dose of UV radiation were reported.

The objectives of this study were to test: (a) The physiological and morphological insensitivity of both the drug- and fiber-types of *C. sativa* to UV-B radiation; and (b) to correlate this insensitivity with a change in production of Δ^9-THC or CBD in drug- and fiber-type plants, respectively.

MATERIALS AND METHODS

Two populations of pistillate *Cannabis sativa* L., one a high Δ^9-THC producing drug-type of Jamaican origin and the other a high CBD producing fiber-type of Czechoslovakian origin were cloned from vegetative cuttings as described by Coffman and Gentner (1979) in a greenhouse located at USDA, Beltsville, MD. Rooted cuttings were transferred to 0.6-ℓ plastic pots containing Metromix 300 potting mixture (composed of composted bark, peat moss, vermiculite, perlite, granite, and sand) and watered daily, alternating between tap water and a dilute solution of Peters 20–20–20 general purpose fertilizer providing 7.3 mmol N, 0.7 mmol P and 1.0 mmol K per pot. To prevent root binding, approximately every two months plants were transferred to successively larger pots with an additional 0.3-ℓ vol. Vegetative or flowering plants were exposed to UV-B radiation for 30 days prior to the start of the experiment. At that time, plants were trimmed to a uniform height. Vegetative and floral meristems developed while plants were irradiated for an additional 40 days.

UV-B radiation was suplied by filtered Westinghouse FS-40 sunlamps as described by Mirecki and Teramura (1984). All lamps were fitted with either presolarized 0.08 or 0.13 mm cellulose acetate (UV-B transmitting) or 0.13 mm Mylar Type S (control) filters. Treatments were separated by 0.26 mm Mylar shields. Individual plant heights were adjusted to maintain a uniform canopy, while the lamp/plant distance was maintained at 0.25 m (0.08 mm CA filtered lamps) or 0.35 m (0.13 mm CA and Mylar filtered lamps). The spectral irradiance was determined with an Optronic Laboratories, Inc. Model 742 Spectroradiometer interfaced with a Hewlett Packard 85 printing calculator, and weighted with a generalized plant action spectrum (UV-B_{BE}, Caldwell, 1971), normalized at 300 nm. Ultraviolet-B radiation was provided for 3 h on either side of solar noon, resulting in a daily UV-B_{BE} dose of 0, 6.7, or 13.4 effective kJ m^{-2} UV-B_{BE}. The highest dose was equivalent to the daily weighted UV-B dose received under clear sky conditions and minimum solar zenith in Colombia, South America (0° latitude, 3 km elevation, Julian date 80, Green *et al.*, 1980). There was no natural solar contribution to the daily UV-B_{BE} dose due to the absorptivity of greenhouse glass.

Vegetative drug-type plants (10 plants per treatment) were exposed to UV-B radiation during the months of November and December 1983 when the plants were 9 months old; average total daily photosynthetic photon flux density (PPFD between 400–700 mm), recorded with a LI-COR LI-550 printing calculator equipped with a LI-COR LI-185 quantum sensor, was 11.4 mol m^{-2}, and average daily maximum/minimum air temperature were 25/20°C. The natural photoperiod was extended to 16 h with a 1000 W General Electric Hg vapor lamp to inhibit flowering. Reproductive drug-type plants (10 plants per treatment) were irradiated during the months of February and March 1984 when the plants were 11 months old, the average total daily PPFD was 13.5 mol m^{-2}, and average daily maximum/minimum air temperatures were 28/23°C. Reproductive fiber-type plants (10 plants per treatment) were irradiated during the months of November and December 1984 when the plants were 9 months old, average total daily PPFD was 10.7 mol m^{-2}, and average daily maximum/minimum air temperatures were 29/20°C, while vegetative fiber-type plants (8 plants per treatment) were irradiated during the months of April and May 1985 when the plants were 13 months old, average total daily PPFD was 21.2 mol m^{-2}, and average daily maximum/minimum air temperatures were 28/23°C. Thus, data for vegetative and reproductive tissues (within a chemotype) were collected from the same plants at different developmental stages.

After the 40-day irradiation period, CO_2 assimilation (A), transpirational water loss (E), dark respiration (R_d), and leaf conductance to water vapor (g_s) were measured on single, fully expanded, attached leaves in temperature-controlled cuvettes in an open gas exchange system using a dual gas detector Anarad 600R infrared gas analyzer. Gas exchange measurements for drug-type plants were conducted at an ambient CO_2 concentration (C_a) of 340 $\pm$ 6 $\mu\ell\ \ell^{-1}$. Air was bubbled through water at 18°C and circulated over a leaf maintained at 28°C, resulting in an average leaf-to-air vapor pressure deficit (VPD) of 1.45 $\pm$ 0.03 kPa. The flux of H_2O vapor was measured with an EG&G Model 911 and a General Eastern Systems 1100 DP condensation dew point hygrometer located immediately before and after the leaf chamber. Natural insulation was supplemented with a General Electric 1000 W Hg vapor lamp to provide saturating irradiances of 1500 μmol $m^{-2}\ s^{-1}$ (measured with a LI-COR LI-185 quantum sensor). Gas exchange measurements for the fiber-type plants were conducted at similar conditions except C_a was 310 $\pm$ 2 $\mu\ell\ \ell^{-1}$ and VPD was 1.84 $\pm$ 0.03 kPa. To correct for differences in C_a and VPD of drug- and fiber-type plants at the time of measurement, reported A and E data for fiber-type plants were standardized for average intercellular CO_2 and VPD values of drug-type plants (i.e. 235 $\mu\ell\ \ell^{-1}$ and 1.45 kPa, respectively). Carbon dioxide assimilation and E were calculated in a manner similar to that described by Nobel (1976). Leaf conductance to water vapor (g_s) was calculated following conventional resistance analyses using 1.56 as the coefficient relating the diffusivities of CO_2 and water vapor (Nobel, 1976).

The photosynthetic light response was determined at a uniform temperature (28°C) by covering the leaf cuvette with successive layers of cheesecloth and ultimately covering the cuvette with a light-tight cloth for estimates of R_d. The temperature response of photosynthesis was determined at a saturating PPFD of 1500 μmol $m^{-2}\ s^{-1}$ by increasing leaf temperature (*via* cuvette air temperature) by 1 $\pm$ 0.2°C from 26 to 32°C. Both light saturation and temperature response of photosynthesis were measured on attached, mature leaves which expanded while irradiated with a daily UV-B_{BE} dose of either 0 or 13.4 effective kJ m^{-2} (one leaf per plant, three plants per treatment, and two treatments, N=6).

Samples for cannabinoid analysis were harvested after 40 days of irradiation at the same time of day (3 h after solar noon) to avoid sample differences due to possible diurnal fluctuations in cannabinoid content (Turner *et al.*, 1975) lyophilized, and stored at −5°C. Five hundred milli-

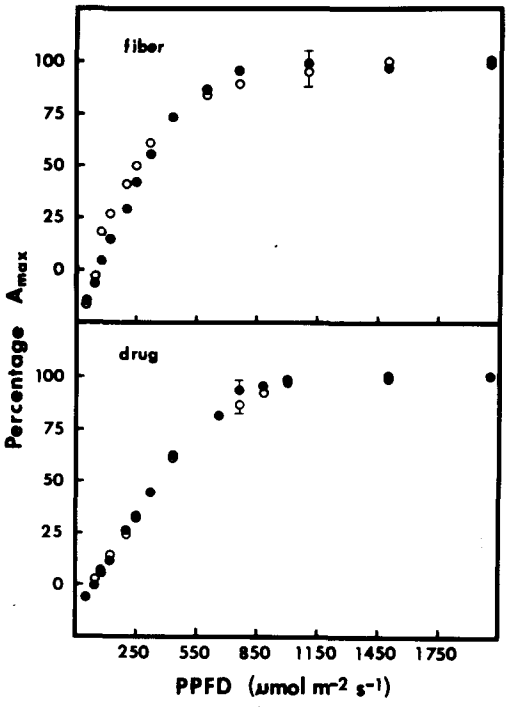

Figure 1. The effects of photosynthetic photon flux density (PPFD, 400–700 nm) on CO_2 assimilation of fiber-type and drug-type *Cannabis sativa* irradiated with a daily UV-B_{BE} dose of 0 (○) and 13.4 (●) effective kJ m^{-2}. Vertical lines represent ±1 SE.

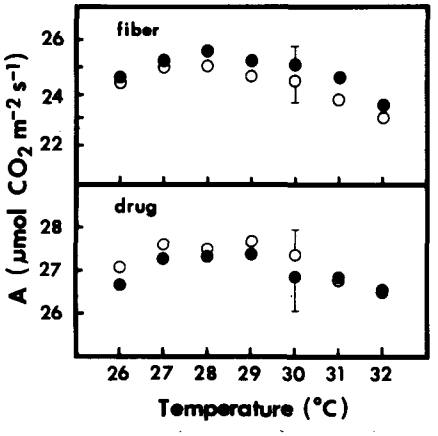

Figure 2. The effects of temperature on CO_2 assimilation of fiber-type and drug-type *Cannabis sativa* irradiated with a daily UV-B_{BE} dose of 0 (○) and 13.4 (●) effective kJ m^{-2}. Vertical lines represent ±1 SE.

grams of floral tissue (or 100 mg leaf tissue) were ground in a mortar with pestle and extracted in 100 mℓ chloroform for 24 h with sonication at 0, 12 and 24 h. The extract was then filtered, evaporated to dryness under vacuum at room temperature, redissolved in 1 mℓ hexane containing 5 μg $\mu\ell^{-1}$ of n-hexacosane (the internal standard) and centrifuged at 30 000 ***g*** for 10 min before analysis by gas chromatography.

Chemical analysis of the supernatant was performed using a Varian 3700 gas–liquid chromatograph (with a Varian CDS 401 data processor) equipped with a hydrogen flame-ionization detector and a 6.4 mm OD, 2 mm ID by 2.43 m glass column packed with 3% SP-2100 on 100/120 mesh Supelcoport. The inlet and detector temperatures were 240 and 260°C, respectively, while the column was operated isothermally at 190°C. Helium was the carrier gas flowing at 0.33 mℓ s^{-1}. The system was calibrated using varying concentrations of the following pure standards (purchased from Applied Sciences) dissolved in *n*-hexane containing 5 μg $\mu\ell^{-1}$ *n*-hexacosane: Δ^9-THC, Δ^8-THC, CBD, cannabichromene (CBC), cannabinol (CBN) and cannabigerol (CBG). Cannabinol and CBG co-chromatograph and were calibrated as the combined peak. Results were expressed as mg cannabinoid g^{-1} dry weight (DW) leaf or floral tissue.

A comparison among treatments was made with analysis of variance with three treatments, eight or ten plants per treatment, and the average of three replicate samples per plant (N=24 or 30, Kleinbaum and Kupper, 1978). Linear regression analysis was used where UV-B treatment effects were found to be significant ($P < 0.05$) by analysis of variance.

RESULTS

Light saturation of CO_2 assimilation (A) in the drug- and fiber-type controls occurred at PPFDs greater than 850 μmol m^{-2} s^{-1} (Fig. 1). There was no significant difference in A from 26 to 32°C in either clone, although A tended to decrease at temperatures >30°C (Fig. 2). The light and temperature response of A and A_{max} were unaffected by UV-B radiation in both drug- and fiber-type plants (Figs. 1 and 2). Data on the physiological and morphological parameters of the drug- and fiber-type controls are presented in Table 1. None of these physiological parameters were significantly affected by UV-B radiation in either chemotype (data not shown).

The cannabinoid levels and profiles of the drug- and fiber-type controls (Table 1) were similar to those reported for other Jamaican and Czechoslovakian populations of *C. sativa* (Holly *et al.*, 1975). The major cannabinoid (CBD) in the fiber-type plants and the minor cannabinoids in drug- and fiber-type plants were not significantly affected by UV-B radiation (data not shown). Only the Δ^9-THC content in leaf and floral tissues of drug-type plants increased significantly with UV-B radiation. Regression analyses indicated that there was a significant linear increase in Δ^9-THC with UV-B dose in these tissues (Fig. 3). The concentration of Δ^9-THC in leaf tissue increased by 22% and 48% with a total daily UV-B dose of 6.7 and 13.4 effective kJ m^{-2}, respectively, as compared to controls. The same levels of UV-B radiation resulted in a 15% and 32% increase in Δ^9-THC, respectively, in floral tissues. The R^2 values, based on regression (see Fig. 3 for equations) about all sample observations were 0.32 and 0.31 for floral and leaf tissues, respectively. However, R^2 values based on regression about the sample means were 0.99 for both tissues. Thus the regression equations provide a suitable model for UV-B induced Δ^9-THC production in these tissues.

Table 1. Physiology, leaf morphology and cannabinoid production of greenhouse-grown drug- and fiber-type control plants

Parameter	Drug	Fiber	Units
A	25.2 ± 0.5	24.2 ± 1.0	μmol CO_2 m^{-2} s^{-1}
E	3.29 ± 0.19	3.38 ± 0.30	mmol H_2O m^{-2} s^{-1}
R_d	5.80 ± 0.38	8.23 ± 0.71	μmol CO_2 m^{-2} s^{-1}
g_s	225 ± 9.9	200 ± 6.5	mmol H_2O m^{-2} s^{-1}
Lf A	2.2 ± 0.13	2.1 ± 0.12	$m^2 \times 10^{-3}$
Lf DW	113 ± 5.5	116 ± 7.2	mg
Lf DW/Lf A	53 ± 2.3	57 ± 2.5	g m^{-2}

	Cannabinoids (mg g^{-1} DW)			
	Drug		Fiber	
Δ⁹-THC	4.45 ± 0.46	25.1 ± 1.11	—	1.36 ± 0.07
CBC	2.54 ± 0.23	5.39 ± 0.29	0.30 ± 0.06	1.77 ± 0.14
CBD	—	—	5.74 ± 0.52	23.1 ± 0.68
CBN/CBG	0.11 ± 0.05	0.33 ± 0.03	0.41 ± 0.12	0.55 ± 0.03

Each value represents the mean of 10 sample means (10 plants with 3 subsamples per plant) ± 1 SE, except for fiber-type vegetative tissue, where each value represents the mean of 8 sample means. A = CO_2 assimilation; E = transpiration; R_d = dark respiration; g_s = leaf conductance to water vapor; Lf A = leaf area; Lf DW = leaf dry weight; Lf DW/Lf A = leaf dry weight to leaf area ratio; Δ⁹-THC = Δ⁹-tetrahydrocannabinol; CBC = cannabichromene; CBD = cannabidiol; CBN/CBG = cannabinol and/or cannabigerol.

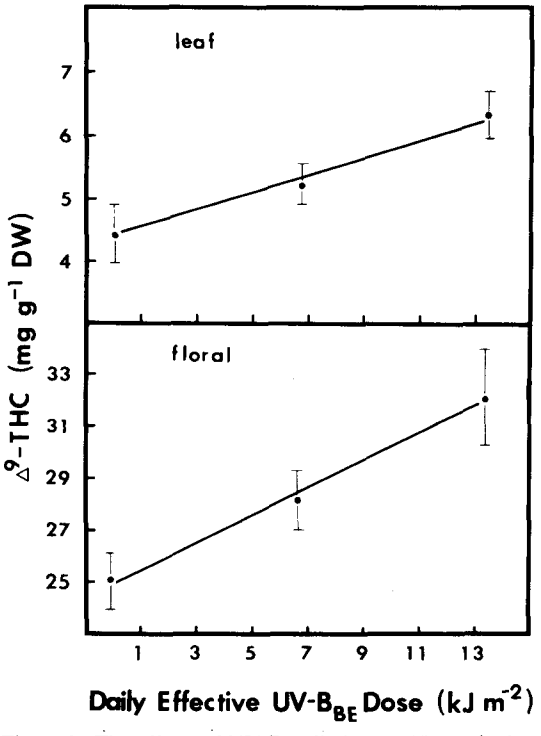

Figure 3. The effects of UV-B radiation on Δ⁹-tetrahydrocannabinol (Δ⁹-THC) content of leaf and floral tissue from drug-type *Cannabis sativa*. Vertical lines represent ±1 SE. Regression equations are: $Y = 25.0 + 0.5149\ X$ for floral tissue; and $Y = 4.39 + 0.1402\ X$ for leaf tissue.

DISCUSSION

The chemotypes used in the present study could be representative of tropical (Jamaican) and temperate (Czechoslovakian) populations. A_{max} and g_s for these populations were within the range reported for other herbaceous C_3 plants (Korner *et al.*, 1979). Light saturation of photosynthesis in both populations occurred at irradiances typical of plants grown in intermediate PPFDs (Boardman, 1977), which were the approximate conditions in the greenhouse (i.e. about 62% of ambient, Solar Radiation Measurements 1980–1981, Smithsonian Radiation Biology Laboratory, Rockville, Maryland). In a study of four different populations of *C. sativa* from two distinct climates (tropical and temperate), Bazzaz *et al.* (1975) reported that all populations had similar photosynthetic light responses, failed to light-saturate and A_{max} was 7.9 to 9.7 μmol CO_2 m^{-2} s^{-1} (at 80 000 lux). The A_{max} values reported by Bazzaz *et al.* (1975) were 2 to 3.5 times lower than those measured in the drug- and fiber-type populations used in this study. The latter is possibly due to differences in the PPFD between their growth chamber study and this greenhouse study.

Pate (1983) reported that *C. sativa* originating from high UV-B environments produced high levels of Δ⁹-THC and low levels of CBD, while the reverse was true in low UV-B environments. He concluded that Δ⁹-THC functioned as a UV-B filter, and that

UV-B radiation was the selective force that resulted in the present distribution of these populations. The results presented here indicate that both types of vegetative *C. sativa* are physiologically and morphologically insensitive to UV-B radiation. The increased level of Δ^9-THC found in leaf tissues upon UV-B irradiation may account for this insensitivity in the drug-type plants. However, fiber-type plants showed no comparable change in the level of CBD (which has similar UV-B absorption characteristics). Thus, the contribution of cannabinoids to the UV-B insensitivity in vegetative *C. sativa* is equivocal. Perhaps the background levels of CBD present in the fiber-type tissues were sufficient to protect the plant from UV-B radiation. Alternatively, other UV-B absorbing compounds such as flavonoids may account for this UV-B insensitivity. Flavonoids are the principle pigments associated with UV radiation screening in plants (Caldwell *et al.*, 1983; Flint *et al.*, 1985). Barrett *et al.* (1985) reported that the concentration of Cannflavin A (a flavonoid from *C. sativa*) was similar in drug- and fiber-type leaf tissue, whereas Gellert *et al.* (1974) reported relatively more flavonoids in drug- than fiber-type plants. Whether the quality and quantity of flavonoids in leaf tissues of the chemotypes in this study were sufficient to account for the observed UV-B insensitivity was not determined.

The results presented in this study, however, do not totally invalidate Pate's hypothesis. Only capitate-sessile and capitate-stalked glands contain cannabinoids, with stalked glands containing > 7 times more cannabinoids than sessile glands (Mahlberg *et al.*, 1984). Capitate-stalked glands are only present on bracts (and associated leaves), cover over 70% of the surface area of bracts, and contain 100% of the cannabinoids in these tissues (Mahlbert *et al.*, 1984). Thus, the UV-B absorbing cannabinoids may be important in preventing this actinic radiation from penetrating ovary tissues. If so, populations of *C. sativa* which express UV-B enhanced cannabinoid production (as demonstrated with the drug chemotype) may have more reproductive success in high UV-B radiation environments. This may account for the distribution of these chemotypes in temperate and tropical environments as reported by Pate (1983). It should be pointed out, however, that one should be cautious when extrapolating from greenhouse to field conditions in UV-B studies. In addition, when considering the distribution of *C. sativa*, one cannot overlook the fact that it is one of the oldest cultivated plants known to man. Thus, its present distribution may possibly be an artifact of man's cultural practices.

In conclusion, the Δ^9-THC content in leaf and floral tissues of greenhouse grown drug-type *C. sativa* increased linearly with UV-B dose. Other cannabinoids in drug- and fiber-type plants were unaffected by UV-B radiation. Both drug and fiber chemotypes were physiologically and morphologically tolerant to UV-B radiation. Thus, this tolerance was observed regardless of the concentration or composition of UV-B absorbing cannabinoids. Therefore, it appears that other factors may contribute to the UV-B insensitivity of vegetative *C. sativa*. Nevertheless, the increased production of Δ^9-THC in floral tissues (where this compound is most concentrated) of drug-type plants upon UV-B irradiation may result in more reproductive success for drug-type than fiber-type *C. sativa* in high UV-B environments as suggested by Pate (1983).

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