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# Photosynthesis and Cannabinoid Content of Temperate and Tropical Populations of *Cannabis sativa*

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**Key Word Index**—*Cannabis sativa*; Cannabinaceae; photosynthesis; cannabinoids; population studies.

**Abstract**—Four populations of *Cannabis sativa* L. grown from seeds collected in Panama, Jamaica, Nepal, and east central Illinois were grown under controlled conditions in growth chambers. One set was grown under warm conditions (32° day and 23° night) and the other set was grown under lower temperatures (23° day and 16° night). $CO_2$ exchange and transpiration were examined under various temperatures and light intensities. Observations on growth, and analyses for chlorophyll and $\triangle_1$THC (tetrahydrocannabinol) content were made. Under warm growth conditions, the central Illinois population had the highest photosynthetic rate at all temperatures investigated. The Nepal population had intermediate rates, while the Jamaica and the Panama populations had the lowest rate. The Jamaica and Panama populations had insignificant changes in photosynthetic response to changes in temperatures between 15° and 30°. Under cool growing conditions the central Illinois population had the highest rate of photosynthesis with a definite peak at 25°. Nepal plants had intermediate rates of photosynthesis, while the Panama and Jamaica populations had the lowest rate. Differences in chlorophyll and drug content were also significant between these populations. From these data it is suggested that the four populations can be grouped into different ecotypes genetically adapted to their respective environments.

## Introduction

Hemp (*Cannabis sativa* L.) is a widely distributed plant in various parts of the world. Originally indigenous to temperate parts of Asia, probably to the desert region near the Caspian Sea [1], it now grows as a weed in a variety of habitats ranging from sea level in tropical areas [2] to 7000 ft in the Himalaya Mountains [3]. The species was first introduced into the United States from England in 1632 by the pilgrims and later from China [4]. Presently, it is a common weed in the eastern and mid-western United States [5].

*Cannabis* has had a long history of association with man. Schultes estimates that it has been used for over 6000 years, and regards it as one of the oldest cultivated plants [6]. The plant is a source of fiber, oil, and a narcotic drug. The illicit use of *Cannabis* as a source of marihuana (plant shoots) and hashish (resin) is widespread throughout the world despite severe legal penalties in some countries. The active principle in the resin, which is found on flowers, leaves and stems, is mostly $\triangle_1$-tetrahydrocannabinol ($C_{21}H_{30}O_2$). This compound is biosynthetically derived from cannabidiol ($C_{21}H_{30}O_2$) and may be biosynthetically oxidized into cannabinol ($C_{21}H_{26}O_6$) [7].

It has been reported that plants from different geographic locations differ in the amount of resin they produce. For example, plants from warm climates produce 10 times as much of the active principle, tetrahydrocannabinol, as plants from central Europe grown under identical conditions [8]. Furthermore, it has been suggested that temperature and humidity affect the resin content; thus there is evidence that the drug content is both genetically and environmentally controlled.

In this paper we examine the differences in photosynthetic capacity and the cannabinoid content of populations of *Cannabis sativa* from four different geographic locations grown under two distinct environmental regimes. On the basis of these differences, we suggest that the populations from these areas may be grouped into temperate and tropical ecotypes.

## Results

Light saturation curves for the net photosynthesis of four populations were similar for all populations under both growing conditions (Fig. 1). Saturation was not reached even at 120 000 lux indicating that all populations belong to the sun plant group. Photosynthetic response to temperature of plants grown under

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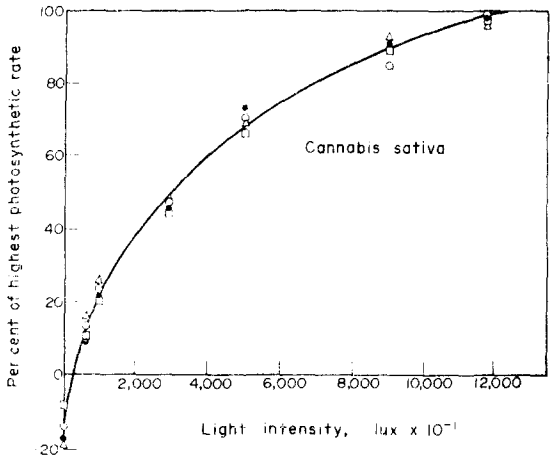


FIG. 1. LIGHT SATURATION CURVES FOR 4 POPULATIONS OF *CANNABIS SATIVA* (△ ILLINOIS, ● NEPAL, ○ PANAMA, □ JAMAICA) GROWN UNDER COOL CONDITIONS.

warm temperature conditions separates the four populations into two distinct groups (Fig. 2a). Photosynthesis of Nepal and Illinois populations increased with air temperature rise from 20° to 25° then declined sharply. Photosynthesis of the Jamaica and Panama plants increased with temperature rise from 20° to 30° (Fig. 2a). Maximum net photosynthesis was 15·4, 14·5, 14·5 and 12·5 mg $CO_2$ $dm^{-2}$ $hr^{-1}$ respectively for Nepal, Illinois, Panama and Jamaica plants. The rate of photosynthesis of the two groups were statistically different (5% level) at 25° only.

Photosynthetic rates of plants grown under cool conditions (Fig. 2b) were significantly lower than the rates of those grown under warm conditions, especially for plants of the tropical populations. Rate of photosynthesis of the Illinois plants increased sharply at air temperatures between 15° and 25°, then decreased slightly at 30°. Photosynthesis of the Nepal plants was less sensitive to change in air temperature than that of the Illinois plants and the photosynthetic rates of these two populations were statistically different from each other at 15° only. Photosynthetic rates of the Panama and Jamaica populations increased only slightly with temperature rise from 15° to 30° and were not significantly different from each other at all temperatures investigated. As a group, however, their photosynthetic rates were significantly different (5% level) from the Illinois and Nepal group at all temperatures except that of the Illinois plants at 15° (Fig. 2b). On the basis of these

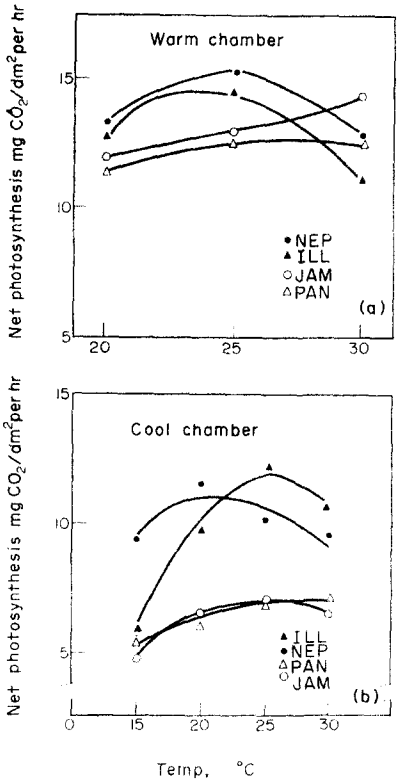


FIG. 2. PHOTOSYNTHETIC RESPONSE TO TEMPERATURE OF 4 POPULATIONS OF *CANNABIS SATIVA* PLANTS GROWN UNDER (a) WARM AND (b) COOL CONDITIONS.

data the plants grown under cool conditions and the plants grown under warm conditions can be grouped into two distinct groups: temperate (Illinois and Nepal) and tropical (Jamaica and Panama).

The chlorophyll content of leaves from plants of different origin grown under cool conditions were markedly diverse but no differences were observed between the plants grown under warm conditions. The chlorophyll data of plants grown under cool conditions again show that the plants can be grouped into temperate (Illinois–Nepal; 8·9 mg and 8·5 mg of chlorophylls a and b per gm dry wt respectively) and tropical (Jamaica–Panama; 2·2 mg and 1·2 mg per gm dry wt) with the former group having significantly more chlorophyll per leaf dry wt than the latter. The relatively low chlorophyll content of the tropical plants may be a cause of their low photosynthetic rates.

Although there were significant differences in cannabinoid content between the popula-

tions under the two growth conditions, these differences were generally of less use in separating the populations into temperate and tropical groups (Table 1). Contrary to available

TABLE 1. CANNABINOID CONTENT OF LEAVES OF PLANTS FROM FOUR LOCALITIES GROWN UNDER WARM 32°/23°) AND COOL (23°/16°) CONDITIONS

| Origin of plant | Condition | mg $g^{-1}$ dry wt | | | |
|---|---|---|---|---|---|
| | | CBD | THC | CBN | Total cannabinoid |
| Panama | warm | 0·64 | 4·80 | 0·80 | 6·24 |
| Panama | cool | 0·65 | 6·18 | 0·43 | 7·26 |
| Jamaica | warm | 0·88 | 4·07 | 1·11 | 6·06 |
| Jamaica | cool | 1·42 | 13·20 | 0·30 | 14·92 |
| Nepal | warm | 0·40 | 2·97 | 1·14 | 4·51 |
| Nepal | cool | 0·88 | 12·94 | 1·57 | 15·39 |
| Illinois | warm | 1·23 | 0·46 | 0·05 | 1·74 |
| Illinois | cool | 2·89 | 2·99 | 0·00 | 5·88 |

Cannabinoids were extracted with light petroleum and analyzed by GLC. Cetyl alcohol was used as internal standard.

information, the total cannabinoid content as well as the euphoric $\Delta_1$-tetrahydrocannabinol were much higher in plants grown under cool conditions than in plants grown under warm conditions. However, under warm growth conditions Nepal and Jamaica plants differ more from Illinois and Panama plants than between each other. Although the photosynthetic rate and cannabinoid content generally separate the populations into two groups, the data from these two parameters do not completely correspond. This suggests that more than one parameter must be used in assessing ecotypic differentiation in *Cannabis* and probably in other species.

## Discussion and Conclusions

It has long been argued that the genus *Cannabis* may consist of more than one species. Certain authors have considered *C. indica* Lam. to be the plant which grows wild in tropical Asia and is used as a source of Marihuana and hashish [1, 6]. *Cannabis sativa* is assumed to be the species which for many years has been under cultivation in various parts of the world as a source of rope. However, current feeling among most botanists is that *Cannabis* is a monotypic genus with many ecotypes and cultivated races [1, 6].

Since the plant has been used as a source of drug, fiber, and oil for thousands of years one can assume a large amount of breeding and selection for various qualities has occurred, making the taxonomy and evolution of *Cannabis* very complicated. In the U.S. the situation is aggravated by the introduction, over long periods of time, of *Cannabis* from various sources including Europe and China and the more recent small-scale introduction of tropical *Cannabis* for illicit use as a source of drug.

Although the plasticity of *Cannabis* has long been recognized, it has not been completely understood [6]. It is known, for example, that the species exhibits morphological variation when grown under various environmental conditions and that the plant grows under a variety of conditions and has been collected in various types of habitats in the United States [5]. Even sex in the species is influenced by various environmental factors such as UV light [9], day-length [10], temperature [11] and nitrogen concentration in the soil solutions [12]. However, our results clearly show that physiological and biochemical ecotypes are present in *Cannabis* and that these ecotypes can be distinguished from each other by their rate of photosynthesis, chlorophyll and cannabinoid content. Cool growth conditions distinguish these ecotypes more clearly than warm growth conditions.

## Experimental

*Cannabis sativa* seeds were collected from mature plants in east central Illinois, near the coast in Panama, at about 2000 ft elevation in central Jamaica, and at about 6000 ft near Katmandu, Nepal.

Seeds from these localities were sown in loam-filled 8-in. clay-pots incubated at 22° for 11 days and then transferred to two growth chambers when the seedlings were about 4 cm tall. The growth chambers were programmed to approximate tropical and temperate temperatures. The temperature of the "warm chamber" was 32° during the day and 23° at night, while that of the cool chamber was 23° and 16°; day length was 15hr. Light intensity, generated by 16 cool white fluorescent and eight 25W incandescent lamps was approximately 40 000 lux near the top of the plants. Since *Cannabis* is a dioecious species and there may be differences between male and female plants, only mature yet sexually undifferentiated plants were used for all measurements.

*Measurement of photosynthesis.* Net photosynthesis, hereafter referred to as "photosynthesis", and transpiration of plants were measured using a semi-closed infra-red gas analysis system. Plant shoots were enclosed in an assimilation chamber in which light intensity, air temperature, windspeed, relative humidity and $CO_2$ concentration were precisely controlled. Air was circulated between the assimilation chamber and a Grubb Parson infra-red gas analyzer which detected changes in $CO_2$ concentrations in the air stream. For a detailed description of the gas analysis system and measurement techniques see Bazzaz and Boyer [13].

Light saturation curves at 25°, 70 ± 2% relative

humidity, 5 m $sec^{-1}$ windspeed and 300–310 ppm $CO_2$ were established for plants from the four populations. After a steady rate of $CO_2$ release in the dark was achieved, light intensity was increased stepwise by removing layers of cheesecloth placed between the assimilation chamber and the light source above it. At each of several intensities, repeated photosynthetic measurements were made after a steady rate of $CO_2$ exchange had been achieved between the enclosed plant shoot and the air circulating in the system. Under 80 000 lux light intensity and the conditions specified above for relative humidity, windspeed, and $CO_2$ concentration, photosynthesis of plants from the four populations from both growth chambers was measured at 20°, 25° and 30° ambient temperature. Additional photosynthetic measurements were made at 15° on plants grown in the "cool chamber". At each set of environmental conditions about 10 readings on each fo 4 different individual plants from each population were made.

*Determination of chlorophyll and cannabinoids.* Chlorophyll from mature leaves was extracted with acetone, and optical density measurements were made at 645 and 663 nm with a Beckman DU spectrophotometer. Total chlorophyll was calculated according to the method of Arnon [14]. Cannabinoids [cannabidiol (CBD), tetrahydrocannabinol (THC) and cannabinol (CBN)] were extracted with petroleum ether from whole shoots of sexually undifferentiated plants. The extracts were analyzed by GLC on a 3 m × 3 mm stainless steel column packed with 5% SE-30 silicone liquid phase. The chromatograph was programmed to increase temperature from 150° to 250° at 4 deg per min after an 8-min post-injection period. Under these conditions the average retention time for the three cannabinoids was about 17 min. Cetyl alcohol was found to be a suitable internal standard and was used to quantitate the results. The cannabinoid standards were kindly supplied by the National Institute of Mental Health, HEW.

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