The Constituents of *Cannabis sativa* Pollen

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This research concerns the study of various constituents, chiefly the cannabinoids and nitrogenous substances of the pollen of *Cannabis sativa* that had been cultivated under artificial climatic conditions in the C. N. R. S. phytotron at 91 Gif-sur-Yvette, France. It covers principally an examination with qualitative and quantitative analyses of the main cannabinoids found as well as observations on the alkaloids and flavonoids present. The conditioned rooms of the phytotron enabled enough pollen to be produced to carry out these tests.

MATERIALS AND CONDITIONS OF CULTURE

All the experiments were carried out under the entirely artificial conditions of the phytotron where illumination was provided by luminous ceilings consisting of fluorescent tubes and incandescent lamps that gave from 14,000 to 18,000 lux at plant level. The seed was sown in vermiculite and the plants repotted into glass wool (verrane) covered with vermiculite. They were watered for two days in seven with a nutrient solution (Chouard and Tran Thanh Van, 1964) (3) and during the remaining five days received de-ionized water. Seeding was carried out with two lots of *Cannabis sativa* L. seed[2]. UNC 255 from South Africa and UNC 258 from Turkey, while the seed beds were submitted to four different temperate regimes with the same lighting and two different lighting regimes at the same temperature. The results are given in Table I.

TABLE I
CULTURAL REGIMES FOR *CANNABIS SATIVA* L.

Daily temperature (°C)	Night temperature (°C)	Illumination (h)	Summary
32	12	16	32°-12°-16 h
22	12	16	22°-12°-16 h
27	27	16	27°-16 h
24	24	16	24°-16 h
24	24	9	24°-9 h

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Fig. 1. *Cannabis* UNC 255, 36 days after sowing. On the left: 24° C — 9 h (male plant). On the right: 24° C - 16 h.

Fig. 2. *Cannabis* UNC 258, 36 days after sowing. From the left to the right: 24° C - 9 h (male plant); 24° C - 9 h (female plant); 24° C - 16 h.

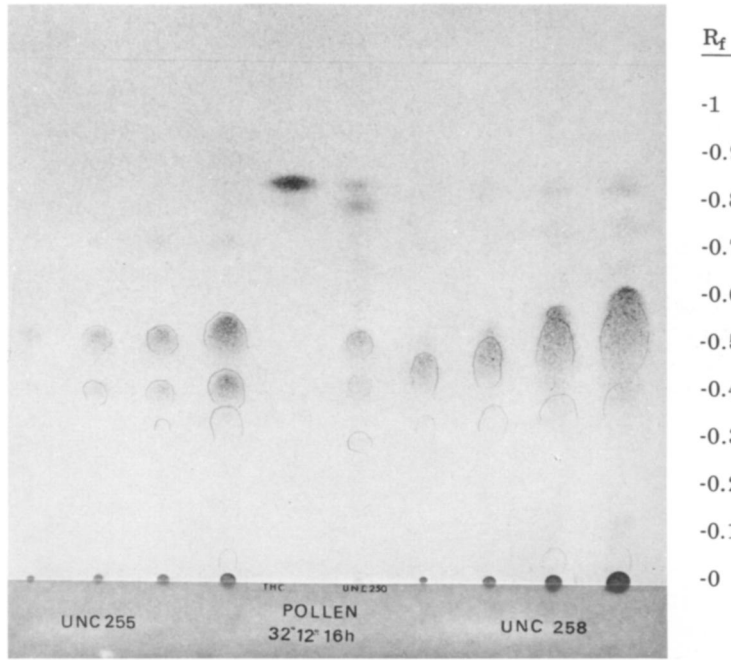

Fig. 3. Thin-layer chromatography of the pollen. Silica gel precoated plates; Solvent: hexane-dioxane 4:1 v/v; visualization by fast blue salt B. THC = Δ_9-THC; UNC 250 = reference sample from U.N. T_2 purple R_f 0.40; CBDA orange 0.45; THCA purple 0.55; CBD orange 0.75; THC purple 0.80.

Observations of the growth under the above conditions revealed an early flowering of the male plants subjected to the 9 h daily illumination regime (Figs. 1, 2 and 3).

TABLE II

PHYSIOLOGICAL CHARACTERISTICS OF THE FLOWERING STAGE UNDER 9 h ILLUMINATION

Particulars	Lot UNC 258	Lot UNC 255
Date of flowering: Number of days after sowing	26	32
No. of pairs of leaves	5	6
Av. height of plant (cm)	7	8

The *Cannabis* would appear to be a short-daylight plant (J. Heslop-Harrison and Y. Heslop-Harrison, 1969) (6). When the 16 h experimental lots were given 10 days at only 9 h illumination, flower buds were observed to have already formed upon the male plants. After the return to the 16 h day an abundant flowering was obtained which enabled pollen to be gathered from each of the lots submitted to the different climatic regimes shown in Table I.

At harvesting this pollen showed the following colour differences:
— lot UNC 255 : pale yellow;
— lot UNC 258 : golden yellow.

Microscopic examination revealed 3 germ pores and an average diameter of 45 to 50μ.

TECHNIQUES

1. **Extraction.** The pollen was submitted to an extractive stabilization by boiling in methanol for half an hour; it was then filtered and the residue re-extracted with fresh boiling methanol. The two filtrates were united and concentrated to one half the weight of the pollen treated. The quantities used for the tests varied from 0.20 g to 2 g, giving a final volume of from 0.10 to 1 ml of extract.

2. **Analysis of cannabinoids.** We employed thin-layer chromatography on silica gel (M. Paris and D. Demesy, 1971) (8), using the methanol extract.

The principal polyphenols were determined by quantitative photodensitometry (M. Paris and G. Faugeras, 1971) (5), using a Joyce-Loebl Chromoscan.

3. **Analysis of the alkaloid type compounds.** The extractive liquor was purified as follows: evaporated *in vacuo* below 40°C; taken up in 1% sulphuric acid and filtered; rendered alkaline with ammonia and the nitrogenous compounds extracted with agitation by chloroform which was then evaporated; the dry residue was taken up in a precisely known quantity of distilled chloroform (R. R. Paris and L. Cosson, 1965) (10). This chloroform solution was then deposited with a micropipette as a range of spots on the chosen supports.

Three different solvent systems and two types of support were used successively and systematically: the supports were either silica gel or cellulose layers, the latter proving the more effective; the solvent systems were:

system I : acetone, chloroform and 25% ammonia (160:10:10);
system II : butanol, acetic acid and water (4:1:5);
system III : isobutanol, fuming HCl and water (7:1:2): used with cellulose, this gave the best chromatographic analysis of the extract.

Three detection reagents were used (11):

the Dragendorff reagent (Munier and Dragendorff formula);
the Trabert reagent (Saint Firmin, R. R. Paris);
potassium iodoplatinate reagent.

4. **Analysis of the flavonoids.** Thin-layer chromatography on silica gel was used with solvent II. The detection reagent was aluminium chloride used after the layers had been observed by ultraviolet light. With crude methanol extracts, the "cyanidin reaction" was carried out.

EXPERIMENTAL RESULTS

A. *Cannabinoids.*

1. **Qualitative study.** This was carried out using the techniques already described. Spraying the chromatograms with blue salt B revealed the presence of cannabinoids of which certain ones were easily identified with the indicators we had: tetrahydrocannabinol (THC) and cannabidiol (CBD).

Calculating the R_f of the other spots detected enabled an identification of the acids of these two compounds: tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA).

In the extracts of one of the two lots of plants we also observed the constant presence of a spot which is now in course of identification, but which we named T_2 and of which the R_f value is given in Table III which will be illustrated by Fig. 3.

TABLE III

QUALITATIVE COMPOSITION OF THE POLLEN OF UNC 255 AND UNC 258 (FIG. 3)

Pollen UNC 255	Cannabinoids-colour-R_f*		Pollen UNC 258
+ +	Δ_9-THC purple	0.80	+
+ + +	THCA purple	0.55	possible
o	CBD orange-yellow	0.75	faint traces
traces (?)	CBDA orange	0.45	+
+	T_2 purple	0.40	o

*R_f measured with authentic samples on the chromatograms after double development in the same solvent.

The results showed that whatever the climatic conditions chosen, the pollen from the UNC 255 plants was both qualitatively and quantitatively (as will be seen later) much richer than the pollen from the UNC 258 plants. Furthermore, they confirmed the following characteristics appropriate to each lot that had already been demonstrated in the leaves (M. Paris, 1971) (9):

— the presence mostly of THC and THCA in UNC 255;
— the presence of CBD and CBDA in UNC 258.

2. **Quantitative study.** These results show that:
— the highest THC and THCA levels for UNC 255 pollen were obtained at 24° C 16 h daily illumination and 50% relative humidity;
— the THCA level was in all cases higher than that of THC;
— T_2 was consistently present and at a higher level than THC under conditions of 32°C 12°C 16 h and 27°C 16 h, but at concentrations lower than THC under the other two regimes.

Pollen from UNC 258 was not tested quantitatively because THC was not present and only traces of THCA were occasionally observed. CBDA was the only major constituent of this pollen and showed its maximum concentration under the 32° 12° 16 h regime.

B. *Alkaloid type substances.*

These substances were studied by the thin-layer chromatographic techniques already described, the compounds producing the general reactions characteristic of alkaloids having given very clear results (1), (2), (7), (12). The evaluations of the spots appearing on the chromatograms are given in Table V which also shows particulars for the plant at the 7th pair of leaves stage and for the male and female flower heads respectively. The R_f values are given for system III solvent. The (+) signs show the importance of the

TABLE IV

RESULTS OF ANALYSIS OF CANNABINOIDS PRESENT IN THE POLLEN OF LOT UNC 255 UNDER EACH CLIMATIC REGIME*

UNC 255: Cannabinoids g% in fresh pollen	24° 16 h 50% RH	32° 12° 16 h 80% RH	22° 12° 16 h 80% RH	27° 16 h 80% RH
THC	0.32	<0.01	0.06	0.016
THCA	0.60	0.04	0.16	0.08
T_2	0.04	0.028	0.023	0.06

*The THCA and T_2 levels were calculated by using the ratios $\frac{THCA}{THC}$ and $\frac{T_2}{THC}$ the value of THC having been previously determined.

spots, after use of the detection reagent, and judged according to the following scale:

++++ and +++ : positive reactions for 20 µl of a 1/2 solution;

++ and + : for 20 µl of a very concentrated solution: in the latter case a distinction must be made between the pollen extracts and the plant extracts.

A very concentrated pollen solution for lot UNC 255 corresponded to 1 g of fresh pollen per 1 ml of chloroform, and for lot UNC 258 to 2 g of fresh pollen per 1 ml of chloroform. The average concentration of the chloroform extracts of the non-flowering and the flowering plants was 15 g of fresh plant per 1 ml of solvent.

The results are entered in Table V.

This analysis revealed substances in the pollen and the different aerial organs of *Cannabis sativa* L. that were very probably of an alkaloid type. The pollen and aerial parts of UNC 255 appeared to be richer in these substances than those of the same organs of UNC 258. The most favourable climatic regime seemed to be 24° constant temperature and this also appeared to be the optimal temperature if the THC content of the pollen was taken into consideration.

In order to complete this study of the nitrogenous compounds of the pollen, we tried a further analytical technique: gas-liquid chromatography using a Girdel apparatus with temperature programming. The operative conditions were:

— stainless steel column 1/8", 1.5 m, 5% SE 52 on gas-chrom Q 80/100;
— nitrogen flow: 20 ml min.;
— programmed temperature of 90°C to 250°C at the rate of + 10°C/min.;
— temperature of injector 220° C;
— temperature of detector 250°C.

The chloroform extracts were injected before and after silylation by the BSTFA. This technique proved to be by far the best method of analysis for these products. The analysis of pollen extracts after silylation at programmed temperature between 90° and 250°C enabled us to observe chromatograms presenting interesting constants. In the first place, there did not appear to be any qualitative difference between the chromatograms of UNC 255 and UNC 258. In both lots there were two dominant peaks: the first appeared at 170°C and was constantly the most abundant whatever extract was injected; the second appeared at 190°C. Under the operative

TABLE V

ALKALOID TYPE SUBSTANCES PRESENT IN THE POLLEN AND AERIAL ORGANS OF *CANNABIS SATIVA* L. (USING SOLVENT III AND A CELLULOSE LAYER)

Part Analyzed	Plant at 7th pair of leaves stage Flower heads: male and female				Pollen: UNC 255			Pollen: UNC 258		
R_f	UNC 255 24° 16 h	UNC 258 24° 16 h	UNC 255 24° 9 h	UNC 258 24° 9 h	24° 16 h	32° 12° 16 h	22° 12° 16 h	24° 16 h	32° 12° 16 h	22° 12° 16 h
0,10 - 0,20				♀ + + ♂ +	+ + +	+ + +	+ + +	+ + +		+
0,35										
0,65 - 0,70	+		♀ + + ♂ + +		+ + + +	+ + +		+ + +		
0,80 - 0,90					+ + +	+ + +		+ + +		

conditions THC appeared between 230°C and 245°C and CBD between 220°C and 230°C.

A similar study of a silyl extract of UNC 250 used as a reference sample in research on *Cannabis sativa* L. did not invalidate, *a priori*, the hypothesis that these two peaks were attributable to substances of an alkaloid nature.

CONCLUSION

The pollen of *Cannabis sativa* L. was rich in cannabinoids and particularly in THC and THCA, the latter being able to be transformed into physiologically active THC. Climatic factors and particularly temperature played an important role, since the THC content at 24°C 16 h was 30 times as great as at 22°C 12°C 16 h.

Determinations of the phenol compounds in the corresponding flowering heads had not been completed, but those that had been finished showed that the optimum content was given by plants cultivated at 24°C 16 h.

The highest concentration of alkaloid type substances was also given under this climatic regime: these substances were different from choline and trigonelline and studies were under way to identify them.

With regard to the flavonoids that were examined by two-dimensional paper chromatography, two principal spots were detected corresponding to two glycosides. After hydrochloric acid hydrolysis two different genins were identified as apigenin and luteolin respectively. These flavone glycosides were also found in the leaves together with several others, and a further study is being made of them the results of which will be published shortly.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Aguar, O. 24th May, 1971. Examination of *Cannabis* extracts for alkaloidal components. United Nations Document ST/SOA/SER. S/28.
2. Bercht, C. A. L. and Salemink, C. A. 22nd August, 1969. Recherches sur les principes de base du *Cannabis*. United Nations Document ST/SOA/SER. S/21.
3. Chouard, P. and Tran-Thanh Van M. 1964. Vernalisation sans réfrigération par les facteurs trophiques de la vigueur, grande luminosoté et niveau élevé de la nutrition minérale shez le *Geum urbanum* L. *C. R. Ac. Sc. 259*, 4783-4786.
4. Cosson, L. 1972. Influence de l'éclairement sur la teneur en alcaloides tropaniques des *Datura*: analyse des processus pouvant en expliquer les effets. D. Sc. thesis. Paris VI.
5. Faugeras, G. and Paris M. 1971. Dosage des tétrahydrocannabinols du *Cannabis* par densitométrie après séparation par chromatographie sur couche mince. *Plantes méd. et Phyto., 5*, 224-233.

6. Heslop-Harrison, J. and Heslop-Harrison, Y. 1969. In Induction of flowering: Some Case Histories. Evans L.T.I., Cornell University Press, 205-226.
7. Klein, F. K., Rapoport, H. and Elliott, H. W. 1971. *Cannabis* alkaloids. *Nature. 232*, 258.
8. Paris, M. and Demesy, D. 1971. Nouvelles méthodes d'identifications et d'évaluations de l'activité du Cannabis. *Pl. méd. et Phyto. 5*, 28-38.
9. Paris, M. 7-12 September, 1971. Communication presented to the 31st International Congress of Pharmaceuticals Sciences in Washington, D.C.
10. Paris, R., Cosson, L. and Chouard, P. 1966. Influence de l'éclairement sur les variations ontogéniques des alcaloides de *Datura tatula. Lloydia. 29*, 19-25.
11. Saint Firmin, A. and Paris, R. 1967. A new method for determining alkaloids of the atropine group by thin-layer chromatography. *J. chromatography. 31*, 252-254.
12. Samrah, H. 11th December, 1970. Etude préliminaire sur la présence éventuelle de substances alcaloidiques dans le *Cannabis*. United Nations Document ST/SOA/SER. S/27.

Book Reviews *(continued from page 244)*

copied without credit to source, e.g., *Lemna trisulca, Spirodela,* and the flowers of *Ruppia* have obviously been lifted from Mason's *A Flora of the Marshes of California.*

The general observations rarely include any data on such subjects as pollination (*Ruppia* is an exception), but there are notes on edible species and, often, on weedy ones (with the disclaimer that a pest in one region may not be so in another), or other aspects of economic significance.

For a volume with no halftones and printed from cold-type composition with unjustified margins, this seems grossly over-priced even in these inflationary times. But anyone with $50.00 to spare and with a curiosity about the genera of freshwater bryophytes, charophytes, and vascular plants is sure to find something in this comprehensive tome. It would have added more to its usefulness than to its bulk to have added the handful of marine genera.

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Natural Regions of the United States and Canada. Charles B. Hunt. 725 pp. illus. W. H. Freeman and Company, San Francisco, 1974. $14.95

The purposes of this book are "to remind us that we are greatly favored over the rest of the world by our natural environment, and to introduce non-earth scientists to the art of appreciating landscape." The book is a result of Dr. Hunt's course at The Johns Hopkins University in which students not majoring in earth sciences are presented a broad view of natural features and resources of the United States and Canada. It is a new and enlarged version of his *Physiography of the United States* (1967).

The book is divided into two parts. Part one, "General Features and Processes," contains nine chapters discussing natural regions, structural framework of the continent, landforms, climate, water, surface deposits, land sculpture, biogeography, and resources-conflicts of interest. The author introduces the reader to eleven physiographic provinces of the United States and Canada in part two, "The Provinces." For each he explains the structural framework, climate, soils, water and mineral resources, vegetation or agriculture, and other conditions important to that area. Parts one and two are profusely illustrated with good photographs, maps, line drawings, cross sections, and physiographic and block diagrams. Each chapter ends with a list of selected references. The four appendixes inform the reader about topographic maps of the United States and Canada, altitudes of the 25 largest cities of the countries, altitudes of named summits over 14,000 feet, and extreme and mean altitudes of states and provinces. A place index and a subject index complete the book.

Although the book is geologically oriented, botanists should find the sections on vegetation, forests, and agriculture of interest. Many readers will find Chapter nine, "Resources—Conflicts of Interest," especially informative in this period of concern for the environment. One is made to pause and think by statements such as "Some think the country has gone dam crazy. For example, water losses by evaporation from reservoirs now exceed the amount used for

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