

CANNABINOID FORMATION IN *CANNABIS SATIVA* GRAFTED INTER-RACIALLY, AND WITH TWO *HUMULUS* SPECIES

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Abstract—Inter-racial grafts between high and low Δ^1 -THC strains of *Cannabis sativa*, as well as cross-grafts with two *Humulus* (hop) species have been effected. *C. sativa* strains continue to produce essentially their own characteristic mixtures of cannabinoids, with undiminished vigour, whatever part of the graft system they form. There is no evidence of transport of intermediates or factors critical to cannabinoid formation across the grafts.

Cannabis is a monotypic genus containing only the species *C. sativa*. The position of the genus has been the subject of debate [1]. Within the Order Urticales the modern tendency is to place it, together with *Humulus*, the genus of the hop plant, into a separate family, the Cannabidaceae. *Cannabis* and *Humulus* have, however, also been classified as members of the Urticaceae (nettle family) or the Moraceae (fig family) [1, 2]. The single species *Cannabis sativa* has diversified, and there are a considerable number of ecotypes and cultivated races. In our laboratory, strains of Thai and S. African origin produce a high proportion of Δ^1 -tetrahydrocannabinol, the major psychotomimetic component, whilst a third strain of Kew origin produces mainly cannabidiol (Table 2).

In view of the alliances between *Cannabis* and *Humulus* we have explored the cross-grafting of *C. sativa* with *H. lupulus* and *H. japonica* (*H. scandens*). GLC analysis of the latter two species shows no production of characteristic cannabinoids (though chemically different natural products are well known) [3–5]. Seedlings of the two species to be grafted were planted side by side, and when they were about 4 weeks old a diagonal cut was made approximately half-way through each stem, at the same level. The cut portions were slipped into each other and the joints held with

adhesive cellulose tape. After 1–2 weeks the unwanted top portion was cut away, followed in a few days by the unwanted lower portion, thus completing the graft. At least 20 specimens of each graft-type were made. The successful graft-types are shown in Table 1 and include inter-race *Cannabis* grafts as well as *Cannabis*/*Humulus* types. Success rates were >30%, considerably higher if the grafts were made early in the season (May–June). All successfully grafted systems continued to grow well, and the *Cannabis* upper parts flowered in both male and female cases, the latter fruiting.* Differences in stem diameter between *C. sativa* and *H. japonicus* and *H. lupulus* presented no barrier to satisfactory union (cf. Figs. 1(1) and 1(2)). *H. lupulus* grew well on *Cannabis*, but as the grafting was done late in the season, the former did not flower. Attempts to graft *C. sativa* on the common

Table 1. Successful grafts employing *Cannabis sativa*

Scion*	Stock*
<i>Cannabis sativa</i> (Thailand)† on <i>Cannabis sativa</i> (Kew)‡	
<i>Cannabis sativa</i> (S. Africa)† on <i>Cannabis sativa</i> (Kew)‡	
<i>Cannabis sativa</i> (Thailand)† on <i>Humulus japonicus</i>	
<i>Cannabis sativa</i> (S. Africa)† on <i>Humulus japonicus</i>	
<i>Cannabis sativa</i> (Kew)‡ on <i>Humulus japonicus</i>	
<i>Cannabis sativa</i> (Thailand)† on <i>Humulus lupulus</i>	
<i>Cannabis sativa</i> (Kew)‡ on <i>Humulus lupulus</i>	

* In every case reverse grafts (as between scion and stock) were also successful.

† High Δ^1 -THC strain.

‡ Low Δ^1 -THC strain (high CD).

* Monoecious grafts were occasionally found.

Table 2. Percentage composition and total amounts of cannabinoids in *C. sativa* (Kew) *C. sativa* (Thailand) grafts*

Cannabinoids	Thai Control		Thai Top**		Kew Control		Kew Top††	
	A†	B‡	A	B	A	B	A	B
CD-C ₅	—	—	—	—	83	95	82	88
Δ ¹ -THC-C ₃	1	—	trace	—	—	—	—	—
CC-C ₅ + CG-C ₅	5	7	9	10	3	2	4	trace
Δ ¹ -THC-C ₅	94	93	91	90	14	3	14	12
Wt. mg/g.‡	3.1	2.3	1.7	4.1	0.6	5.1	0.7	8.2

* Grafted on base of stem: analysis 10 weeks after completion of graft: Kew strain at fruiting stage, Thailand vegetative.

† % Composition of non-carboxylic acid cannabinoids.

‡ % Composition of carboxylic acid cannabinoids, after decarboxylation.

§ CD-C₅ = cannabidiol, Δ¹-THC-C₃ = Δ¹-tetrahydrocannabinol, CC-C₅ = cannabichromen, CG-C₅ = cannabigerol, Δ¹-THC-C₅ = Δ¹-tetrahydrocannabinol, CN-C₅ = cannabinol.

‡ Wt of total cannabinoids mg/g of dry weight.

** Kew stock.

†† Thai stock.

nettle (*Urtica dioica*) failed, but this is not unexpected as the botanical relationship is not particularly close.

After at least 8 weeks growth from completion of the graft (frequently longer), leaves and flowers or fruit were stripped from the plant, dried (50°) and extracted at 20° with *n*-pentane, followed by methylene chloride, and separated into uncarboxylated and carboxylated cannabinoids. Components of each fraction were identified by using three TLC methods: (a) silica gel, eluting with CHCl₃, (b) silica gel, eluting with C₆H₆-MeOH-AcOH (88:10:2%) (particularly suited to acids) [6], (c) Korte's system [7], together with cannabinoid reference standards.

Fast Blue Salt B colours were used as a guide to the qualitative composition. The cannabinoids were then estimated [8], after trimethylsilylation, by GLC using OV225 (50' SCOT column) or OV17 (2% on Chromosorb W, 5' column). Results are reported for uncarboxylated cannabinoids in columns A of the Tables. Acid cannabinoids were estimated after decarboxylation by heating in pyridine and are reported in columns B in the Tables. Data for control plants, planted at the same time as those used for the grafts, and treated identically except for the grafting procedure, accompany all results.

The salient characteristics of the two strains of *Cannabis* employed are as follows:

Kew Strain. Cannabidiol (CD-C₅) contents are high, usually >90–95% of the carboxylated cannabinoids and >70–75% of the uncarboxylated cannabinoids. The cannabigerol (CG-C₅) and canna-

bichromen (CC-C₅) mixture (determined together) is normally 5% or less of the total, being higher in the uncarboxylated cannabinoid fraction. The Δ¹-tetrahydrocannabinol (Δ¹-THC-C₃) content is usually <25% of the uncarboxylated cannabinoid fraction, and frequently <5% of the carboxylated cannabinoid fraction. No Δ¹-tetrahydrocannabinol (Δ¹-THC-C₃) is produced.

Thai Strain. No cannabidiol is produced. The cannabigerol-cannabichromen mixture usually forms a higher percentage of the mixture than in the Kew strain and in contrast to the latter the figures are usually higher for the carboxylated than the uncarboxylated cannabinoid fraction. The Δ¹-tetrahydrocannabinol content is high, usually >90% for the uncarboxylated and >~85% for the carboxylated cannabinoids. Small amounts of Δ¹-tetrahydrocannabinol are usually formed.

Total cannabinoid production in the Kew strain plants was 3.6 mg/g. of dry weight with much larger amounts of carboxylated than uncarboxylated cannabinoids being produced. The Thai strain produced 5–9 mg/g. of total cannabinoids but the relative amounts of carboxylated and uncarboxylated cannabinoids were more variable. As the techniques used were not designed for a rigorous study of the carboxylated/uncarboxylated cannabinoid balance we have not used this as a criterion in the graft studies. The cannabinoid acids are known to be easily decarboxylated, and information such as the relative ease of decarboxylation of the different members of the group under various conditions would be required to obtain a proper perspective on the situation.

Table 3. Percentage composition and total amount of cannabinoids in *C. sativa* (Kew) *C. sativa* (Thailand) grafts*†

Cannabinoid	Thai Control		Thai Top‡		Thai Bottom§		Kew Control		Kew Top¶		Kew Bottom**	
	A	B	A	B	A	B	A	B	A	B	A	B
CD-C ₅	—	—	—	—	—	—	75	95	70	92	79	98
Δ^1 -THC-C ₃	2	2	2	2	—	2	—	—	—	—	—	—
CC-C ₅ + CG-C ₅	7	14	4	7	4	12	5	2	5	—	2	—
Δ^1 -THC-C ₅	91	84	94	91	96	86	20	3	25	8	19	2
Wt mg/g.	3.7	4.1	0.6	6.0	0.2	6.9	0.3	4.0	0.3	5.2	0.5	4.9

* Grafted higher on stem than in Table 2: analysis 8 weeks after completion of graft. Kew strain at fruiting stage, Thailand vegetative.

† For abbreviations see Table 2.

‡ On Kew stock.

§ Leaves from stock shoots of a Thai stock with a Kew scion graft.

¶ On Thai stock.

** Leaves from stock shoots of a Kew stock with a Thai scion graft.

Table 2 shows the cannabinoid analysis for *C. sativa* (Thailand) grafted on *C. sativa* (Kew), and vice versa, together with data from controls. It is apparent that there is no important change in the characteristic composition of the cannabinoid mixtures of the two strains when cross grafted, and production of total cannabinoid in terms of mg/g. of dry weight is unimpaired. Table 3 gives figures for a similar experiment in which grafts were made higher up the stem of the stock so that the stock itself produced its characteristic foliage, which was also analyzed (cf. Figs. 1 (1) and 1 (2)). Again, no important change in cannabinoid content or composition has occurred.

The grafting of *C. sativa* (Kew or Thailand strains) on *Humulus japonicus* stock (Table 4)

caused no significant alteration in the composition of the mixture of cannabinoids. Total cannabinoid production, on the basis employed, was in fact stimulated by grafting relative to the control plants. Table 5 shows that a similar situation applies to the *C. sativa* (Thailand) on *Humulus lupulus* stock. There was some modification of the usual analytical characteristics in the base shoot from a *C. sativa* (Thailand) stock on which an *H. lupulus* specimen had been grafted. Δ^1 -Tetrahydrocannabinol content was lowered whilst the figures for Δ^1 -tetrahydrocannabivarol and the cannabichromen-cannabigerol mixture were higher than usual: the change, though interesting, is not drastic. All the plants used in the work reported in Table 5 were rather old, and the incursion of can-

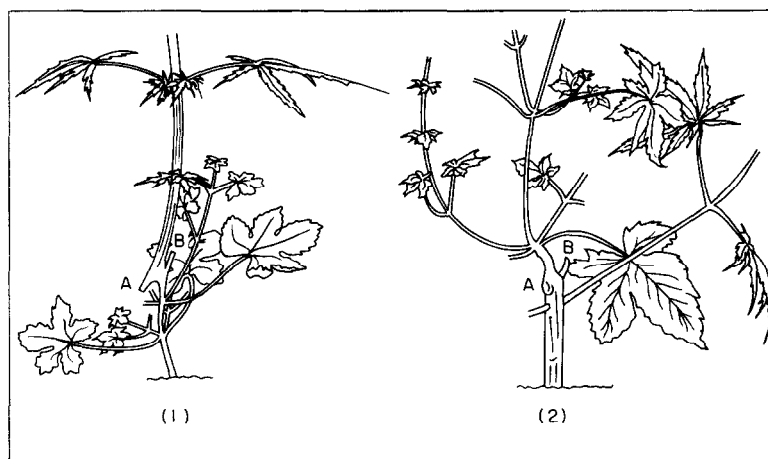


Fig. 1. Hop - *Cannabis sativa* grafts. (1) Kew strain of *Cannabis sativa* grafted onto *Humulus lupulus* scion. (A) Remains of lower part of *Cannabis* plant; (B) Remains of upper part of hop plant. (2) *Humulus japonicus* scion grafted onto Thailand strain of *Cannabis sativa* stock. (A) Remains of lower portion of hop; (B) Remains of upper part of *Cannabis* plant.

Table 4. Percentage composition and total amount of cannabinoids in *C. sativa* (Kew and Thailand) scions on *Humulus japonicus* stock*†

Cannabinoid	Thai control		Thai top		Kew control		Kew top	
	A	B	A	B	A	B	A	B
CD-C ₅	—	—	—	—	87	98	83	97
Δ ¹ -THC-C ₃	4	2	2	2	—	—	—	—
CC-C ₅ + CG-C ₅	5	6	8	14	5	1	5	1
Δ ¹ -THC-C ₅	91	92	90	84	8	1	12	2
Wt mg/g.	0.8	5.0	5.3	5.8	0.9	2.2	0.8	5.6

* Twelve weeks after completion of graft. Kew fruiting, Thailand vegetative.

† For abbreviations see Table 2.

Table 5. Percentage composition and total amounts of cannabinoids in *C. sativa* (Thailand)/*Humulus lupulus* grafts*†

Cannabinoid	Thai control		Thai top‡		Thai bottoms	
	A	B	A	B	A	B
Δ ¹ -THC-C ₃	1	1	1	1	5	10
CC-C ₅ + CG-C ₅	3	3	6	9	15	23
Δ ¹ -THC-C ₅	93	82	92	82	80	63
CN-C ₅	3	14	1	8	—	4
Wt mg/g.	2.3	5.7	8.6	4.0	3.6	2.3

* Eighteen weeks after completion of graft: Thailand fruiting.

† For abbreviations see Table 2.

‡ Thailand scion on *H. lupulus* stock.§ Single bottom shoot from the *C. sativa* (Thailand) stock on which *H. lupulus* had been grafted.

nabinol into the analysis occurred for the first time in these experiments.

As mentioned earlier, no cannabinoids were found in *H. japonicus* or *H. lupulus* control plants, nor in the scions grafted on *Cannabis* stock. Leaves produced on *H. japonicus* stock from below a *Cannabis* graft showed no cannabinoids, and neither did a fruiting specimen of *H. japonicus* grown on Kew strain *Cannabis* for 17 weeks. Compounds typical of *H. lupulus* [3] were not found in *Cannabis* scions grafted on hop stock.

The success of the grafting experiments between *C. sativa* and the two *Humulus* species is thus consistent with their close botanical relationship. *C. sativa* strains however, continue to produce essentially their own characteristic mixture of cannabinoids whether they are grafted with other strains of the same species, or with the two *Humulus* species, and whatever part of the graft system they form. There is no evidence of loss of vigour in the quantitative production of cannabinoids in the grafts—if anything, the reverse. Transport of special precursors or factors upwards or downwards

across the graft has apparently little influence on the composition and total amount of cannabinoid mixture which is generally considered to be formed and stored in the green leaves and bracts of *Cannabis*.

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