JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, July 2012, Vol. 57, No. 4
doi: 10.1111/j.1556-4029.2012.02068.x
Available online at: onlinelibrary.wiley.com

PAPER

CRIMINALISTICS

Benjamin De Backer,[1] *M.Sc.; Kevin Maebe,*[2] *M.Sc.; Alain G. Verstraete,*[2] *M.D., Ph.D.; and Corinne Charlier,*[1] *Ph.D.*

Evolution of the Content of THC and Other Major Cannabinoids in Drug-Type Cannabis Cuttings and Seedlings During Growth of Plants*

ABSTRACT: In Europe, authorities frequently ask forensic laboratories to analyze seized cannabis plants to prove that cultivation was illegal (drug type and not fiber type). This is generally done with mature and flowering plants. However, authorities are often confronted with very young specimens. The aim of our study was to evaluate when the chemotype of cannabis plantlets can be surely determined through analysis of eight major cannabinoids content during growth. Drug-type seedlings and cuttings were cultivated, sampled each week, and analyzed by high-performance liquid chromatography with diode array detection. The chemotype of clones was recognizable at any developmental stage because of high total Δ^9-tetrahydrocannabinol (THC) concentrations even at the start of the cultivation. Conversely, right after germination seedlings contained a low total THC content, but it increased quickly with plant age up, allowing chemotype determination after 3 weeks. In conclusion, it is not necessary to wait for plants' flowering to identify drug-type cannabis generally cultivated in Europe.

KEYWORDS: forensic science, forensic toxicology, cannabis, plant analysis, chemotype, tetrahydrocannabinol, high-performance liquid chromatography–diode array detection

Cannabis is the most frequently used illicit drug in the world. The cannabis plant can be cultivated in a wide range of environments, and it grows wild in many parts of the world. It is currently estimated that cannabis is cultivated in 172 countries and territories (1).

The chemistry of *Cannabis sativa* L. (Cannabaceae) has been studied extensively, and more than 525 different chemical substances have been isolated from the plant (2–6). The most interesting constituents of the plant are terpenophenolic compounds unique to cannabis, the cannabinoids. Approximately 109 cannabinoids are known to exist, concentrated in a resinous secretion that is produced by glandular trichomes distributed across most aerial surfaces of the plant, and particularly concentrated at the female inflorescence (7). The main biologically active cannabinoid is Δ^9-tetrahydrocannabinol, commonly referred as Δ^9-THC, known for its psychoactive properties (7–9). Another intensely studied cannabinoid is the nonpsychoactive cannabidiol (CBD) (10,11), characteristic of fiber-type cannabis.

The cannabinoids are actually biosynthesized in a carboxylated form in plant tissues. The most common types of acidic cannabinoids are Δ^9-tetrahydrocannabinolic acid A (THCA-A), cannabidiolic acid (CBDA), and cannabigerolic acid (CBGA). THC acid exists under two forms: THCA-A and THCA-B. Only traces of THCA-B can be detected in cannabis samples (12). THCA-A is therefore the major form and will be further referred to as THCA. CBGA is the direct precursor of THCA, CBDA, and cannabichromene acid (Fig. 1). Almost no neutral cannabinoid can be found in fresh plant material. However, the carboxyl group is not very stable and easily lost under influence of heat or light, resulting in the corresponding neutral cannabinoids (7). Most of these are formed slowly upon heating and drying of harvested plant material, during storage, and when the cannabis products are heated before or during consumption (i.e., smoking, vaporizing, making tea, or baked products) (7,9,13,14). In this article, the total content of THC or CBD present in the plant material corresponds to the sum of the free cannabinoid and its respective acidic precursor.

The variable conditions met during growing, processing, storage, and use of cannabis can also induce the formation of cannabinoids breakdown products. The most commonly found in aged cannabis is cannabinol (CBN), produced by oxidative degradation of THC (7,15). THC can also be transformed by isomerization to Δ^8-THC, which is an artifact (7).

According to total THC and total CBD contents, three main chemotypes (chemical phenotypes) of cannabis are recognized: drug-type plants (chemotype I) have a high [total THC/total CBD] ratio (>>1.0) with a total THC content over 0.3% of inflorescence dry weight and a CBD level lower than 0.5%; intermediate-type plants (chemotype II) show an intermediate ratio (close to 1.0, typically 0.5–2.0); and fiber-type plants (chemotype III) exhibit a low

[1]Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology, CIRM, CHU Sart-Tilman, University of Liège, B-4000 Liège, Belgium.

[2]Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium.

*Financial support provided by the Belgian Science Policy (Belspo), GEOCAN Project DR-00-48.

Received 22 Dec. 2010; and in revised form 22 April 2011; accepted 30 April 2011.

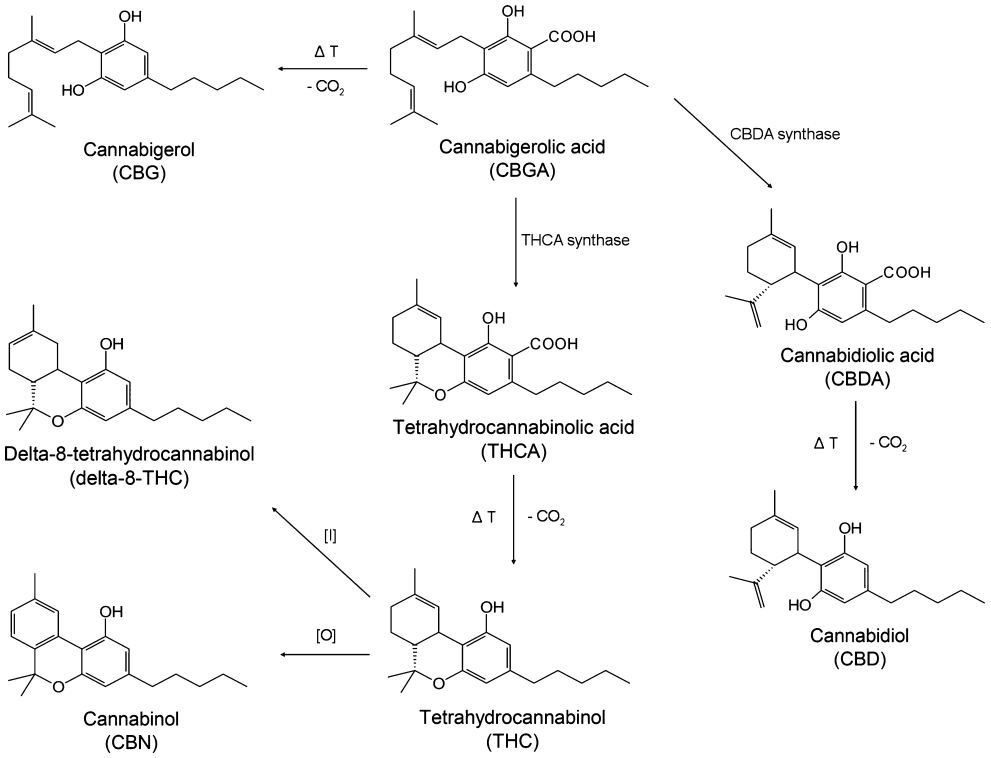

FIG. 1—*Biosynthetic pathway for acidic and neutral forms of THC, CBD, and CBG, and main THC breakdown products.* ΔT = heating, [O] = oxidation, [I] = isomerization.

[total THC/total CBD] ratio (<<1.0) (13,16). The latter, usually called "hemp," thus refers to varieties that have low THC concentrations (usually lower than 0.3%) but contain CBD as major compound. However, hemp includes also two later defined nonpsychoactive chemotypes: chemotype IV contains cannabigerol (CBG) as main cannabinoid (>0.3%); and chemotype V shows practically undetectable amounts of all cannabinoids (16).

In countries where hemp cultivation is allowed, the cultivars are tested to verify that the psychoactive potency is below an acceptable level. For granting permits to fiber hemp growers, the European Union requires that the total THC amount does not exceed 0.2% (dry weight of the inflorescence) (16,17).

Regarding drug-type cannabis, the last decades of the 20th century saw the appearance of a new problem in Europe: high-THC-yielding varieties of cannabis are now cultivated and produced locally, generally indoor under controlled conditions. Consequently, an increase of cannabis plant seizures was observed during the last years, for example, *c.* 15,000 cases were reported in Europe in 2007 with 2.4 million plants seized (18). In Belgium, law enforcement authorities frequently ask forensic laboratories to determine the chemotype of seized plants to prove that the cultivation is illegal. In general, the above-mentioned chemotype determination is done with female inflorescences of mature plants. However, police forces and laboratories do not always have adult plants for analysis. It is therefore interesting to consider chemotype identification in young specimens.

The aim of the study was to observe the evolution of major cannabinoid levels in high-THC-yielding varieties all along the growth of plants and therefore to evaluate when the chemotype of plantlets can be safely determined. Cannabis seedlings and cuttings were cultivated under controlled environmental conditions, and sampling was performed each week. According to a previously published method, total THC (THCA + THC), total CBD (CBDA + CBD), and CBN were quantitatively determined, whereas Δ^8-THC was qualitatively determined (19). Samples were analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD). HPLC-DAD was chosen because the thermal conversion of acidic cannabinoids occurring in gas chromatography (GC) systems was demonstrated to be incomplete and irreproducible (19,20). As only neutral forms of cannabinoids are detected after GC analysis, the partial conversion of acidic cannabinoids may cause an underestimation of the total cannabinoid content (20). With HPLC, samples are not heated. Acidic and neutral cannabinoids are therefore detected, and their concentrations are summed to obtain the total cannabinoid content.

Materials and Methods

Plant Material

Plants of *C. sativa* were provided by the Belgian Federal Police. The shoots were received *c.* 1 week after germination or after cutting from a mother plant and were cultivated from the earliest stage of development until harvest. We received three sets of plants coming from different seizures. The plants belonged to the same cannabis variety within each group. The first set (A) contained 13 young shoots grown from seeds. Five were female plants, five were males, and three of undetermined sex (harvested before flowering). The second set (B) consisted of seven young female clones coming from the same mother plant. The last set (C) comprised 45 female clones taken from another mother plant, among which 12 were cultivated until senescence stage.

The plants were cultivated in an indoor grow box in the laboratory during the year 2008. Environmental conditions were rigorously controlled. The cultivation conditions are listed in Table 1. The plants were grown under an indoor vegetative lights cycle

TABLE 1—*Cultivation conditions met in the grow room.*

Grow room	Home Box® L[a] (100 × 100 × 200 cm)	
Air extractor	Ruck® RVK 125L[b]; flow: 355 m^3/h	
Lighting	HPS (high-pressure sodium) lamp: Philips Green Power® 400 W[c]	
Potting soil	Sets A and B:	2/3 of Plagron® Light Mix[d] 1/3 of worm castings Plagron®
	Set C:	Plagron® All Mix
Nutrients	Deionized water added with organic, liquid fertilizers, and additives for growth and flowering (Plagron® Alga-Grow, Alga-Bloom, and Green Sensation)	
Temperature	17–32°C. Mean: ±25°C during the day	
Hygrometry	45–95%. Mean: ±60%	

[a]HomeBox, Berlin, Germany.
[b]Ruck Ventilatoren GmbH, Boxberg, Germany.
[c]Philips Belgium NV, Brussels, Belgium.
[d]Plagron, Ospel, The Netherlands.

(18-h light and 6-h dark) during 4–9 weeks depending on the set. When their sizes were appropriate, plants were then exposed to a flowering light cycle (12-h light and 12-h dark) until harvest. All along the development, the plants were gradually transplanted in increasingly large pots when it was necessary.

All the plants were periodically analyzed for their cannabinoid content. The samples, collected each week all along the growth, consisted of young shoots and leaves during the growing phase and inflorescences during flowering.

Sample Preparation and HPLC-DAD Conditions

Sample preparation and HPLC-DAD analysis were performed following the study by De Backer et al. (19). In accordance with ISO17025 and the guidelines of the French Society of Pharmaceutical Sciences and Techniques, this method was fully validated using the total error approach (21–24).

Briefly, plant material samples were dried for 18 h in a 35°C forced ventilation oven and then powdered, and 200 mg was extracted with 20 mL of methanol/chloroform (v/v: 9/1) by agitation during 30 min. The extract was filtered, diluted, then evaporated under a gentle stream of nitrogen and finally redissolved in 100 μL of a mixture of water/methanol (v/v: 5/5). Prazepam was used as internal standard.

All chromatographic runs were carried out using a Hewlett-Packard (HP) 1100–1200 series HPLC System (Agilent Technologies, Böblingen, Germany) coupled with a Waters (Zellik, Belgium) 2996 photodiode array detector (DAD). The wavelengths monitored were 211 and 220 nm for the neutral and the acidic cannabinoids, respectively. Chromatographic separations were achieved using a Waters XTerra® MS C18 analytical column (5 μm, 250 × 2.1 mm i.d.) protected by a Waters XTerra® MS C18 guard column (5 μm, 10 × 2.1 mm i.d.). The mobile phase consisted of a mixture of methanol/water containing 50 mM of ammonium formate (adjusted to pH 5.19). Initial setting was 68% methanol (v/v), which was linearly increased to 90.5% methanol over 25 min, then increased to 95% in 1 min. After maintaining this condition for 3 min, the column was set to initial condition in 1 min and re-equilibrated under this condition for 6 min. Flow rate was set to 0.3 mL/min, and the injection volume was 30 μL. All experiments were carried out at 30°C.

The levels of seven major cannabinoids (THCA, THC, CBDA, CBD, CBGA, CBG, and CBN) were quantified, and Δ^8-THC was determined qualitatively. THC, CBD, and CBN were purchased from LGC Standards (Molsheim, France); THCA, CBDA, CBGA, CBG, and Δ^8-THC were from Echo Pharmaceuticals BV (Weesp, the Netherlands). The limits of quantification (LOQ) and detection (LOD) are listed in Table 2. For more details and all validation results, see De Backer et al. (19).

TABLE 2—*Limits of quantification (LOQ) and limits of detection (LOD) of the method.*

	LOQ		LOD	
	%*	ng	%*	ng
THCA	0.05	12.5	0.025	6.25
THC	0.05	12.5	0.025	6.25
CBDA	0.05	12.5	0.05	12.5
CBD	0.075	18.75	0.075	18.75
CBGA	0.05	12.5	0.05	12.5
CBG	0.15	37.5	0.1	25
CBN	0.05	12.5	0.025	6.25

CBD, cannabidiol; CBDA, cannabidiolic acid; CBG, cannabigerol; CBGA, cannabigerolic acid; CBN, cannabinol; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid.
*Percentage of weight of dry plant material.

Results

Total THC Content During Growth

The evolution of the total THC content at different developmental stages during growth is shown in Fig. 2 for the three sets of cannabis plants. In set A, we received plants that were just germinated but at different developmental stages. The age of the plants was not exactly known, but we estimated it as comprised between 1 and 3 weeks postgermination. We therefore expressed, in Fig. 2*A*, the time as the number of weeks following the reception of the plants and not following the germination. In sets B and C, the time is expressed in weeks following the cutting of the clones.

One week after the reception, the young shoots of set A contained a total THC concentration (mean ± SD) of 0.21 ± 0.06% (percentage of weight of dry plant material) at the beginning of the vegetative state. Total THC content then increased to values (mean ± SD) comprised between 1.62 ± 0.08% and 2.08 ± 0.60% before the onset of flowering. The cannabinoid content was stable throughout the vegetative stage, before the appearance of flowers (Fig. 2*A*). In sets B and C, the total THC content was directly higher than in set A (week 1) because clones contain the same THC content as the mother plant from which they are taken. Total THC concentration values (mean ± SD) were comprised between 2.24 ± 0.99% and 3.33 ± 1.28% in set B, and between 2.16 ± 0.39% and 2.69 ± 0.35% in set C. In clones, total THC content was also stable all along the vegetative stage (Fig. 2*B*,*C*). During the reproductive stage, total THC content increased strongly with plant age and reached the highest level during the fifth or the sixth week postflowering in each set. Highest total THC concentrations (mean ± SD) were 18.91 ± 0.86%, 16.52 ± 3.02%, and 22.50 ± 2.65% in sets A, B, and C, respectively (Fig. 2). After a short plateau, the peak concentrations were followed by a decline in total THC content that is considered the onset of senescence of the plants. Only in set C, total THC content showed a decrease followed by an increase during the 14th and 15th weeks. This is probably due to discrepancies in the THC content between different parts of the plants sampled, and it would likely represent a plateau between the 13th and the 15th weeks.

Other Major Cannabinoids

Cannabidiol—Studying drug-type cannabis, we expected to find very small amounts of total CBD. However, CBD and CBDA were

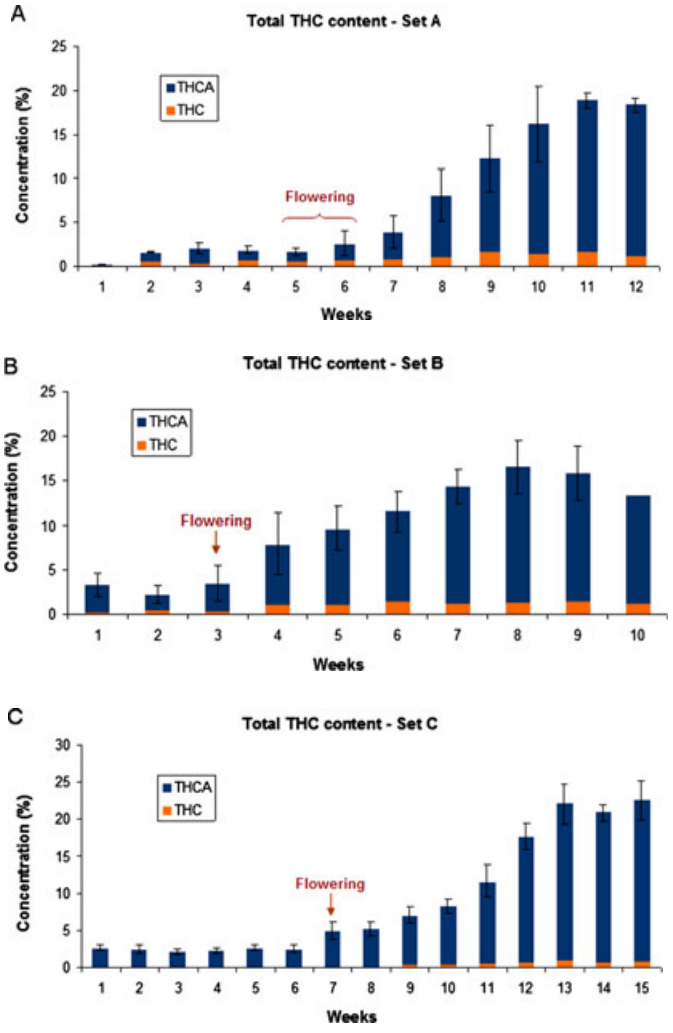

FIG. 2—*Evolution of the total THC (THC + THCA) content during the cannabis plant growth in the three sets. The bars represent the mean values of the THC and THCA concentrations at each time point ± the standard deviations of the total THC values. The onset of flowering is indicated by a bracket or an arrow. Set A:* n = *2 (weeks 1–2),* n = *8 (week 3),* n = *11 (week 4), n = 10 (week 5),* n = *7 (week 6), n = 5 (week 7), and* n = *4 (weeks 8–12). Set B:* n = *6 (weeks 1–2),* n = *5 (week 3),* n = *4 (week 4),* n = *3 (week 5),* n = *6 (weeks 6–8), n = 5 (week 9), and* n = *1 (week 10). Set C: n = 6 (week 1), n = 10 (week 2), n = 7 (week 3),* n = *8 (week 4),* n = *6 (week 5),* n = *14 (week 6),* n = *4 (week 7),* n = *12 (weeks 8–11),* n = *10 (week 12),* n = *11 (week 13),* n = *4 (week 14), and* n = *8 (week 15).*

never detected in the plants of sets A and B. If some CBD/CBDA was present, the levels reached were always below the LOD of the method for these two sets of plants. This is not surprising because in the Netherlands, the best varieties are selected for their high THC content and their low CBD production which may almost be suppressed in some cases after years of cross-breeding. Nevertheless, small amounts of CBDA were found in a few samples of set C at the end of the reproductive stage. At that moment, the plant produces more resinous secretion and the cannabinoid levels increase. During the 12th week, two samples of 12 contained CBDA levels above the LOD but below the LOQ. During the 15th week, five samples were positive. Only one sample contained a CBDA concentration equal to the LOQ (0.05%), whereas its total THC concentration was 26.4%.

Breakdown Products—CBN was found in only three samples, in very small amounts. Mean ± SD values were 0.09 ± 0.02% of CBN. On the other hand, Δ^8-THC was never detected in the samples. This indicates that our drying and storage methods were appropriate.

Cannabigerol—Similar to THC, an increase in total CBG content was observed in all sets of plants with plant growth. However, the concentrations found were low in comparison with total THC levels. For example in set C, CBG was not detected during the vegetative stage and appeared during the ninth week (third week of flowering) at concentrations below the LOQ (0.15%), which was never reached. CBGA was found from the fourth week until the end of the cultivation in higher concentrations. CBGA levels rose up from 0.11 ± 0.08% to 1.93 ± 0.22% during the flowering (12th week). A slight decline was observed during the three last weeks of cultivation. Results in the two other sets were similar.

Discussion

Cannabis cultivation in Europe is widespread and possibly increasing. In 2007, 19 European countries mentioned domestic cannabis cultivation, with big variations in the scale of the phenomenon (18). In this study, we analyzed drug-type cannabis varieties usually cultivated in Belgium and representative of Belgian high-scale illegal cultivations. We reproduced the cultivation conditions used by illegal indoor growers in Europe (Belgium, the Netherlands, the United Kingdom, etc.) as closely as possible. For example, we used the same growth material, the same type of lighting and nutrients, and we cultivated high-THC-yielding varieties. The plants were seized in Belgium, but these varieties were probably coming from the Netherlands because the vast majority of Belgian growers are buying their clones or seeds in that country. Another phenomenon is high-scale cannabis cultivation by Dutch traffickers on Belgian soil, using material coming from the Netherlands. Those Dutch varieties are mainly intended to be cultivated indoor for the production of sinsemilla ("Nederwiet").

It was previously reported that total THC content generally increases with cannabis plant age up to the highest level during the peak budding stage, reaching a plateau that is followed by a decrease during the onset of senescence (8). The results we obtained in this study are in accordance with those of Chandra et al. (8), even though they used a different cannabis variety originating from Mexico. The three sets of plants we studied were comparable in terms of yield of cannabinoids content. Changes in total THC concentration followed a similar pattern in the different sets during the vegetative and reproductive states. Although peak total THC content was somewhat different among the sets, the overall trend was the same and the discrepancies observed were probably due to various potencies of the cultivated varieties. The most important difference was seen at the very early stage of development between clones (sets B and C) and seedlings (set A). In the first case, cuttings have the same cannabinoid content as the mother plant from which they are taken. The mother plants used by growers are well developed, and therefore, the clone chemotype is immediately recognizable. In the case of drug-type cannabis clones, the maximum THC content allowed (0.2%) is exceeded at any developmental stage. Conversely, seedlings contain very low total THC concentration right after the germination. Vogelmann et al. (25) showed that THC appeared at very low concentrations after 60 h in seedlings of a drug strain of Mexican origin grown under vegetative greenhouse conditions. The THC level increased gradually during development and reached only 0.04 ± 0.01% after 6 days. In the seedlings we cultivated, the total THC concentration also took a few weeks to increase above 0.2%.

THC and THCA were the only cannabinoids detected when we received the seedlings. At that moment, the total THC level was already near 0.2% (0.21 ± 0.06%). Unfortunately, the exact age of the seedlings was not known, and they were probably not all sowed at the same moment. The seedling size was between 7 and 15 cm, and we evaluated their age as comprised between 1 and 3 weeks after germination. It is interesting to note that even though the plants did not have the same age, their total THC levels were very close. After 1 week of cultivation in our growth room, the total THC content increased sharply to a mean value (±SD) of 1.62% (±0.09%), and therefore, there was no doubt concerning the plant's chemotype because no CBD/CBDA was found.

It may be difficult to determine the plant's chemotype during or right after the germination. But in high-potency varieties, the maximum THC content allowed for fiber-type cannabis cultivation can be quickly reached. We can consider that 3 weeks after germination, the chemotype of a high-THC-yielding variety can be safely determined. This corresponds to plantlets being at third or fourth leaf stage, under our cultivation conditions. This means that it is not necessary to wait for plants' flowering to analyze female buds and identify drug-type cannabis.

The results of this study are in accordance with previous reports despite discrepancies in the described procedures, for example, a different type of cannabis cultivated (16), plantations in greenhouses under environmental conditions or analysis of plant samples after only 28 days of growth (16,26). In this study, we cultivated highly potent cannabis varieties ("Nederwiet") and reproduced indoor controlled cultivation conditions used in Belgian illegal plantations. The plants were sampled and analyzed weekly, from the first week following seedling or clone cutting until senescence. We agree with Pacifico et al. (16) who postulated that no plant intended for the effective production of THC and marijuana will develop a low THC/CBD ratio typical of fiber hemp, irrespective of the development stage; vice versa, no fiber-type plant will show a high THC/CBD ratio typical of drug plants whatever the moment of the analysis.

In conclusion, the data presented in this study show that the chemotype of high-THC-yielding varieties can be determined at a young age and is stable throughout the life of the plants. In addition, it has to be pointed out that the high total THC concentrations observed in this study likely represent the strong potency of cannabis varieties cultivated in Western Europe at the present time.

Acknowledgment

The authors are very grateful to the Belgian Federal Police for providing seizures of cannabis products and growth material.

References

1. United Nations Office on Drugs and Crime. World drug report. New York, NY: United Nations Publications, 2009.
2. ElSohly MA, Slade D. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci 2005;78(5):539–48.
3. Radwan MM, Ross SA, Slade D, Ahmed SA, Zulfiqar F, Elsohly MA. Isolation and characterization of new cannabis constituents from a high potency variety. Planta Med 2008;74(3):267–72.
4. Ahmed SA, Ross SA, Slade D, Radwan MM, Zulfiqar F, Matsumoto RR, et al. Cannabinoid ester constituents from high-potency *Cannabis sativa*. J Nat Prod 2008;71(4):536–42.
5. Radwan MM, Elsohly MA, Slade D, Ahmed SA, Khan IA, Ross SA. Biologically active cannabinoids from high-potency *Cannabis sativa*. J Nat Prod 2009;72(5):906–11.
6. Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, et al. Potency trends of Delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. J Forensic Sci 2010;55(5):1209–17.
7. Hazekamp A. Cannabis; extracting the medicine [thesis]. Leiden (the Netherlands): Universiteit Leiden, 2007.
8. Chandra S, Lata H, Mehmedic Z, Khan IA, ElSohly MA. Assessment of cannabinoids content in micropropagated plants of *Cannabis sativa* and their comparison with conventionally propagated plants and mother plant during developmental stages of growth. Planta Med 2010;76(7):743–50.
9. Giroud C. Analysis of cannabinoids in hemp plants. Chimia 2002;56(3):80–3.
10. Russo E, Guy GW. A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses 2006;66(2):234–46.
11. Zuardi AW, Crippa JAS, Hallak JEC, Moreira FA, Guimaraes FS. Cannabidiol, a *Cannabis sativa* constituent, as an antipsychotic drug. Braz J Med Biol Res 2006;39(4):421–9.
12. Lehmann T, Brenneisen R. High performance liquid chromatographic profiling of Cannabis products. J Liq Chromatogr 1995;18(4):689–700.
13. Hillig KW, Mahlberg PG. A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). Am J Bot 2004;91(6):966–75.
14. Doorenbos NJ, Fetterman PS, Quimby MW, Turner CE. Cultivation, extraction, and analysis of *Cannabis sativa* L. Ann N Y Acad Sci 1971;191:3–12.
15. Stolker AAM, van Schoonhoven J, de Vries AJ, Bobeldijk-Pastorova I, Vaes WHJ, van den Berg R. Determination of cannabinoids in cannabis products using liquid chromatography-ion trap mass spectrometry. J Chromatogr A 2004;1058(1–2):143–51.
16. Pacifico D, Miselli F, Carboni A, Moschella A, Mandolino G. Time course of cannabinoid accumulation and chemotype development during the growth of *Cannabis sativa* L. Euphytica 2008;160(2):231–40.
17. Hewavitharana AK, Golding G, Tempany G, King G, Holling N. Quantitative GGMS analysis of delta(9)-tetrahydrocannabinol in fiber hemp varieties. J Anal Toxicol 2005;29(4):258–61.
18. European Monitoring Center for Drugs and Drug Addiction. Annual report 2009: the state of the drugs problem in Europe. Luxembourg: European Monitoring Center for Drugs Addiction, 2009.
19. De Backer B, Debrus B, Lebrun P, Theunis L, Dubois N, Decock L, et al. Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877(32):4115–24.
20. Dussy FE, Hamberg C, Luginbuhl M, Schwerzmann T, Briellmann TA. Isolation of delta(9)-THCA-A from hemp and analytical aspects concerning the determination of delta(9)-THC in cannabis products. Forensic Sci Int 2005;149(1):3–10.
21. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007;24(10):1962–73.
22. Boulanger B, Chiap P, Dewe W, Crommen J, Hubert P. An analysis of the SFSTP guide on validation of chromatographic bioanalytical methods: progress and limitations. J Pharm Biomed Anal 2003;32(4–5):753–65.
23. Hubert P, Nguyen-Huu JJ, Boulanger B, Chapuzet E, Chiap P, Cohen N, et al. Harmonization of strategies for the validation of quantitative analytical procedures. A SFSTP proposal—part I. J Pharm Biomed Anal 2004;36(3):579–86.
24. Hubert P, Nguyen-Huu JJ, Boulanger B, Chapuzet E, Cohen N, Compagnon PA, et al. Harmonization of strategies for the validation of quantitative analytical procedures. A SFSTP proposal—part III. J Pharm Biomed Anal 2007;45(1):82–96.
25. Vogelmann AF, Turner JC, Mahlberg PG. Cannabinoid composition in seedlings compared to adult plants of *Cannabis sativa*. J Nat Prod 1988;51(6):1075–9.
26. Broseus J, Anglada F, Esseiva P. The differentiation of fibre- and drug type cannabis seedlings by gas chromatography/mass spectrometry and chemometric tools. Forensic Sci Int 2010;200(1–3):87–92.

Additional information and reprint requests:
Benjamin De Backer, M.Sc.
Centre Hospitalier Universitaire Sart-Tilman B 35
B 4000 Liège
Belgium
E-mail: bdebacker@chu.ulg.ac.be