TETRAHYDROCANNABINOL LEVELS IN HEMP (*CANNABIS SATIVA*) GERMPLASM RESOURCES[1]

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Small, Ernest *(Eastern Cereal and Oilseed Research Centre, Research Branch, Agriculture and Agri-Food Canada, Central Experimental Farm, Ottawa, ON, Canada K1A 0C6; email: smalle@agr.gc.ca),* **and David Marcus** *(Natural Hemphasis, 43 Melville Ave., Toronto, ON, Canada M6G 1Y1).* TETRAHYDROCANNABINOL LEVELS IN HEMP (*CANNABIS SATIVA*) GERMPLASM RESOURCES. *Economic Botany 57(4):000–000, 2003. In most of the western world where industrial hemp,* Cannabis sativa, *is licensed for cultivation, the plants must not exceed a level of 0.3% tetrahydrocannabinol (THC), the principal intoxicating constituent of the species. Because there are no publicly available germplasm hemp collections in North America and only a very few, recent North American cultivars have been bred, the future breeding of cultivars suitable for North America is heavily dependent on European cultivars and European germplasm collections. Based mostly on material from Europe, this study surveyed THC levels of 167 accessions grown in southern Ontario, making this the largest survey to date of germplasm intended for breeding in North America. Forty-three percent of these had THC levels 0.3% and, therefore, are unsuitable for hemp development in North America. Discrepancies were found between THC levels reported for some germplasm holdings in Europe when they were grown in Canada and, accordingly, verification of THC levels developed in North America is necessary.*

Key Words: Hemp; industrial hemp; marijuana; *Cannabis sativa*; Cannabaceae; tetrahydrocannabinol; THC; germplasm.

The ancient cultigen hemp (*Cannabis sativa* L.), grown under license in Canada, is the most prominent ''new'' crop in North America. Until very recently the prohibition against drug forms of the plant prevented cultivation of fiber and oilseed cultivars in Canada. However, in the last 5 years, three key developments occurred: 1) recent advances in the legal cultivation of hemp in western Europe, especially for new value-added products, were widely publicised; 2) enterprising farmers and farm groups became convinced of the agricultural potential of hemp in Canada, and obtained permits to conduct experimental cultivation; and 3) lobby groups convinced the Parliament of Canada that narcotic forms of the hemp plant are distinct and distinguishable from fiber and oilseed forms. In March 1998, new regulations under the Controlled Drugs and Substances Act were adopted to allow the commercial development of a hemp industry in Canada, and since then hundreds of licenses have been issued and thousands of hectares have been cultivated. Information on the commercial potential of hemp in Canada is found in Blade (1998), Marcus (1998), and Pinfold Consulting (1998). In the U.S., experimental hemp cultivation was recently carried out in Hawaii, but despite widespread interest in reestablishing the industry, there is strong official opposition. Nevertheless, there have been several feasibility analyses, for example Ehrensing (1998) and McNulty (1995), of the possibility of growing hemp in the U.S. Small and Marcus (2002) reviewed the potential of hemp and new hemp products for both Canada and the U.S.

Both in Canada and the U.S. a critical problem to be addressed for commercial exploitation of *C. sativa* is the possible unauthorized drug use of the plant. Indeed, one of the reasons hemp cultivation ceased in North America was concern that the hemp crop was a drug menace. The drug potential is, for practical purposes, measured by the presence of the constituent ((6aR,10aR)-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol), or

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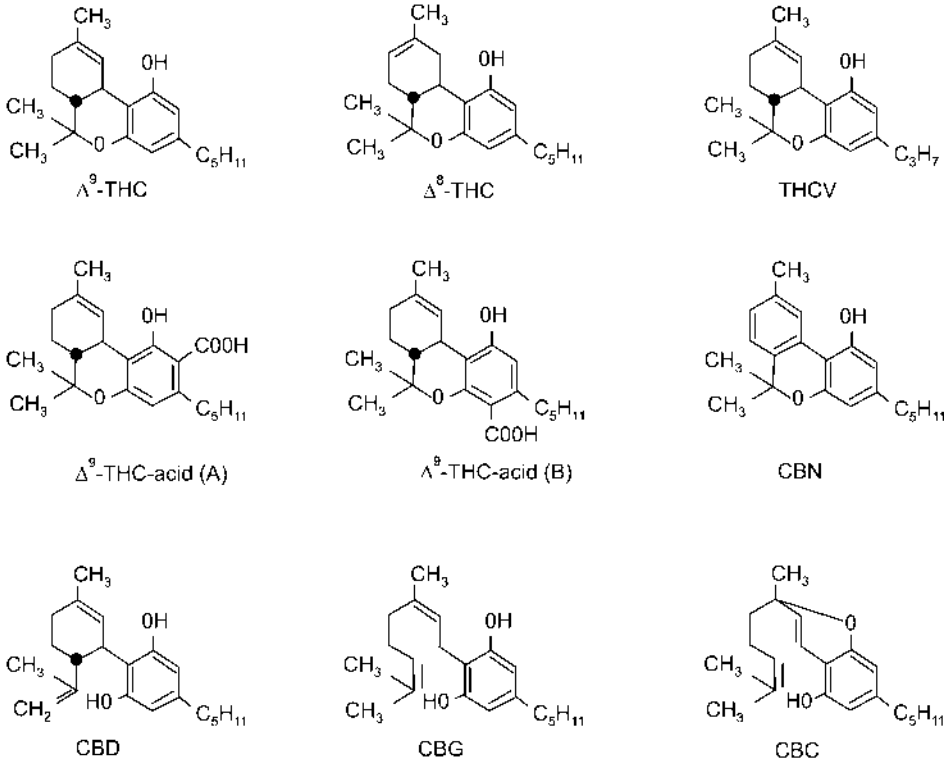

Fig. 1. Molecular diagrams of cannabinoids mentioned in this paper. Δ^9-THC = delta^9-tetrahydrocannabinol, Δ^8-THC = delta^8-tetrahydrocannabinol, THCV = tetrahydrocannabiverol, Δ^9-THC-acid = delta^9-tetrahydrocannabinolic acid (forms A and B shown), CBN = cannabinol, CBD = cannabidiol, CBG = cannabigerol, CBC = cannabichromene.

more simply delta^9-tetrahydrocannabinol (Δ^9-THC, hereafter simply THC, Fig. 1). The designation delta-9 employs formal chemical nomenclature for pyran-type compounds. In an alternative nomenclature system often employed in Europe, based on regarding the cannabinoids as substituted monoterpenoids, this is known as Δ^8-THC (Fig. 1). A second intoxicating isomer, Δ^8-THC, is much less abundant in *C. sativa*, occurring only in trace amounts, if at all, and is somewhat less potent than Δ^9-THC. Still another homologue, occasionally found in large amounts, is tetrahydrocanabiverol (THCV, Fig. 1), which has also been reported to be less psychoactive than THC. Cannabinol (CBN, Fig. 1), a degeneration or transformation product produced when THC ages, has been said to have some limited euphoriant activity (Clarke 1998a), although most literature states that this cannabinoid is not euphoriant. Cannabichromene (Fig. 1) is a frequent minor constituent of highly-intoxicating strains of *C. sativa*, especially from Africa, and cannabigerol (CBG, Fig. 1) rarely dominates the resin of *Cannabis*; neither is considered euphoriant. Potential interactions of the cannabinoids, particularly between THC and cannabidiol (CBD), and also perhaps with various terpenes, modify the psychological and physiological effects of cannabis drugs (see reviews by Clarke 1998a, McPartland 2000). In the living plant, the cannabinoids are mostly carboxylated (i.e., a carboxyl group is attached) and not effective psychologically. Heat (as provided when marijuana is smoked or cooked in brownies) and/or aging decarboxylate the cannabinoids.

A variety of terms, none of which is completely satisfactory, has been used to denote the euphoric psychological effects of marijuana in general and THC in particular. Psychoactive is widely used, but is so general it applies to a very wide variety of psychological states. Narcotic is also widely used, but has several distinctive meanings, referring alternatively to substances

of abuse, substances defined in law as abusive, and substances that produce sleep. The resin of intoxicant types of plant is dominated by THC, whereas non-intoxicant types have resin dominated by CBD, and while THC is not narcotic in the sense of producing sleep, CBD has been shown to have sleep-inducing properties (Carlini and Cunha 1981), consequently the term narcotic is rather contradictorily applied exclusively to the intoxicant types (at least in a pharmacological sense). Psychotomimetic (mood-altering) is perhaps the most appropriate pharmacological term. Psychotropic, meaning mind-altering, is also used, but both intoxicant and non-intoxicant types of *Cannabis* can influence the mind by virtue of the properties of THC and CBD. Hallucinogenic is also used, but less appropriately since true hallucinogens are rarely produced.

Industrial hemp is a phrase that has become common to designate hemp used for commercial non-intoxicant purposes. Small and Cronquist (1976) split *C. sativa* into two subspecies: *C. sativa* subsp. *sativa*, with less than 0.3% (dry weight) of THC in the upper (reproductive) part of the plant, and *C. sativa* subsp. *indica* (Lam.) E. Small & Cronq. with more than 0.3% THC. This classification has since been adopted in the European Community and Canada, and most areas of Australia, as a dividing line between cultivars that can be legally cultivated under licence and forms that are considered to have too high a drug potential. In the U.S., the 0.3% dividing line has been used to discriminate some hemp products that can be imported from products that can not; however, a U.S. Treasury Department/ Customs Service memo dated 30 December, 1999, suspended the policy in effect up to that time allowing the importation into the U.S. of sterilized hemp seed and other hemp products containing no more than 0.3% THC. Marijuana in the illicit market typically has a THC content of 5 to 10% (levels as high as 25% have been reported), and as a point of interest, a current Canadian government experimental medicinal marijuana production contract calls for the production of 6% marijuana. A level of about 1% THC is considered the threshold for marijuana to have intoxicating potential (Grotenhermen and Karus 1998), so the 0.3% level is conservative, and some countries (rarely in Australia, commonly in Switzerland) have permitted the cultivation of cultivars with higher levels. It should be appreciated that there is considerable variation in THC content in different parts of the plant (THC content increases in the following order: achenes (excluding bracts), roots, large stems, smaller stems, older and larger leaves, younger and smaller leaves, flowers, perigonal bracts covering the female flowers and fruits), and that it is well known in the illicit trade how to screen off the more potent fractions of the plant in order to increase THC levels in resultant drug products. Nevertheless, a level of 0.3% THC in the flowering parts of the plant is reflective of material that is too low in intoxicant potential to actually be used practically for illicit production of marijuana or other types of cannabis drugs.

A much lower level of THC is allowed in human food products manufactured from the seeds—currently 10 ppm in seeds and oil products used for food purposes in Canada and in much of the European Community. This is because of alleged toxicity and conjectured interference with drug tests, and because hemp food products are considered to have great economic potential, there is great pressure on the hemp industry in North America to reduce THC levels. Clearly THC analyses are critical to considering germplasm that can be used for the future development of hemp.

Breeding for low THC cultivars in Europe has been reviewed by Bócsa (1999), Bócsa and Karus (1998), and Virovets (1996). Some researchers have claimed to have produced essentially THC-free strains, although at present no commercial cultivar seems to be 100% free of THC. There is certainly a need to utilize available germplasm sources in order to breed suitable cultivars for North America. However, the introgression of novel genes from diverse kinds of hemp into commercial cultivars is hampered by the fact that most hemp germplasm sources almost inevitably will have some THC. Nevertheless, the goal of producing THC-free cultivars does not eliminate the value of germplasm sources that retain their capacity for THC production.

Relatively little information is available on the inheritance of THC, although it is clear that it is polygenic. Based on over two dozen combinations of different accessions, Small and Beckstead (1979) found that the majority of first generation hybrid crosses were intermediate in THC content between their respective parents, showing no dominance toward either parent (cf.

TABLE 1. *CANNABIS* ACCESSION AND THC CONTENT.

Code[a]	Source	Cultivar or status	Percent THC: Canada 1999	Percent THC: Canada 2000	Percent THC: Russia[b]
1	Vavilov[c] 141 (Russia)	Land race	<0.1		**0.582**[d]
2	Vavilov 152 (Russia)	Land race	<0.1		
3	Vavilov 155 (Russia)	Land race	<0.1		0.192
4	Vavilov 167 (Russia)	Land race	<0.1		
5	Vavilov 497 (Ukraine)	'YUSO-22'	<0.1		**0.94**
6	Vavilov 499 (Ukraine)	'YUSO-14'	<0.1		0.18
7	Vavilov 501 (Ukraine)	'YUSO-19'	<0.1		0.18
8	Vavilov 503 (Ukraine)	'YUSO-21'	<0.1		0.14
9	Vavilov 541 (Ukraine)	'YUSO-31'	<0.1		
10	Vavilov 542 (Ukraine)	'YUSO-33'	<0.1		
11	Gatersleben[e] D2244 (Slovak Republic)	Land race	**0.3**		
12	Gatersleben D2890 (Hungary)	Land race	<0.1		
13	Gatersleben D4176 (Italy)	Land race	<0.1		
14	Gatersleben D4217 (Korea)	Land race	**0.4**		
15	Gatersleben D4140 (Romania)	Land race	<0.1		
16	Gatersleben D4554 (Georgia)	Land race	<0.1		
17	Gatersleben D4738 (Korea)	Land race	**0.4**		
18	Gatersleben D5055 (Italy)	Land race	0.1		
20	Gatersleben D5564 (Turkey)	Land race	<0.1		
21	Gatersleben D5747 (unknown)	Land race	**0.5**		
22	Gatersleben D5802 (Romania)	Land race	0.2		
24	Gatersleben D6407 (Romania)	Land race	0.2		
25	Gatersleben D6409 (Romania)	Land race	<0.1		
26	Gatersleben D6416 (Romania)	Land race	<0.1		
27	Gatersleben D6417 (Romania)	Land race	<0.1		
28	Gatersleben D6419 (Romania)	Land race	<0.1		
29	Gatersleben D6858 (unknown)	Land race	**0.7**		
30	Gatersleben D6866 (France)	Land race	**0.4**		
31	Gatersleben D6406 (Romania)	Land race	<0.1		
32	Gatersleben D7160 (China)	Land race	<0.1		
33	Carolinae University Botanical Garden (Czech Republic)	Land race	<0.1		
34	Agritec Ltd. (Czech Republic)	'Beniko'	<0.1		
35	Agritec Ltd. (Czech Republic)	'Bialobrzezski'	<0.1		
36	Agritec Ltd. (Czech Republic)	'YUSO-11'	<0.1		
37	Escola Superior d=Agricultura de Barcelona (Spain)	'Delta 405'	<0.1		
38	J.D. Spanring, Ljubljana (Slovenia)	'Rudnik A16'	**0.3**		
39	J.D. Spanring, Ljubljana (Slovenia)	'Rudnik T17'	0.1		
40	J.D. Spanring, Ljubljana (Slovenia)	'Rudnik A16'	0.2		
41	J.D. Spanring, Ljubljana (Slovenia)	'Pesnica'	<0.1		
42	J.D. Spanring, Ljubljana (Slovenia)	'Gazvoda'	<0.1		
43	Agra Seeds. Inc. (Canada)	'Fasamo'	<0.1	0.09	
44	Utrecht University Botanic Garden (Netherlands)	Land race	**0.3**		
45	GEN-X Research Inc. (Canada)	'Fin 314' (= 'Finola')	<0.1	0.06	
46	Agric. Res. Inst., Kompolt (Hungary)	'Kompolti'	<0.1	0.08	
47	Agric. Res. Inst., Kompolt (Hungary)	'Uniko-B'	<0.1		
48	Agric. Res. Inst., Kompolt (Hungary)	'Kompolti' (hybrid TC)	<0.1		
49	Agric. Res. Inst., Kompolt (Hungary)	'Fibriko'	<0.1	**0.68**	
50	Agric. Res. Inst., Kompolt (Hungary)	Hybrid: 'Lipko' ('Fibr' × 'Uniko-B')	<0.1		

TABLE 1. Continued.

Code[a]	Source	Cultivar or status	Percent THC		
			Canada		
			1999	2000	Russia[b]
51	Agric. Res. Inst., Kompolt (Hungary)	Hybrid: F × T, F1	<0.1		
52	Agric. Res. Inst., Kompolt (Hungary)	Hybrid: K. monecious × K. unisex	0.2		
53	Agric. Res. Inst., Kompolt (Hungary)	Hybrid: Fibrimon × (F×T)	<0.1		
54	Agric. Res. Inst., Kompolt (Hungary)	Hybrid: 'Fibrimon' × K. unisex	<0.1		
55	Kenex (Canada)	'Ferimon 12'	<0.1	0.14	
56	Kenex (Canada)	'Fedora 19'	<0.1	0.16	
57	Kenex (Canada)	'Felina 34'	0.1	0.13	
58	Kenex (Canada)	'Fedrina 74'	<0.1	0.18	
59	Kenex (Canada)	'Futura 77'	<0.1	0.28	
60	Kenex (Canada)	'Uniko B'	<0.1	**0.31**	
61	CGP (Canada)	'Zolotonosha 11' (super elite)	<0.1	0.06	
62	CGP (Canada)	'Zolotonosha 15' (elite)	<0.1	<0.05	
63	CGP (Canada)	'Fedora 19'		0.24	
64	CGP (Canada)	'YUSO 14' (super elite)	<0.1	<0.05	
65	CGP (Canada)	'YUSO 31' (elite)	<0.1	<0.05	
66	Norddeutsche Pflanzenzucht, Hohenlieth (Germany)	'Fasamo'		0.05	
68	Kunming Institute Botany (China)	Land race		0.06	
70	Brighton, Ont. (Canada)	Spontaneous		**0.65**	
72	Vavilov 58 (Russia)	Land race		0.14	
73	Vavilov 65 (Ukraine)	Land race		0.13	
74	Vavilov 66 (Russia)	'Krasnodarskaya 14'		0.14	
75	Vavilov 74 (Russia)	Land race		0.22	**0.636**
76	Vavilov 81 (Latvia)	Land race		**1.17**	
77	Vavilov 82 (Ukraine)	'Glukhovshaya 2'		**0.33**	
78	Vavilov 83 (Ukraine)	''Hybrid19''		**0.38**	
79	Vavilov 84 (Ukraine)	''Hybrid-3''		**1.76**	
80	Vavilov 90 (Russia)	Land race		0.21	
81	Vavilov 140 (Russia)	Land race		**0.85**	
82	Vavilov 147 (Ukraine)	Land race		0.15	**1.115**
83	Vavilov 190 (Ukraine)	'Zolotonoshskaya1'		**0.35**	
84	Vavilov 191 (Ukraine)	'Zolotonosha' (Hybrid 49)		**1.16**	
85	Vavilov 192 (Ukraine)	'Zolotonosha'–'Chuiskaya' × 'Toguchinsk'		**0.85**	
86	Vavilov 193 (Ukraine)	'Zolotonosha'–'Ferraloniya' × 'Toguchinsk'		**0.37**	
87	Vavilov 196 (Russia)	Land race		**0.87**	
88	Vavilov 206 (Ukraine)	Land race		**0.64**	**1.840**
89	Vavilov 209 (France)	'Chenevis'		**0.34**	**0.965**
90	Vavilov 210 (Romania)	'I.C.A.R.42/118'		**1.55**	**0.400**
91	Vavilov 211 (Germany)	''Monoecious 78142''		0.18	
92	Vavilov 212 (Germany)	''G-3''		0.23	
93	Vavilov 226 (Germany)	''Monoecious 4555''		0.11	0.192
94	Vavilov 294 (Ukraine)	'Pavlogradskaya'		**0.41**	**2.300**
95	Vavilov 307 (Ukraine)	'Poltavskaya'		**1.92**	
96	Vavilov 308 (Ukraine)	'Glukhovskaya 1'		**0.49**	
97	Vavilov 309 (Ukraine)	'Glukhovskaya 3'		**0.98**	
98	Vavilov 312 (Ukraine)	Land race		**0.68**	
99	Vavilov 340 (Bulgaria)	''G-3''		**0.98**	1.22
100	Vavilov 342 (Russia)	'Odnodomnaya Yuzkhnaya'		**1.08**	1.103
101	Vavilov 364 (Hungary)	'Kompolti'		**2.43**	
102	Vavilov 365 (Hungary)	''B-7''		0.24	0.635

TABLE 1. Continued.

Code[a]	Source	Cultivar or status	Percent THC: Canada 1999	Percent THC: Canada 2000	Percent THC: Russia[b]
103	Vavilov 366 (Romania)	'Lovrin'		**0.37**	**1.416**
104	Vavilov 367 (Germany)	'Bernburg' (monecious)		0.09	
105	Vavilov 371 (Sweden)	'Svalefskaya 55/703'		**0.64**	
106	Vavilov 391 (Ukraine)	'YUSO-6'		0.28	**0.499**
107	Vavilov 392 (Ukraine)	''Monecious 12''		0.19	
108	Vavilov 394 (Estonia)	'Iygeva 525'		0.09	
110	Vavilov 396 (Hungary)	'Kompolti 13-7' (hybrid)		**0.41**	**0.609**
111	Vavilov 398 (Turkey)	'Fatza'		**0.38**	**0.870**
112	Vavilov 399 (Turkey)	'Unya'		0.11	**0.450**
113	Vavilov 400 (France)	'Fibrimon'		0.11	**0.384**
114	Vavilov 407 (Hungary)	'Vengerskaya'		0.15	0.105
115	Vavilov 409 (France)	'Fibrimon'		**0.53**	
117	Vavilov 426 (Ukraine)	'YUSO-13'		**0.69**	
118	Vavilov 427 (Ukraine)	'Zolotonoshskaya 1'		**0.49**	
119	Vavilov 428 (Ukraine)	'Zolotonoshskaya 2'		**0.69**	
120	Vavilov 429 (Ukraine)	'YUSO-1'		**0.49**	
121	Vavilov 431 (Russia)	'Krasnodarskaya 2'		**0.52**	
122	Vavilov 432 (Russia)	'Krasnodarskaya 3'		**0.36**	
123	Vavilov 434 (Ukraine)	'Zolotonoshskaya'		**0.74**	
124	Vavilov 435 (France)	'Fibrimon'		**0.41**	**1.148**
125	Vavilov 436 (Hungary)	'Fertodi'		0.12	0.093
126	Vavilov 437 (Hungary)	'Szegedi 9'		0.14	0.123
127	Vavilov 440 (Germany)	'Fibridia 4'		**2.10**	
128	Vavilov 441 (Italy)	'Carmagnola'		**0.38**	**0.302**
129	Vavilov 442 (Yugoslavia)	'Novosadska'		**1.70**	0.230
130	Vavilov 444 (China?)	'Vengerskaya?'		**0.48**	**1.98**
131	Vavilov 445 (Hungary)	'Uniko'		**1.23**	0.220
133	Vavilov 447 (France)	'Fibrimon'		**0.56**	0.240
134	Vavilov 448 (Hungary)	'Kompolti'		**1.52**	0.101
135	Vavilov 449 (Hungary)	'Szegedi 9'		**0.43**	0.150
136	Vavilov 452 (France)	'Fibrimon 56'		0.20	
137	Vavilov 451 (France)	'Fibrimon 24'		0.15	**0.980**
138	Vavilov 452 (France)	'Fibrimon 56'		**0.40**	
139	Vavilov 453 (Hungary)	''B-7 hybrid''		**0.50**	
140	Vavilov 454 (Hungary)	''B7 ES hybrid''		0.20	
141	Vavilov 458 (Ukraine)	'Glukhovskaya-10'		0.24	**0.650**
142	Vavilov 472 (Armenia)	Land race		0.18	
143	Vavilov 484 (Kazakhstan)	Wild		**0.41**	0.08
144	Vavilov 492 (Russia)	'Maikopskaya-2'		**1.03**	
145	Vavilov 493 (Ukraine)	'Glukhovskaya-10'		**1.27**	0.05
146	Vavilov 495 (Ukraine)	'YUSO-11'		**0.30**	0.10
147	Vavilov 496 (Ukraine)	'YUSO-12'		**0.79**	**0.51**
148	Vavilov 504 (Ukraine)	'YUSO-23'		0.15	**0.67**
149	Vavilov 505 (Ukraine)	'Sozrevayushchaya-24'		**1.73**	
150	Vavilov 507 (Russia)	'Krasnodarskaya 10 FB FB'		**0.64**	**1.90**
151	Vavilov 508 (Russia)	'Krasnodarskaya 56'		0.28	
152	Vavilov 511 (Ukraine)	'Dneprovskaya 5'		**0.44**	
153	Vavilov 518 (Russia)	'Dneprovskaya 1'		**0.88**	
154	Vavilov 520 (Ukraine)	'YUSO-28'		0.17	
155	Vavilov 521 (Russia)	'Maikop 2' × 'Odnod K232'		0.28	
156	Vavilov 540 (Ukraine)	'YUSO-29'		**0.40**	
157	Vavilov 543 (Ukraine)	'YUSO-35'		0.20	

TABLE 1. Continued.

Code[a]	Source	Cultivar or status	Percent THC Canada 1999	Percent THC Canada 2000	Russia[b]
158	Vavilov 544 (Ukraine)	'YUSO-36'		**0.74**	
159	Vavilov 545 (Ukraine)	'YUSO-37'		0.26	
160	Vavilov 546 (Ukraine)	'YUSO-38'		0.20	
161	Vavilov 550 (Russia)	'Vengriya' × 'Odnod K-432'		**0.34**	
162	Vavilov 554 (Daghestan)	Land race		**0.36**	
163	Vavilov 555 (Ukraine)	'Zolotonoshskaya-11'		0.17	
164	Vavilov 556 (Hungary)	Land race		0.25	
165	Vavilov 558 (Ukraine)	''Zolotonoshskaya-11' × 'YUSO'		**0.49**	
166	Vavilov 559 (Ukraine)	'Zolotonoshskaya-19'		**0.89**	
167	Vavilov 560 (Ukraine)	'Dneprovskaya 1'		**0.47**	
168	Vavilov 561 (Ukraine)	'Dneprovskaya 8'		0.18	
169	Vavilov 562 (Ukraine)	'Dneprovskaya 84'		**0.43**	
170	Vavilov 566 (Ukraine)	'Dneprovskaya'		0.07	
171	Vavilov 572 (Russia)	Wild		0.26	
172	Vavilov 575 (Russia)	Land race		0.09	
173	Xinjiang Institute Ecology & Geography (China)	Land race		**0.43**	
174	Arnprior, Ont. (Canada)	Ruderal		0.27	
175	Cobourg, Ont. (Canada)	Ruderal		0.12	

[a] Herbarium vouchers of E. Small were deposited at the herbarium of the Canada Department of Agriculture Herbarium, Ottawa (DAO). Detailed accession and other information is on the labels.
[b] From Anonymous, 1975.
[c] Detailed hemp accession information for the Vavilov gene bank is available in Anonymous, 1975.
[d] THC levels 0.30%, the generally accepted limit for industrial hemp, are in bold letters.
[e] Detailed hemp accession information for the Gatersleben gene bank is available on the Web at http://fox-serv.ipk-gatersleben.de/.

Bócsa (1999), suggesting evidence of dominance of high THC content in the F_1 generation).

About two dozen cultivars are approved for cultivation in Canada (http://www.hc-sc.gc.ca/hecs-sesc/ocs/pdf/cultivar_e.pdf). Most of these are regulated by the European Organization of Economic Cooperation and Development (OECD). These cultivars are approved for use in Canada not on agricultural criteria, but merely on the basis that they meet the THC criterion. Indeed, most of these are unsuitable or only marginally suitable for Canada (Small and Marcus 2000), and only a very few Canadian cultivars to date have been created. In Canada, every acquisition of hemp grown at a particular place and time must be tested for THC content by an independent laboratory, and under the industrial hemp regulations, fields of hemp with more than 0.3% THC may be destroyed. Importation of experimental hemp lines (i.e., other than the approved cultivars, = cultigens) requires importation licenses as well as phytosanitary clearance of the shipment by the Canadian Food Inspection Agency, and the importation licenses require an indication that the THC contents are low. As will be evident, reported levels can not always be trusted.

There are no publicly available germplasm collections of *C. sativa* in North America. The hundreds of seed collections acquired for Small's studies (Small 1979) were destroyed in 1980 because Canadian government policy at that time envisioned no possibility that hemp would ever be developed as a legitimate crop; voucher specimens, however, were deposited in five herbaria. An inquiry regarding the 56 United States Department of Agriculture hemp germplasm collections grown by Small and Beckstead (1973) resulted in the reply that there are no remaining hemp collections in USDA germplasm holdings, and, indeed, that were such to be found they would have to be destroyed. While hemp has been and still is cultivated in Asia and South America, it is basically in Europe that germplasm banks have made efforts to preserve hemp seeds. Because hemp is regaining its ancient status as an important crop, a number of private germplasm collections have been as-

sembled for the breeding of cultivars as commercial ventures (de Meijer 1999; de Meijer and van Soest 1992), and of course these are available only on a restricted basis, if at all.

METHODS

Seeds were obtained from overseas sources by licensed importation, from authorized sources in Canada, and by personal collection from wild populations in Ontario. One hundred and eight of the accessions were from the Vavilov Institute of Russia, by far the largest germplasm collection of hemp of any public gene bank, with about 500 collections (detailed information on the majority of hemp accessions of the Vavilov Institute can be found in Anonymous, 1975). We also grew 20 of the collections of the Gatersleben gene bank of Germany, the second largest public gene bank in Europe, which has less than 40 *Cannabis* accessions (detailed information on the hemp accessions of the Gatersleben gene bank are available at http://fox-serv.ipk-gatersleben.de/). Detailed information on all of the accessions reported in this paper are on the labels of the herbarium vouchers at DAO. Summary information on the collections is given in Table 1.

Plants in the field plots were seeded in the first week of June in 1999 and 2000, near Cobourg, Ontario (44°04′N, 77°56′W), after appropriate licenses were obtained. Plots consisted of 15 plants in a 4.68 m row, with four rows spaced 1.25 m apart, and were arranged in a randomized complete block design with four replicates. In 1999, 62 accessions were cultivated (these are among listings 1–65 in Table 1; see Small and Marcus, 2000, for a general report on the 1999 trials). In 2000, 117 accessions were cultivated, of which 14 were the same as grown in 1999. Also, we report here THC measurements for two wild populations sampled at their wild sites in 2000 (174 and 175 in Table 1). In total, 167 different germplasm lines were examined.

A variety of reproductive types was present among the accessions, including exclusively monoecious (male and female flowers on each plant), exclusively dioecious (male and female flowers on separate plants), and various degrees of intermediacy. Male (staminate) plants, which die after shedding their pollen, were not sampled, since they typically are not harvested for seed, stem fiber, or flower essential oil. Previous studies have shown that THC levels in male and female plants are often comparable but sometimes lower in the males (Small 1979).

In Canada, the methodology used for analyses and sample collection for THC analysis is standardized (at the Health Canada/Therapeutics Program/Hemp web site at http://www.hc-sc.gc.ca/hecs-sesc/ocs/hemp/manuals_reports.htm, see Industrial Hemp Technical Manual for procedures on sampling plant materials and chemical procedures for determining THC levels). The regulations require that one of the several independent laboratories licensed for the purpose conduct the analyses and report the results to Health Canada. Sample collection is also normally carried out by an independent authorized firm, but we were licenced to collect samples according to the standard, and we did so. While there is often variability in THC content among plants of a given provenance, de Meijer, van der Kamp, and van Eeuwijk (1992) found that a sample of 20 provided a reliable approximation of average THC content. Most of our samples for THC analysis represented a composite sample of about 50 plants, bulked over the four replicates. As required by Canadian regulations, samples were collected from the upper, reproductive parts of the plant (‘‘the entire, fruit-bearing part of the plant shall be used as a sample . . . normally the top one-third of the plant’’) when the plants were beginning to produce mature seeds (‘‘when the first seeds of 50% of the plants are resistant to compression’’). The collection standard calls for screening the material through a sieve of mesh size 2 mm, which eliminates seeds and all but the smallest twigs, and essentially represents leaves and floral (perigonal) bracts. Since plants came into flower and fruit at different times, collection times varied, and the few accessions that had not yet produced mature seeds were sampled just before frost.

A test of the consistency of THC analyses among laboratories was conducted by Lakefield Research Limited (Lakefield, Ont.), one of the approximately 12 Canadian laboratories authorized by Health Canada to conduct analyses for required reporting purposes. Lakefield Research has provided the data to us for publication. Three standardized samples, as well as a duplicate of one of these that was not identified as such, were submitted for THC analysis to four different laboratories, as well as being analysed by Lakefield Research.

TABLE 2. STATISTICAL SUMMARY OF THC CONTENT.

	All accessions grown in Canada (this paper)	All Vavilov Institute accessions grown in Canada (this paper)	All Vavilov accessions grown in Russia reported by Anonymous, 1975
Sample size	167	108	278
Mean (%)	0.366	0.504	0.597
Variance	0.1988	0.2423	0.4033
Minimum (%)	ca. 0	ca. 0	0.01
Maximum (%)	2.45	2.43	3.26
Accessions with THC 0.3%	72 (43.1%)	63 (58.3%)	152 (54.7%)

RESULTS

THC levels for the 167 different accessions grown over the 2 years are given in Table 1. Also, THC levels are added for the 41 Vavilov Institute collections for which THC measurements were available from published data in Anonymous (1975) as well as from the same collections we grew in Canada. For 19 of these, there was disagreement regarding whether or not the germplasm line had an acceptable level of THC (i.e., <0.0.3%); for 12 of these the Russian measurements were higher than 0.3% and for seven, the Canadian measurements were higher. However, the means were not significantly different. Frequency histograms for THC content, for the 167 different accessions we grew in Canada, and for the 284 Vavilov gene bank accessions for which THC content was reported by Anonymous (1975), are shown respectively in Figs. 2 and 3. We employed one authorized laboratory for THC analyses in 1999, and another in 2000, and of the 14 accessions that were grown in both years the two laboratories were in apparent disagreement regarding whether or not the germplasm line met the acceptable level of ≤0.3% THC for only two accessions.

For comparison purposes, Table 2 presents summary statistical data for all 167 accessions we grew in Canada, the 108 accessions we obtained from the Vavilov Institute, and 278 of the Vavilov accessions for which THC data were reported by Anonymous (1975). Whether evaluated in Canada or in Russia, more than half of the Vavilov accessions exceeded the 0.3% THC criterion. Of the 20 accessions we grew in Canada from the Gatersleben gene bank, the THC content was 0.3% in only six. Of all 167 different accessions we cultivated in Canada, about 43% failed to meet the 0.3% THC criterion. Figures 2 and 3 show, respectively, summary histograms of the Canadian THC data we obtained and summary histograms of the published Russian THC data for the Vavilov Institute hemp germplasm collection.

Entries 43, 45–49, and 55–66 (Table 1) were

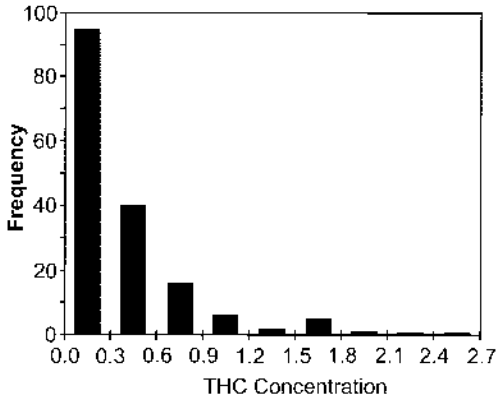

Fig. 2. Frequency histogram of THC concentration based on means of 167 accessions of *Cannabis sativa* grown in Canada.

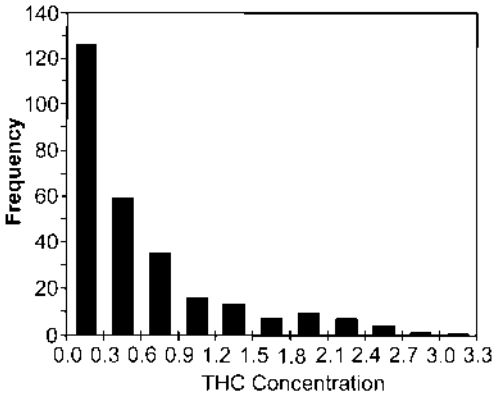

Fig. 3. Frequency histogram of THC concentration based on 278 accessions of *Cannabis sativa* of the Vavilov Institute (St. Petersburg), reported by Anonymous (1975).

TABLE 3. ANALYSES OF THC CONTENT IN STANDARDIZED SAMPLES BY FIVE CANADIAN LABORATORIES[1]

Laboratory	Sample (% THC) 1	2	3	Blind duplicate of 3	Mean relative percent of absolute deviations from means
Lakefield Research	0.38	0.34	0.26	0.26	16.1
Lab B	0.14	0.14	<0.1	0.14	62.8
Lab C	0.41	0.55	0.42	0.50	34.7
Lab D	0.34	0.53	0.34	0.33	10.5
Lab E	0.46	0.56	0.37	0.44	30.8
Means	0.346	0.424	0.298	0.334	31.0

[1] Data provided by Lakefield Research Ltd., Lakefield Ont.

on the list of 23 cultivars authorized for commercial cultivation in Canada in 2000, and were obtained from sources authorized to distribute these commercially. Of these18, two entries exceeded the 0.3% THC criterion: entry 60 ('Uniko B', on probationary status for 2001 for future acceptability; however entry 47, the same cultivar but from an extremely reliable source, was well below the 0.3% THC criterion); and entry 49 ('Fibriko,' exceeded 0.3% in 2000 but not in 1999). This indicates that the Canadian hemp monitoring system is very effective in maintaining commercially available seeds at THC levels below the 0.3% criterion.

The Vavilov Institute gene bank contained the following accessions that bear cultivar identifications identical to those that are authorized for commercial cultivation in Canada: 6, 9, 101, 103, 113, 115, 124, 128, 131, 133, 134, 136, 138, 163 (Table 1). Of these 14, nine exceeded the 0.3% THC criterion when grown in Canada, showing that furnished varietal identification of a germplasm accession can not be relied on to the same extent as with authorized commercial sources.

A comparison of THC analyses of four standardized samples by five different Canadian laboratories is given in Table 3. Of the five laboratories, Laboratory B always produced the lowest measurement of THC. Laboratories C and E always produced measurements that were higher than the mean measurements of all five laboratories. Based on mean deviations of the given laboratories from the overall means, Laboratory D was most consistent with the other laboratories, differing only by 10.5% from the overall means, while Laboratory B was least consistent, differing by 62.8% from the overall means.

DISCUSSION

The Canadian system of monitoring THC content requires that analyses be reported for every cultivar wherever and whenever it is grown in Canada; and this has permitted rigid limiting of hemp cultivation to cultivars that consistently develop THC levels below 0.3%. Nearly all of the authorized cultivars we grew in Canada were below the 0.3% THC criterion.

Almost all of the 23 cultivars authorized for cultivation in Canada were bred in Europe for European conditions and are of limited utility for most of North America so that the future of hemp development in North America is dependent on the use of germplasm sources for breeding. Of the 167 accessions that we grew in Canada, more than 43% proved to have levels of THC $\geq$0.3%; such high levels strongly limit use for breeding of industrial hemp cultivars. It is particularly disappointing that more than half of the accessions of the world's largest (ca. 500 accessions) and most valuable collection of hemp germplasm, that of the Vavilov Institute, develop THC levels that exceed 0.3%. Nevertheless, the value of this collection for future breeding remains outstanding and every effort needs to be made to preserve it. Maintenance and seed generation issues for the Vavilov hemp germplasm collection are discussed in a number of articles in the Journal of the International Hemp Association (e.g. Clarke 1998b; Lemeshev, Rumyantseva, and Clarke, 1993, 1994).

There is a clear pattern of THC levels among *C. sativa* germplasm collected for industrial uses, as shown in Figs. 2 and 3. That is, almost all of the accessions have THC levels far lower than encountered in narcotic cultivars (which generally have more than 3% in the reproductive

Fig. 4. Wild pistillate plants collected at Arnprior, Ontario, Canada, 17 Oct 2000. The plants were photographed the same day in a studio. Voucher: *E. Small 174* (DAO).

portion of the plants), the most frequent type of accession has less than 0.3%, and there is a very strong decrease of the frequency of accessions with higher THC levels. Fiber and oilseed cultivars have epidermal secretory glands that are fairly comparable to those of narcotic cultivars, with the exception that the resin is composed mostly of CBD rather than THC, reflecting millennia of selection for different purposes (Small 1979).

Variability of THC measurements differed somewhat depending on year of cultivation and/or laboratory used for analysis and whether grown in Russia or Canada. For example, of the 41 accessions for which both (1975) Russian analyses of THC and Canadian analyses (1999 and 2000) were available, the mean Canadian analyses was 0.47% and the mean of the Russian analyses was 0.66%; 20 of the accessions were >0.3% by the Canadian analyses, and 25 by the Russian analyses. Such variability is likely due to some combination of laboratory errors, sampling error, and environmental differences at the cultivation sites.

The comparison of THC analyses of standardized samples, conducted by five of the 12 laboratories in Canada authorized to conduct such tests, revealed an overall mean difference among the laboratories of 31%, with some laboratories tending to report higher THC levels than others. This suggests that there is a need to promote more consistency among laboratories.

Soil characteristics and latitudinal and climatic stresses have been found to have significant effects on THC concentrations, as do seasonal and even diurnal variations (Small 1979; Pate 1999). However, the range of THC concentrations developed by low-THC cultivars (those typically with 0.3% THC) under different circumstances on the whole is limited, for the most part generally not varying more than 0.2% when grown in a range of circumstances, and usually less (note information in Scheifle 2000; Scheifle and Dragula 2000; Scheifle et al. 1999). Practi-

cally, this has meant in Canadian experience that a few cultivars have been eliminated from further commercial cultivation because they sometimes exceed the 0.3% level ('Fedora 19' and 'Futura', authorized in 2000, may no longer be grown because some test results in several years exceeded 0.3%; 'Finola' (formerly 'Fin314') and 'Uniko B' are under probation because of elevated levels), but on the whole most of the permitted cultivars have maintained highly consistent development of quite low levels of THC.

A particularly likely source of inconsistency of THC content in given cultivars or accessions is contamination by hybridization during seed reproduction. *Cannabis sativa* is a wind-pollinated plant that is easily hybridized with pollen from plants in the region (Small and Antle 2003). In Canada, farmers may not generate their own hemp seeds, but must buy pedigreed seeds from authorized seed suppliers, who are required to ensure that their lines remain pure and could be liable for losses associated with crops testing above the legal limit.

For practical purposes, this report indicates that for European germplasm collections, having neither previously reported THC levels nor identified as a cultivar that is generally known to be either high or low in THC content can be relied on, and independent examination of THC level of the plants grown in North America is desirable.

We examined three apparently wild collections from the province of Ontario (70, 174 and 175, Table 1). Our accession 70 proved to be a spontaneous collection, i.e. quite recently escaped from cultivation, a conclusion that became evident from the relatively high THC content and from the domesticated morphology of the achenes (discussed in Small 1975). Accessions 174 and 175 proved to be true ruderal collections, based on the relatively low content of THC and their wild seed characteristics that are found only in plants that have lived in nature for many generations. Wild North American hemp is derived mostly from escaped European cultivated hemp imported in past centuries, perhaps especially from a revival of its cultivation during World War II (Small et al. 2003). Wild Canadian hemp is concentrated along the St. Lawrence and lower Great Lakes, where considerable cultivation occurred in the 1800s. In the U.S., wild hemp is best established in the American Midwest and Northeast, where hemp was grown historically in large amounts. Decades of eradication have exterminated many of the naturalized populations in North America. In the U.S., wild plants are rather contemptuously called ditch weeds by law enforcement personnel. However, the attempts to destroy the wild populations are short-sighted, because they are a natural genetic reservoirs, mostly low in THC. Wild North American plants have undergone many generations of natural adaptation to local conditions of climate, soil and pests, and accordingly it is safe to conclude that they harbour genes that are invaluable for the improvement of hemp cultivars. We have encountered exceptionally vigorous wild Canadian plants which could prove valuable (Fig. 4). Nevertheless, present policies in North America require the eradication of wild hemp wherever encountered.

Conclusions

THC concentration is sufficiently high in many European germplasm collections as to effectively eliminate these from incorporation into hemp breeding programs for North America. However, THC levels reported for particular accessions in the literature need to be accepted cautiously and verification of concentrations developed when European germplasm accessions are grown in North America is advisable. As demonstrated by the Canadian system of THC monitoring, extremely reliable restriction of cultivation to cultivars with highly consistent low levels of THC is achievable.

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