Variations of .9-THC Content in Single Plants of Hemp Varieties.

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Abstract

Within a given plant population, the concentration of any constituent is expected to vary within a certain bandwidth. To test the distribution of .9-Tetrahydrocannabinol (THC) in hemp populations, a number of single plants were taken from populations of 5 well-known hemp accessions (Fasamo, Beniko, Bialobrzeskie, Félina 34, Kompolti) and a Hungarian provenance. The quantitative analysis of single plants delivered a set of 30 to 61 THC and Cannabidiol (CBD) values for each of the six hemp accessions under consideration. The distribution of THC within a number of hemp plants often shows no Gaussian distribution, the different varieties have quite characteristic distributions of THC. Most single plant values are close together, the variation, however, differing from variety to variety. In addition, single plants are found with THC-values far outside this bandwidth. Keywords: Hemp; Cannabis; .9-Tetrahydrocannabinol (THC); Cannabidiol (CBD)

1. Introduction

The presence of psychoactive components in the hemp plant has no adverse affects on fibre quality, but gives rise to some practical problems, mainly concerning the public acceptance. A stimulant level low enough to make the cultivars unsuitable for drug use has been a breeding goal for years, and therefore, modern hemp varieties are not only superior in vegetative growth characteristics and resistance to pests and diseases, but also their psychoactive potency is well below acceptable limits. The psychoactive potency of hemp cultivars is expressed in the .9-THC content of a sample prepared by collecting the upper part of hemp plants, drying and removing stems and seeds, and grinding. The THC-content of this preparation is limited to 0.2% by EU regulations (VO (EG) Nr. 1420/98) and settled by law also in many countries outside the European Union. Within a given plant population, the concentration of any constituent is expected to vary within a certain bandwidth. A sample representative for the whole population is obtained if the number of plants taken is big enough to assure the average concentration of the constituent in the sample is the same as in the whole population. The minimum number of plants to be sampled is easily calculated from data on the variability of the constituent, presuming the variation follows a normal, Gaussian, distribution. Based on literature (de Meijer 1992), a sample size of 20 plants was expected to be sufficient for reliable analyses. When these results were questioned, we decided to repeat the experiment with more varieties and more single plants per variety.

2. Material and Methods

2.1. Plant material:

The test was performed with 5 well-known hemp accessions and one variety with THC content above the tolerated level. The accessions used vary with respect to their vegetative growth characteristics, and, despite the fact that most modern accessions used are monoecious, there is also a dioecious variety under test.

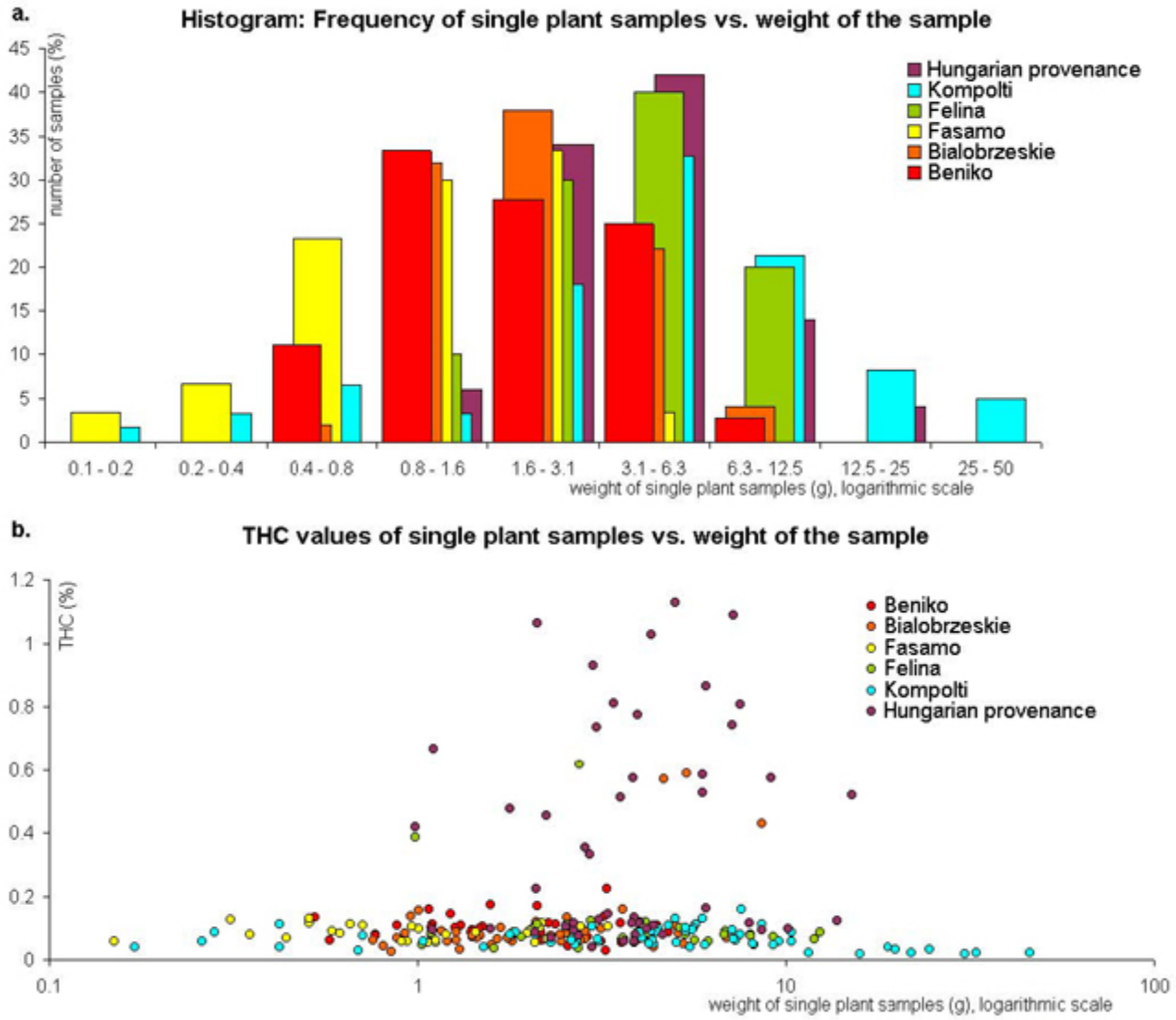
1. Fasamo (Germany), monoecious, very early.
2. Beniko (Poland), monoecious, early.
3. Bialobrzeskie (Poland), monoecious, early to medium.
4. Félina 34 (France), mainly monoecious, medium.
5. Kompolti (Hungary), dioecious, late.
6. Hungarian provenance.

The plants were grown in 1999 on test fields of the research centre in two different regions in Austria. Fasamo, Beniko, Félina and the Hungarian provenance were grown in Fuchsenbigl (dry growth area), Kompolti and Bialobrzeskie in Grabenegg (more humid growth area). Both trial sites are located in Lower Austria. When the plants reached the end of flowering, a number of approx. 30 to 60 plants were sampled. Each plant was cut to the upper third and air-dried at ambient temperature to a residual humidity of less than 12% before it was brought to the laboratory. With the aid of test sieves (mesh 1.4mm), stems and seeds were separated from the dried plant material manually, the resulting material (“laboratory sample”) being weighed and grinded in a mortar.

2.2. THC determination:

The chemical analysis followed the former Austrian national method for THC screening (Flachsbeihilfeverordnung, BGBl. II Nr. 156/1999). 100mg laboratory sample with 4-Androsten-3,17-dion as internal standard was extracted 3 times with 5ml hexane with an ultrasonic disintegrator for 10 min (Branson Sonifier, the sample dipped into a beaker with cold tap water for cooling), the solvent phases combined in a volumetric flask and brought to 25ml with hexane. GC instrument settings: HP 5890 II+, Column HP-5MS, 30m x 0.25mm, film thickness 0.25µm, mobile phase helium 1 ml/min, oven program gradient from 200° to 280°C. Direct injection of the diluted extract,

injection volume 2 µl, auto injector HP 6890, injection block temperature 280°C, split 1:50. A quadruple mass spectrometer (HP 5989 B) was used for detection, EI-mode, transfer capillary 260°C, ion source 250°C, quadrupole 120°C, SIM-mode using the fragments 314 and 299 to quantify THC, 314 and 231 for Cannabidiol (CBD) and 286 and 124 for the internal standard.



3. Results and discussion

3.1. Variation of THC with plant size

The weight of dried single-plant samples without stems and seeds mainly varied from 1 to 10g, with Fasamo reaching only 3g and Beniko only 8g, but Kompolti 20g and more. The smallest sample sizes, less than 0.2g, were found in Fasamo and Kompolti, Kompolti showing by far the greatest variability in size, maybe due to it's dioecious nature. Average sample size was 3.8g, with Fasamo yielding only 1.3g per single plant on an average, and Kompolti 7.5g. Since the figures vary over two orders of magnitude, they are given on a logarithmic scale in fig. 1. No correlation (Spearman correlation coefficient) of plant size and THC content was observed. Samples from very big and very small plants were low in THC, while plants showing higher THC values were of average size. It is clear that, when analysed together in a bulk sample containing many plants, big plants will contribute more to the overall THC value than small plants. However, since there is no correlation between the size of the plant and its THC content, a variation in size of the plants constituting the sample will not affect the result. As shown in Tab. 1, the differences between an "average concentration" (average values of single-plant measurements without regard of plant size) and "weighed average concentration" (calculated with regard to the size of each single-plant sample) are small and vary in direction of deviation.

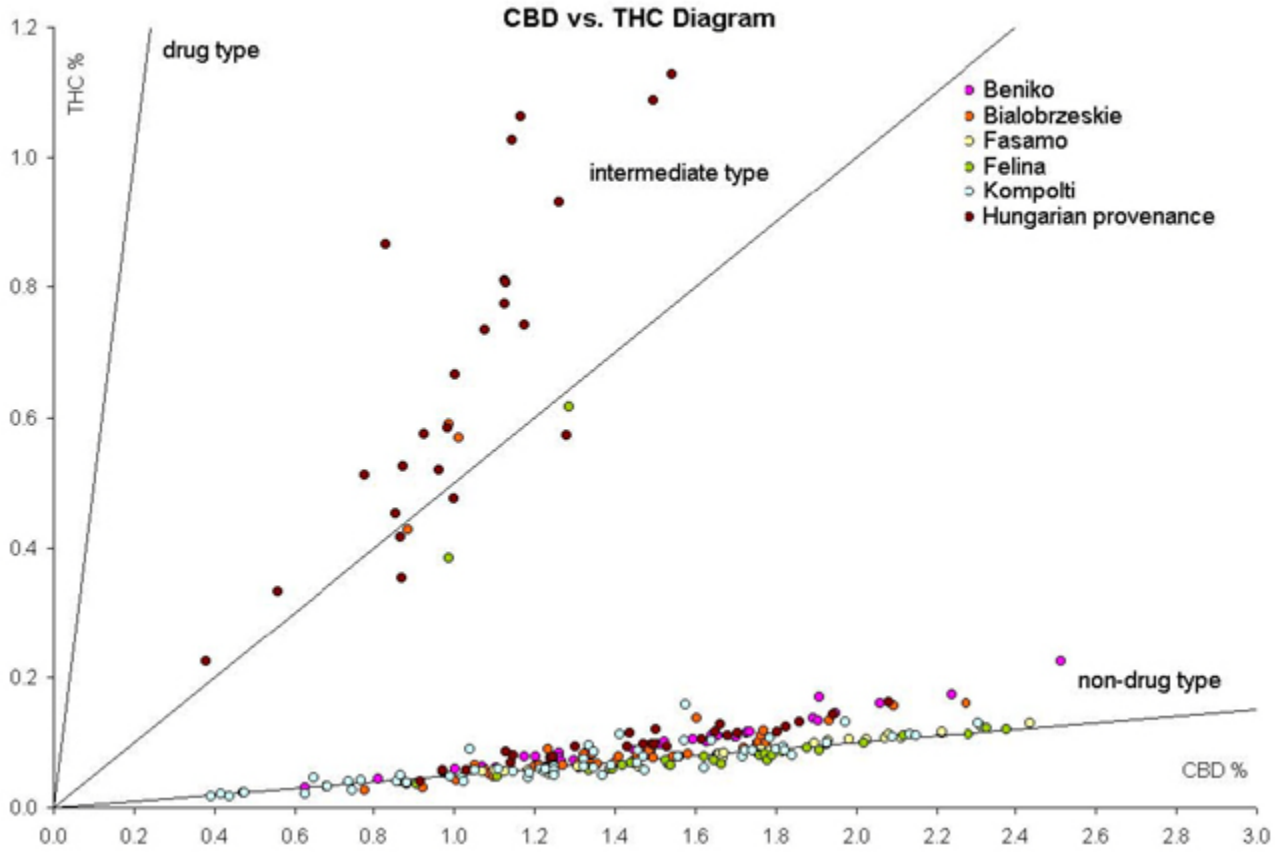


Fig. 2. THC/CBD-Ratio. The pattern shows aggregation in two distinct areas, representing interme diate and non-drug type hemp. The position of the lines crossing the drug type, intermediate type and non-drug type areas is based on experience and not derived from the data set shown.

3.2. THC/CBD-Ratio

The THC/CBD ratio in hemp samples is widely used to assign the hemp under survey to one of three chemo types, drug type, intermediate, or non-drug type (Höppner 1996). Originally proposed as a tool for breeders when hemp generally showed rather high THC-values, experience shows that it gives useful information also with modern fibre hemp low in THC. As shown in fig. 2, virtually all single-plant samples from modern hemp varieties are found on a line representing a THC/CBD-ratio of about 1/20 (non-drug type). This result is consistent with the view achieved over years of routine hemp control. A limited number of single-plant samples from modern fibre hemp varieties and roughly half of the plants from the Hungarian provenance show THC/CBD-ratios around 1/2, of the intermediate chemo type. The pattern showing accumulation of points in two distinct regions, representing two different chemo types, with plants from the same accessions to be found in both areas, suggests the existence of genetic variation in this character.

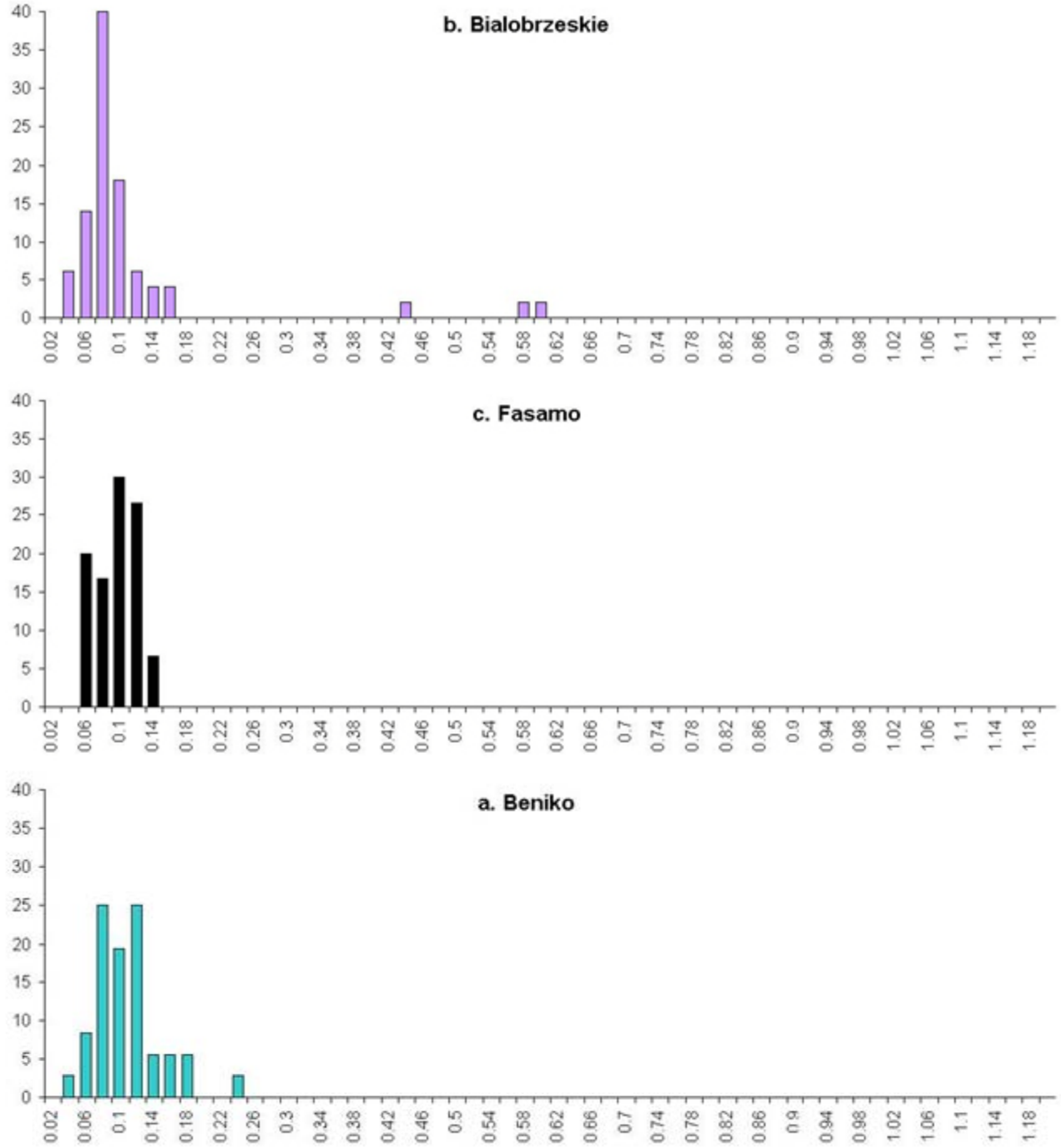


Fig. 3 (first part). THC-Histogram. Frequency of single plant samples vs. THC concentration (%).

3.3. THC Distribution

The distribution of THC within a number of hemp plants often is not a Gaussian one and is best demonstrated in the form of histograms, depicting as bars the number of samples with THC values within a given interval (Fig. 3). In this representation, the different accessions show quite characteristic THC distributions. The THC-values of almost all single-plant samples are found in a peak area between 0.02 and 0.15% THC, with only a few plants of established varieties, but half of the plants from the Hungarian provenance showing values outside this area. Values outside the peak area do not aggregate to a second peak. They are shattered evenly over a broad range, with neither value nor frequency predictable. Beniko has one single-plant sample outside a very broad peak area (1 of 36, 3%), but with 0.22% THC rather close, and three or four more data points at the edge. In Félina, two single-plant samples are far outside an otherwise pretty narrow peak (2 of 30, 7%), and three in Bialobrzeskie (3 of 50, 6%). Fasamo and Kompolti have no single-plant samples outside the peak area (sample size was 30 and 61, respectively).

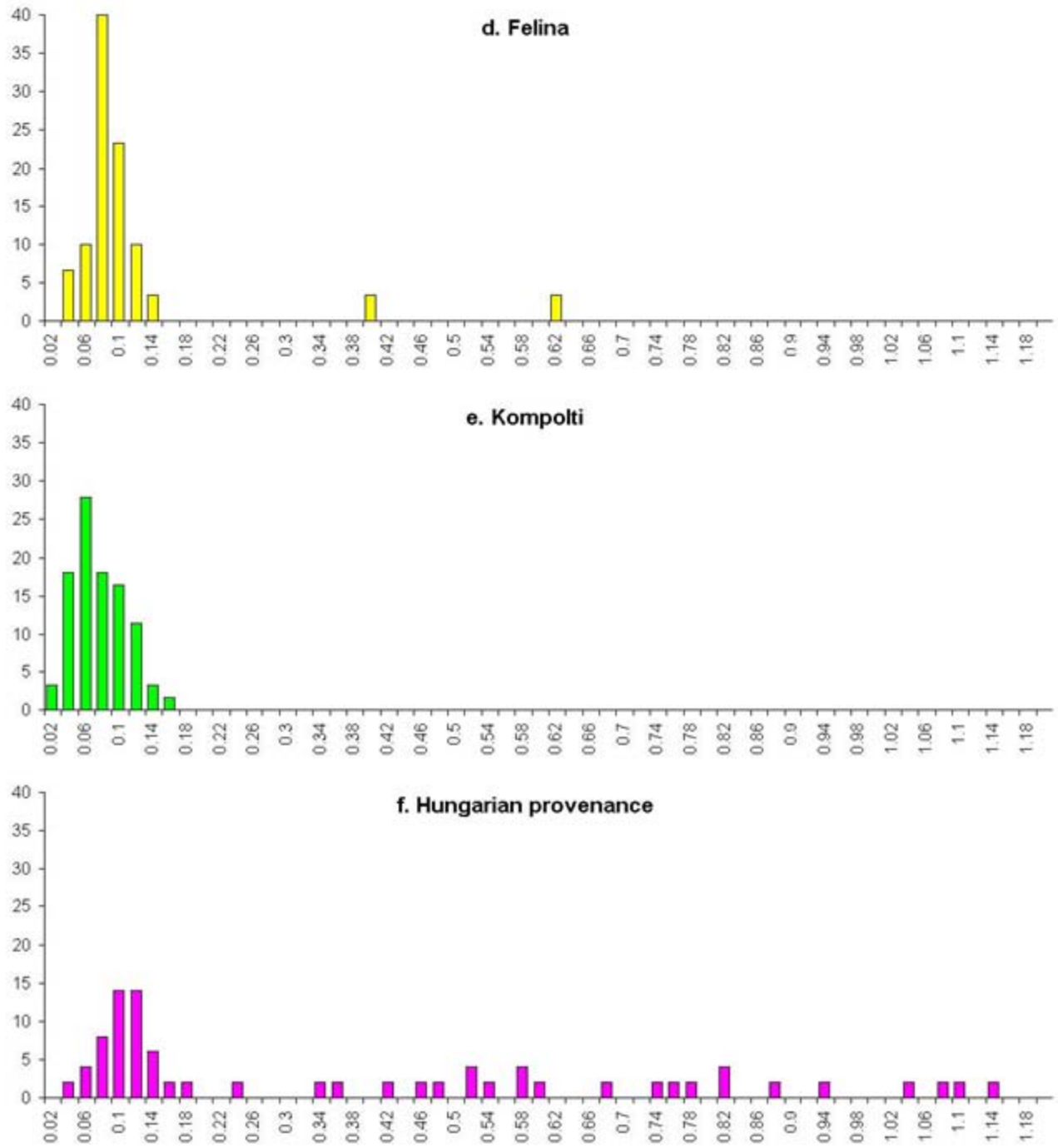


Fig. 3 (continued). THC-Histogram. Frequency of single plant samples vs. THC concentration (%).

Within the peak area, the distribution differs in peak width and shape. Fasamo shows the narrowest THC distribution, around a peak value of 0.09% THC. Beniko has the peak value at 0.09% as well, and the Hungarian provenance at 0.10%, but these peaks are much broader and show a gentle slope towards higher THC-values. Therefore, while, with Fasamo, the peak value coincides with the average value established for this variety (0.09% THC), the average value for Beniko exceeds the peak value slightly (0.10% compared to 0.9% THC), and is much higher for the Hungarian provenance (0.38% compared to 0.10% THC). The average value of the Hungarian provenance is also affected by the numerous values outside the peak area. Bialobrzeskie and Félina both have peak values of 0.07% THC, but, similar to Beniko, broad and asymmetrical peaks. Despite the low peak value, the average values, 0.12% and 0.11% THC, exceed Fasamo and Beniko. On the other hand, the variety with the lowest peak value observed in this study, Kompolti, also showed the lowest average value (0.05% peak value, 0.07% THC average value), despite its very asymmetrical peak shape.

Table 1

Table 1
Averaged weights and analytical values of single-plant samples

Accession	Number of plants used	Sample weight average[1] (min – max)	Average[1] THC	CBD	Weighed average [2] THC	CBD
Fasamo	30	1,3 g (0.2-3.3 g)	0,09 %	1,73 %	0,09 %	1,74 %
Beniko	36	2,3 g (0.5-8 g)	0,10 %	1,50 %	0,10 %	1,45 %
Bialobrzeskie	49	2,5 g (0.8-8.6 g)	0,12 %	1,34 %	0,16 %	1,31 %
Félina	30	4,5 g (1-12.3 g)	0,11 %	1,64 %	0,09 %	1,66 %
Kompolti	61	7,5 g (0.2-46.2 g)	0,07 %	1,28 %	0,05 %	1,01 %
Hung. provenance	50	4,5 g (1-15.1 g)	0,38 %	1,25 %	0,40 %	1,31 %

[1] average values of data measured on single-plant samples
[2] calculated by multiplying the THC and CBD values of each sample by the respective weight of the sample and dividing by the total weight of samples, thus yielding a value close to an analysis of the whole bulk.

4. Conclusion

A main objective of the study was to estimate the sample size necessary for routine control tasks. But the particular THC distribution found in this study made a parametrical approach to estimate necessary sample sizes impossible. Any apparently homogeneous hemp field may contain a serious number of plants behaving irregularly, with respect to their THC values. Therefore, a representative sample will be rather big. A sample size of 50 plants is fixed by EU regulations (VO (EG) 1177/2000) for routine analysis, and bigger samples are collected where results are not clear. When the sample size is smaller than desired, conclusions based on the data generated are limited. Data based on the analysis of a single plant will not necessarily give correct information on chemo type or provenance of the variety.

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