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Study to Develop a Value for Cultivation and Use (VCU) Field Trial Protocol for *Cannabis sativa* L. Flower Varieties

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Abstract: Variety testing systems in Europe do not account for cannabis varieties selected specifically for flower and cannabinoid production. These "flower varieties" are morphologically distinct from industrial varieties, with significant implications for agronomic characterization in the Value for Cultivation and Use (VCU) testing system. However, they are not considered as drug-type varieties due to their low Δ^9-tetrahydrocannabinol (Δ^9-THC) content. Identifying specific traits that can objectively describe these varieties is integral to establishing stable and high-quality production standards. We evaluated specific traits tailored to the VCU testing of flower varieties in two field trials. The assessed phenological traits showed significant differences between varieties ($p < 0.0001$) for all traits except ease of harvest (EH) and lodging, with significant differences also found in all yield-related traits. The number of branches per plant (NBP), flower and leaf yield (FLY), harvest index (HI) and raceme compactness index (RCI) could therefore be considered for VCU testing. The varieties differed significantly in their cannabinoid content, with all falling below the THC limit under Swiss regulation (1%) but not all meeting the 0.3% limit set by European countries. Variations in THC content were dependent on the testing year, the timing of sampling and the number of plants sampled, underscoring the need to clarify VCU testing methodologies. Incorporating cannabinoid content along with morphological and phenological traits is crucial in introducing a new "flower" category within the VCU system for cannabis.

Keywords: *Cannabis sativa* L.; varieties; flower varieties; VCU; flower yield; cannabinoids; phenological trait

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1. Introduction

The genus *Cannabis*, thought to have originated in Central or East Asia [1,2] and domesticated in the early Neolithic period [2], is a crop with a wide range of applications. The main uses of *Cannabis* include fiber production, food, medicine, and the preparation of psychoactive substances [3]. *Cannabis* has been used medicinally for thousands of years, particularly for its anti-inflammatory, antiseptic, and anticonvulsant properties [4]. In this study, we use the term "cannabis" or *Cannabis sativa* L. to refer to all forms of hemp (industrial use) and drug or resin-type cannabis, which is classically known as marijuana.

Until the end of the 19th century, cannabis was cultivated globally. However, at the beginning of the 20th century, restrictions steadily increased in many legal frameworks,

limiting cannabis cultivation, sale, and possession [4,5]. In Switzerland, cannabis was classified under the Swiss Narcotics Law in 1951, subjecting it to the same restrictions and controls as other regulated drugs [6]. Despite these restrictive measures, interest in the use of cannabis for medicinal purposes persisted in several countries. This expanded knowledge about the plant, particularly with the identification of the chemical structure of cannabinoids, such as cannabinol (CBN) and cannabidiol (CBD) in the 1940s, and Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent of cannabis, in 1964 [4,7–9]. The medicinal and recreational use of cannabis has increased intensively in recent years. Global regulations are shifting away from a complete ban on cannabis consumption. Examples include Canada, which legalized access and regulated the production, distribution, and sale of cannabis in 2018 [10]; South Africa, which decriminalized it for personal private consumption in 2018 [11,12]; Morocco, which legalized its use for medical, cosmetic, and industrial purposes [13]; and Germany, which partially legalized cannabis for recreational use in April 2024 [14,15]. In 2021, the Swiss government enacted a series of regulatory amendments authorizing the agricultural production of cannabis provided that it complies with the 1% THC threshold, effectively removing the cannabis variety catalog from the Variety Ordinance [16]. Therefore, as of 2021, cannabis is no longer subject to agricultural seed legislation in Switzerland [17]. Furthermore, the government permitted pilot studies on the use of cannabis for recreational purposes within a research framework [18], making Swiss legislation permissive relative to that of other European countries. Low-THC cannabis is already being retailed throughout Switzerland and Europe, creating a growing market, where the distinction between high- and low-THC cannabis can be subtle [19]. Such pilot studies on its use for recreational purposes aim to provide the base knowledge needed for future regulatory decisions.

The global movement toward a legalization framework for cannabis that facilitates better public health necessitates a clearer understanding of the existing genetics and a description of the varieties found on the market. However, even the terminology and classification of different forms of the genus *Cannabis* remain unclarified. The distinctions between *Cannabis sativa* and *Cannabis indica* have been challenged and shown to be inadequate to represent the underlying genetic variability [20,21]. Classification into "chemotype" or "chemovar" based on the cannabinoid content and other biochemical attributes can differentiate "THC-dominant", "balanced", and "CBD-dominant" types [22]. However, a comprehensive investigation of commercial cannabis in the United States found that commercial labels do not consistently align with the observed chemical diversity [23]. Additionally, chemovar-based classifications seem to overestimate the diversity and do not inform patients regarding chemical properties [24]. Another study suggests a new classification based on genome-wide variation, supplemented by secondary metabolite and morphological information [25]. These approaches focus on general classification and are more research-oriented, but they neglect existing national variety testing systems, such as the Distinctness, Uniformity and Stability (DUS) and Value for Cultivation and Use (VCU) tests that define the national lists of plant varieties and, subsequently, the Common Catalogue of Varieties of Agricultural Plant Species (CCA). DUS and VCU trials aim to provide unique, homogeneous and stable varieties that are robust, efficient and site-adapted to improve agricultural productivity. In Europe, in contrast to Switzerland, cannabis varieties can be marketed only when registered on the national list of an EU member state or the CCA, as long as they pass DUS and VCU tests for cannabis and respect the Δ^9-THC content thresholds of the respective countries. While all cannabis varieties listed in the EU catalog can be traded in Switzerland, the reverse is not true, as there has been no Swiss cannabis variety list since the legislation changed in 2021. As such, seeds and planting materials need only comply with the 1% threshold for Δ^9-THC content [16,17,26].

These two contrasting legislations have resulted in divergent market developments. In Switzerland, varieties bred for CBD production are freely marketed for flower sales but cannot be traded in the EU as they are unregistered, and a 0.3% THC threshold cannot officially be guaranteed. A near-term solution would be to test these varieties in an EU country. However, the VCU protocols for cannabis in European countries are mainly focused on industrial varieties [27], which can be considered to be fiber and seed types. Furthermore, cannabis varieties optimized for maximum flower yields, with chemically rich resins and, therefore, high cannabinoid and terpene content, are morphologically distinct from industrial varieties [28,29]. We propose to refer to these varieties as "flower varieties" based on the harvested product, although they can also be classified as medical or CBD cannabis.

The cannabinoid and terpenoid composition, production stability, flowering time and reduced resource inputs are important criteria for the breeding of flower varieties [30]. Additionally, plant selection criteria, such as compact and healthy racemes and the flower color and aroma, can be considered and are driven by consumer preferences. The development of flower varieties through these breeding criteria can significantly influence the plant's morphology (such as size and structure) and phenology (such as flowering time) [28], as well as agronomic cultivation practices, which in turn affects the characterization processes used in VCU trials. Since flower varieties are morphologically the same as drug-type cannabis and share the same goal of enhancing the flower yield and cannabinoid content, they can exhibit traits such as increased bushiness, reduced height and a greater number of branches [29]. Consequently, cultivation practices are also adapted to enhance the intended use. For example, a reduction in plant density can affect the shoot architecture, potentially resulting in broader growth and branching, which maximizes the flower yield [31]. Flower varieties are usually cultivated in greenhouses but can also be grown outdoors. In both cases, clones derived from a mother plant are either nurtured before being transplanted into the field [32] or they can be cultivated from feminized hybrid seeds. Recommendations on plant density for outdoor conditions are not common, but some studies have shown that it can vary between 5 and 15 plants m^{-2} [32,33]. In Swiss fields, it was observed that even as few as 0.5–1 plant m^{-2} are used. This is in strong contrast to industrial cannabis, which is typically sown using seed drills [32], with sowing densities reaching up to 300 plants m^{-2}, as specified in the official French cannabis cultivation protocol [34]. Due to the effects of breeding and cultivation practices on plant appearance and development, and because the categories in the VCU protocols are predominantly applied to industrial varieties, flower varieties would likely be miscategorized and therefore misjudged by the current VCU trial protocol.

The *Cannabis sativa* varieties registered in the CCA (consolidated version 27.01.2023) are mainly from France, followed by Hungary, Italy, Romania, Poland, the Netherlands and Bulgaria. To our knowledge the variety testing systems of Austria, Bulgaria, Latvia and the Netherlands do not quantify flower production or the cannabinoid content [27]. The French VCU testing system, which classically assesses the straw, fiber and seed yield, lacks flower yield and specific flower characterization, except for a flowering time point trait [35]. Only recently, a regulation section for "cannabis VCU for other uses" was added, where the precocity of female flowers, feminization rate, flower yield, CBD, cannabigerol (CBG) and terpene content are tested [35]. The THC content and feminization rate are the decisive acceptance criteria [35]. Nevertheless, the trial protocols still lack detailed specifications for the assessment of flower yields or the necessity of different cultivation techniques (plants m^{-2}, harvesting technique, etc.) [34]. The Italian protocol includes a category for vegetative propagation, in which the biomass, divided into stems and leaves plus tops, is assessed at the end of flowering, when all styles have darkened. This protocol targets five plants per

m^{-2}, but the trial is conducted in a greenhouse, failing to reproduce the field conditions of common agricultural practices [36]. The above-mentioned protocols minimally address specific traits for flower varieties and generally lack detailed traits relevant to the needs of the farmer, the producer and the final consumer, particularly relating to the flower quantity and quality. The agronomic characterization of cannabis varieties should ideally match their intended use.

As discussions surrounding the legalization or partial legalization of cannabis persist at the political level in numerous countries, the necessity of an official classification system for varieties that are valued for high flower yields and cannabinoid content becomes increasingly apparent. Particularly for medical use, a stable and detailed description of varieties is essential to prove the effectiveness of treatments and guarantee consistent potency [37]. The absence of a registration system in Switzerland provides flexibility to breeders but also brings the likelihood of the cultivation of varieties that do not meet the minimum standards, which are not guaranteed to be quantitatively and qualitatively high-performing and site-adapted.

To address the current knowledge gap in the characterization of cannabis flower varieties, a preliminary study was conducted to define a more precise evaluation protocol and identify specific traits relevant for flower-type cannabis. The aim was to support future legal frameworks by providing clearer criteria for the categorization and assessment of these varieties. The proposed protocol integrates standard traits commonly used in VCU trials—specifically, tailored traits for flower varieties and cannabinoid content. It was first applied in 2020 to two varieties developed by the Swiss cannabis breeder Puregene AG and one commercial variety. In 2021, the refined protocol was implemented on a broader set of ten Puregene AG flower-type varieties to further assess its practicality and robustness. Cannabinoids were evaluated at various time points to assess the relevance of harvest time. In 2021, the cannabinoid content in two varieties was evaluated at the individual plant level to assess the inter-plant variability and therefore the impact of the sample size. A methodological and feasibility study of the characteristics in the context of VCU testing was performed to prepare for possible regulatory changes.

2. Materials and Methods

2.1. Field Trial Specificities

Two field trials were conducted at Zurich-Affoltern (Agroscope-Reckenholz Research Station, Zürich, Switzerland) in 2020 and 2021 to develop a VCU protocol for flower varieties. In the exploratory study in 2020, two flower varieties and one industrial variety were tested, followed by the application of the protocol on ten flower varieties in 2021 (Table 1). With the exception of the industrial variety Eletta Campana, which was sourced from commercially available seeds [38], the flower varieties used in this study originated from the breeding program of Puregene AG and are not currently registered in any official variety catalog. The varieties were propagated from male and female plants producing seeds or through vegetative cuttings. For Eletta Campana, a single plant was selected, and clonal propagation was used for subsequent transplanting. To ensure comparability across propagation methods, all seedlings were transplanted to the field at a similar developmental stage in both trial years. Different cannabinoid-dominant chemotypes were selected based on the cannabinoid content ratio of CBD:THC or CBG:THC previously assessed by the breeder. Based on the chemotype categorization of Mandolino and Carboni [39], where chemotype II is characterized by CBD > 0.5% and THC $\geq$ 0.3% and chemotype III by CBD > 0.5% and THC < 0.3%, multiple varieties were assigned to chemotypes according to the mean THC and CBD values obtained from the 2021 trial. Varieties P6 and P9 fall into the

category of chemotype IV, which is characterized by the dominant presence of cannabigerol (CBG), typically with CBG > 0.3% and CBD below 0.5%.

Table 1. Characterization of *Cannabis sativa* L. flower varieties at the trial in Zurich-Affoltern in 2020 and 2021.

Code	Name	Propagation Type	Dominant Chemotype	Testing Year
P1	Puregene FM Auto	Seed	CBD (Chemotype III)	2021
P2	Puregene Red 3	Vegetative cutting	CBD (Chemotype III)	2021
P3	Puregene Silver 6	Vegetative cutting	CBD (Chemotype III)	2021
P4	V1	Vegetative cutting	CBD (Chemotype II)	2020/2021
P5	Puregene FX	Seed	CBD (Chemotype III)	2021
P6	Puregene CBG 2	Vegetative cutting	CBG (Chemotype IV)	2021
P7	Puregene FQ	Seed	CBD (Chemotype III)	2020/2021
P8	Puregene Silver 3	Seed	CBD (Chemotype III)	2021
P9	Puregene CBG	Seed	CBG (Chemotype IV)	2021
P10	Puregene Red 3	Seed	CBD (Chemotype II)	2021
P11	Eletta campana	Vegetative cutting	CBD (Chemotype III)	2020

In both years, a randomized complete block design (RCBD) was used. However, in the first year, larger plots and more replicates were utilized to assess whether within-plot variability could be accounted for (Table 2). The soil type for both trials was a heterogeneous drained Gleysol with a sandy clay loam texture in the topsoil and a sandy loam texture in the subsoil. The organic matter content of the topsoil was between 4 and 8%. The gravel content of the topsoil ranged from 3 and 8%. The fields were located at an altitude of 420 m a.s.l.

Table 2. Agronomic characterization and experimental information of *Cannabis sativa* L. flower variety trial in Zurich-Affoltern (CH) in 2020 and 2021.

Year	2020	2021
Altitude	420 m a.s.l	420 m a.s.l
Previous crop	Potatoes	Sorghum
Plot size	32.4 m^2	14.4 m^2
Plot replicates	4	3
Planting density	0.56 plants m^{-2}	0.53 plants m^{-2}
Sum of temperature (planting to harvest, base 0 °C)	1873 °C	1588 °C
Precipitation (planting to harvest)	270 mm	189 mm
Soil cover (weed control)	Plastic net (Aquatex black, Hortima)	Plastic net (Aquatex black, Hortima)
Propagation date (greenhouse)	3 June, clone propagation; 30 May, seed propagation	10 June, clone propagation; 28 June, seed propagation
Fertilization	26 March, 40 kg P_2O_5 ha^{-1} (triple superphosphate)	7 July, 40 kg N ha^{-1} (urea)
Planting date (field)	3 July	15 July
Irrigation	3 July, 4.6 mm; 8 July, 0.1 mm *; 27 July, 0.1 mm *	22 July, 10.2 mm; 23 July, 10.2 mm
Pest control	No insecticides, no fungicides	No insecticides, no fungicides
Harvest date	6 October, P11; 21 October, P4 and P7	13 September, P1–P3; 26 October, P4–P10

Note: * Applied for plant cooling purposes.

Difficulties were encountered in 2020 due to strong winds. Several plantlets had to be replaced, particularly variety P11, which did not root well or had its fragile stem broken by the wind. In 2021, precipitation at the end of June and the beginning of July led to water-saturated soils. Since the plantlets needed to be transferred from the greenhouse to the field, the soil conditions were, from an agronomic point of view, too wet and suboptimal. The mean rainfall from January to mid-July during the period 2011–2019 was 530 mm, compared to 454 mm in 2020 and 732 mm in 2021. Under the coverage of the plastic net,

the soil remained highly moist. The plant density was affected by wind, rain and colder temperatures in the early stages. Therefore, some plantlets that broke or died in the first few days after planting were replaced with new ones no later than five days after planting (20 July 2021). A satisfactory average density of 0.53 plants m^{-2} was achieved. The row spacing was 75 cm, but, due to the alternating planting pattern, the diagonal spacing between plants in adjacent rows—the shortest distance between them—was approximately 141 cm. Such a low planting density, compared to industrial cannabis practices, was chosen because it reflects common practice in flower-type cannabis production, where wider spacing allows for full plant development—an important consideration when using costly plantlets. As plant theft had been a problem in previous trials, a visual barrier of tall plants (*Sorghum bicolor* (L.) Moench) was placed at least three meters from the plots around the trials.

The individual plant harvest was performed by hand on two separate dates each year, as the flowering times varied and could be grouped accordingly. The plants were then placed in fabric bags so that the drying process could be carried out in a Pallox forced-air drying system. No heating was applied, as the system relied on the air temperature. We assumed that there was still a negligible amount of water content in the dried plants, which was not taken into account in the yield results, as it was not quantified.

2.2. Standard VCU Traits and Specific Traits for Cannabis Flower Varieties

To select the traits, we drew on various existing frameworks—including the UPOV guidelines for DUS [40], the French protocol for Cannabis [34], breeder consultations and relevant scientific literature—as well as our own expertise in official variety testing, to propose traits that may be relevant for the future VCU testing of flower-type cannabis.

Standard VCU traits for cannabis from GEVES 2020 [34] were adopted. The qualitative trait of early vigor (EV) was assessed using the Swiss VCU protocol for maize [41], scoring the appearance and size of the growing plants (1–9). The stronger, greener and larger the plants, the better the score (1 = excellent); the weaker, paler and smaller the plants, the higher the score (9 = poor, Table 3). The aim is to have plants that have fast juvenile development in order to quickly cover the bare soil and utilize the sunlight. Another trait assessed was anthesis (days after planting). Cannabis flowers are small and numerous, making a clear flowering time point difficult to assess. Based on Yang et al. [42], anthesis (beginning of flowering) was determined when 50% of the plants within a plot showed the first distinct pistillate flowers, in discrepancy with the GEVES protocol [34] but mostly in accordance with the UPOV specifications for cannabis (code 2201) [40]. After flowering, the plant height and lodging at harvest were measured (1 = absent; 9 = present) and the health status of the plants was visually assessed.

Table 3. Description and importance of standard VCU traits and specific traits for *Cannabis sativa* L. flower varieties used to characterize varieties in the Zurich-Affoltern trial in 2020 and 2021.

Trait	Scale or Unit	Description or Equation	Type of Trait	Importance for VCU Trials
Early vigor (EV)	1 = excellent (large, green, high plants); 9 = poor (narrow, yellowish, small plants)	A trait that is assessed in relation to other varieties.	Standard VCU trait	Moderate
Anthesis	Days after planting	50% of plants within a plot show the first distinct pistillate flowers [41].	Standard VCU trait	Critical
Plant height	cm		Standard VCU trait	Critical
Lodging	1 = absent; 9 = present	The score is increased on the basis of the severity of the plant's lodging.	Standard VCU trait	Critical

Table 3. *Cont.*

Trait	Scale or Unit	Description or Equation	Type of Trait	Importance for VCU Trials
Number of branches per plant (NBP)	counts		Flower variety trait	Moderate
Number of racemes per plant (NRP)	counts	See Figure 1 for definition of a raceme.	Flower variety trait	Minor
Ease of harvest (EH)	1 = easy (short bushy shape); 9 = difficult (tall/narrow shape)	The ease of harvesting depends on the position of the colas on the plants: a short, bushy form is easier to reach than a tall, narrow form.	Flower variety trait	Moderate
Dry matter content (DM)	[%]	$\frac{\text{dry biomass}}{\text{wet biomass}} \times 100$	-	-
Dry flower and leaf yield (FLY)	[t ha^{-1}]	$\frac{\text{wet flower and leaf biomass} \times \text{DM}}{\text{harvested surface}}$	Flower variety trait	Critical
Dry stem yield (SY)	[t ha^{-1}]	$\frac{\text{wet stem biomass} \times \text{DM}}{\text{harvested surface}}$	Flower variety trait	Minor
Harvest index (HI)	[%]	$\frac{\text{dry flower and leaf biomass}}{\text{total dry shoot biomass}} \times 100$	Flower variety trait	Moderate
Raceme compactness index (RCI)	[g cm^{-1}]	$\frac{\text{weight of dried raceme}}{\text{dried raceme lenght}}$ See Figure 1 for definition of a raceme.	Flower variety trait	Critical
CBD yield	[t ha^{-1}]	FLY × % CBD in apical cola [42]	Flower variety trait	Moderate

Figure 1. Displayed are dried racemes (distinct clusters of flowers) of various *Cannabis sativa* L. varieties considered as a single raceme (**left figure**) or as two distinct racemes (**right figure**). The red lines facilitate the identification of the distinct clusters (© Gabriela Brändle, Agroscope).

For specific traits for flower varieties, priority has been given to traits that are relevant to the needs of the farmer, the producer and the consumer and which are needed to describe specific characteristics that may explain the yield and quality. Relevant traits for flower varieties are proposed as follows: number of branches per plant (NBP), number of racemes per plant (NRP), ease of harvest (EH, score 1–9), dry flower and leaf yield (FLY, t ha^{-1}), dry stem yield (SY, t ha^{-1}), harvest index (HI, %), raceme compactness index (RCI, g cm^{-1}) and CBD yield (Table 3). Dry matter (DM, %) is seen as a mandatory measurement for yield calculation if only the fresh weight yield is assessed. "Ease of harvest" depends on the position of the colas on the plant: a short, bushy shape (score = 1) is easier to reach than a tall, narrow shape (score = 9). An importance assessment is given for each trait in Table 3, based on a combination of the feasibility of measuring the trait in the context of VCU and the specific description requirements of flower varieties.

Based on observations made in the exploratory study in 2020, dried racemes were easier to recognize as a unit compared to the identification of racemes on fresh flowers. A dried raceme had to build a visual unit; no larger stem fragment had to be recognized within (Figure 1). Racemes, also called 'buds', are important products for sale and refer to distinct clusters of flowers (Figure 1). The raceme compactness index (RCI), based on the equations of Folina et al. and Kakabouki et al. [44,45], was only assessed on the apical cola (30 cm long), as described by the UPOV protocol [40]. Given the morphological variability across varieties and the difficulty in consistently delineating the raceme length—particularly in less compact inflorescences—all putative racemes present on the apical cola were initially

measured. However, to minimize subjectivity and standardize the comparison across plots, only the ten densest racemes per plot (based on the calculated RCI) were retained for analysis. This approach allowed us to apply a consistent threshold for comparison, without imposing an arbitrary minimum weight or length criterion, which could introduce bias given the diversity in floral structure among varieties. Importantly, this method ensured that each plot contributed a consistent number of observations to the analysis. This selection strategy emphasizes the maximum raceme density potential per variety, which aligns with practical breeding and market objectives. The assessment of the number of racemes per plant was conducted solely in 2020 and was subsequently abandoned, as it proved to be a time-intensive and limited-precision procedure that was not compatible with the practical constraints of VCU testing.

The yield assessment was split into three main parameters: FLY (t ha^{-1}), SY (t ha^{-1}) and HI (%) (Table 3). Harvest time was determined based on the 6–7-week post-anthesis window proposed by Yang et al. [42], which corresponds to the expected cannabinoid peak. This timing was complemented and adapted based on the visual assessment of trichome development and style darkening, both indicators of floral maturity, as well as the potential onset of fungal pathogen development. To assess the flower and stem yield, the plants were hand-trimmed. In the exploratory trial, separating flowers from the stem by hand was found to be feasible, but separating leaves from flowers was difficult, laborious and imprecise. Although the number and size of leaves may still affect the yields of flower varieties differently, an approximation was needed for the feasibility of the trials. As a result, the aimed flower yield in this study also included the leaf biomass. This represents a limitation in accurately assessing the flower yield and should be addressed in future trials. Consequently, the harvest index (HI) was calculated using the flower and leaf yield (FLY) rather than the pure flower yield. The CBD yield was also calculated to combine quantitative and qualitative information, for which only the CBD determined in the apical cola was relevant.

Descriptive information on the occurrence of fungal pathogens was collected. In particular, leaves showing symptoms of powdery mildew (*Erysiphaceae* spp.) and *Septoria* spp. were assessed in the laboratory at the Agroscope-Reckenholz Research Station. On the flowers, *Botrytis cinerea* was visually identified in situ.

All statistical analyses and graphs were created in the R statistical software version 4.3.3 (R Core Team, 2016). A linear regression model was used to examine the relationship between each trait (response variable) and the variety (fixed effect). The F-test gave the global p-value results, followed by Tukey's honestly significant difference test, performed at an α-level of 0.05, to determine the significant effects between varieties on the traits. Adjusted R-values were also calculated to indicate the proportion of trait variation explained by the variety.

2.3. Cannabinoid Analysis

Apical flowers, producing colas about 30 cm long, were sampled from four out of eight plants per plot (i.e., per replicate) and analyzed as a pooled sample for cannabinoid content. In 2021, for varieties P1–P3, due to their small plant height, it was not possible to harvest separate apical colas, so the whole plant was harvested and used for quality analysis. Additionally to the pooled samples, single plants of varieties P2 and P10 were sampled and separately analyzed in all plot replicates to assess the inter-plant variability in cannabinoid content. According to ISO 9001:2021 [46], the SCC-certified cannabis analytics laboratory Puregene AG (Zeiningen, Switzerland) performed a high-performance liquid chromatography (HPLC) analysis (Cannabinoids EVO C18). Cannabinoid extraction from flower material was performed through mechanical homogenization in a VWR Starbeater

mill. Here, 500 mg of plant flower material and 15 mL ethanol (99.6%, Ph. Eur. grade) were added to a disposable 50 mL test tube with zirconia beads (~2 mm diameter), and cannabinoids were extracted by shaking for 5 min at 25 Hz. An aliquot of the crude extract was directly filtered through a 0.2 µm PTFE syringe filter (or a 96-well format filter plate with 0.2 µm PTFE) and diluted as needed with ethanol. The cannabinoid assay was run on a 1290 Infinity II Agilent HPLC system equipped with a DAD, temperature-controlled column compartment, multisampler and quaternary pump. The separation of the analytes was achieved on a Kinetex 1.7 µm EVO C18 100A 100 × 1.2 mm column. Full spectra were recorded from 200 to 400 nm, and the absorbance at 230 nm was used to quantify cannabinoids.

Instrument control, data acquisition and integration were achieved with the OpenLAB CDS version 2.5 (Agilent Technologies, Santa Clara, CA, USA) software, applying an identification and quantification method based on an 8-level external standards calibration curve. To confirm the analyte identity in plant material, the retention time and peak purity were compared with the signals acquired on certified reference materials (CRMs).

The calibration curve used for the quantification of the most common cannabinoids was obtained by analyzing serial dilutions of cannabinoid mixtures produced in-house from commercially available cannabinoid CRMs, namely cannabidiol (CBD), cannabigerol (CBG), cannabidiolic acid (CBDA) cannabigerolic acid (CBGA), delta-9-tetrahydrocannabinol (Δ^9-THC), delta-8-tetrahydrocannabinol (Δ^8-THC), cannabichromene (CBC), tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (CBCA).

The content of cannabinoids was calculated as a percentage of the dry mass of the cannabis flower [% $w\ w^{-1}$]. The total CBD (CBD) was calculated according to the formula CBD [% $w\ w^{-1}$] + CBDA × 0.877 [% $w\ w^{-1}$], with the factor of 0.877 accounting for the decarboxylation of the CBDA molecule [47]. Similarly, the formulas for the other relevant major cannabinoids were total THC (THC) = Δ^9-THC [% $w\ w^{-1}$] + Δ^9-THCA × 0.877 [% $w\ w^{-1}$]; total CBG (CBG) = CBG [% $w\ w^{-1}$] + CBGA × 0.878 [% $w\ w^{-1}$]; and total CBC (CBC) = CBC [% $w\ w^{-1}$] + CBCA × 0.877 [% $w\ w^{-1}$].

Moreover, 10% of the samples were subjected to a second laboratory analysis at CBD-Test (Dietikon, Switzerland) for the third-party verification of the results.

2.4. CBD and THC Content Development During Flowering

In the 2020 trial, additional measurements were taken at three time points to monitor the levels of CBD and THC as the plants matured (Table 4). Post-anthesis intervals were determined based on the CBD concentration curve of Yang et al. [42]. The study showed that the highest concentration of CBD was reached after six to seven weeks post-anthesis. The first sample was therefore taken at anthesis, the second at about four weeks and the third at 6–7 weeks post-anthesis. Therefore, sampling in this trial should have captured the curve of CBD development. The GEVES protocol [34] also recommends to start with the sampling at 20 days post-anthesis until 10 days after the ending of flowering, which would align with the observations of Yang et al. [42]. As the plots had to be harvested at full maturity to determine the flower yield, a fourth observation after the end of flowering could not be performed.

Table 4. Dates of three harvest intervals for qualitative analysis of cannabinoid content of *Cannabis sativa* L. flower varieties during trial at Zurich-Affoltern in 2020.

Variety	Anthesis	4 Weeks Post-Anthesis	6–7 Weeks Post-Anthesis
P4	Sep 14	7 October—23 days post-anthesis	21 October—37 days post-anthesis
P7	Sep 14	7 October—23 days post-anthesis	21 October—37 days post-anthesis
P11	Aug 20	17 September—28 days post-anthesis	6 October—47 days post-anthesis

3. Results

3.1. Comparison of Industrial and Flower-Type Varieties

In the 2020 trial, the flower varieties (P4 and P7) were significantly different from the industrial type P11, but not among each other, for the following traits: anthesis, lodging, number of racemes per plant (NRP) and flower and leaf yield (FLY; Table 5). They matured significantly later than P11, had a clearly higher NRP and could achieve a higher FLY. Interestingly, the harvest index (HI) was not as different among the variety types as the FLY. This can be explained by the rather modest stem yield (SY) of P11 (0.90 t ha^{-1}), which is also reflected in fewer branches per plant ($\overline{x}$ = 16.8 branches per plant). Although P11 still produced a satisfactory flower yield over total shoot biomass (46.2%), the total flower yield (0.76 t ha^{-1}) was significantly lower compared to P4 and P7 (2.07 and 2.7 t ha^{-1}). Between the two flower varieties, P7 achieved the highest flower yield and a higher raceme compactness index (RCI; 0.46 g cm^{-1}), but P4 had the highest number of racemes per plant (455 racemes) and a slightly better harvest index (61.6%).

Table 5. Traits assessed on three *Cannabis sativa* L. varieties (P4, P7 and P11) grown at Zurich-Affoltern in 2020.

Variety	EV [Score 1–9]	Anthesis [Days After Planting]	Lodging [Score 1–9]	NBP	NRP	EH [Score 1–9]	FLY [t ha^{-1}]	SY [t ha^{-1}]	HI [%]	RCI [g cm^{-1}]	CBD Yield [t ha^{-1}]
P4	5.8 (0.8) ^{b}	66.8 (0.4) ^{b}	1.0 (0.0) ^{a}	24.5 (1.1) ^{b}	455.2 (28.9) ^{b}	2.0 (0.0) ^{a}	2.07 (0.62) ^{b}	1.72 (0.65) ^{ab}	61.6 (6.8) ^{b}	0.33 (0.01) ^{b}	0.350 (0.117) ^{b}
P7	1.8 (0.4) ^{a}	67.5 (0.5) ^{b}	1.5 (0.5) ^{a}	28.8 (0.8) ^{c}	417.4 (54.0) ^{b}	3.8 (0.4) ^{b}	2.70 (0.68) ^{b}	2.25 (0.24) ^{b}	57.7 (6.5) ^{ab}	0.46 (0.02) ^{c}	0.323 (0.096) ^{b}
P11	4.3 (0.8) ^{b}	44.0 (0.0) ^{a}	6.0 (2.0) ^{b}	16.8 (1.5) ^{a}	250.3 (41.9) ^{a}	8.0 (0.7) ^{c}	0.76 (0.14) ^{a}	0.90 (0.20) ^{a}	46.2 (3.0) ^{a}	0.16 (0.02) ^{a}	0.003 (0.001) ^{a}
p-value	0.0003	<0.0001	0.0011	<0.0001	0.0005	<0.0001	0.0049	0.0099	0.0233	<0.0001	0.0015
adjusted R^2	0.8037	0.9985	0.7325	0.9355	0.77	0.9574	0.6251	0.5615	0.4698	0.9744	0.7137

Note: EV = early vigor, scored from 1 (excellent) to 9 (poor); anthesis = days after planting; lodging scored from 1 (absent) to 9 (present); NBP = number of branches per plant; NRP = number of racemes per plant; EH = ease of harvest, scored from 1 (easy) to 9 (difficult); FLY = dry flower and leaf yield; SY = dry stem yield; HI = harvest index; RCI = raceme compactness index; CBD yield = cannabidiol yield. Values are presented as mean (standard deviation). Means not sharing any letter are significantly different by the Tukey test at the 5% level of significance. The *p*-values correspond to the global F-test of the differences between varieties for each cannabinoid. The adjusted R^2 value indicates the proportion of the trait that can be explained by the variety.

For early vigor, there was no significant difference between the variety types, with only P7 showing a better score ($\overline{x}$ = 1.8). This variety showed a very leafy structure in the early stages of development and fast growth, covering the ground quicker than P4 and P11 (Figure 2).

Figure 2. Early vigor (EV) [1 = excellent, 9 = poor] visible differences in three *Cannabis sativa* L. varieties (P4, P7 and P11) grown at Zurich-Affoltern in 2020. To help identify plants of the same variety, yellow dashed lines show an example of plot boundaries.

The ease of harvest differed significantly among all varieties, with P11 showing the highest score. The poor ease of harvest scores can be partly explained by the heights of the plants, as P11 was the highest variety, with a mean height value of 153 cm. Overall, in this one-year trial, traits were significantly differentiated between varieties, and flower varieties performed better in traits designed to indicate their potential for flower production.

3.2. Implementation of the Proposed Protocol for Flower-Type Varieties

Standard VCU traits and specific flower variety traits tested in 2020 were applied to a larger set of ten varieties in 2021.

Early vigor (EV) varied between varieties, being the strongest for P10 (score = 2) and the weakest for P3 (score = 8, Figure 3). Difficulties were encountered in balancing the plant height and leaf coverage while annotating the trait, as some varieties were small but covered the ground well, and some were tall but covered the ground poorly, probably due to a less leafy structure.

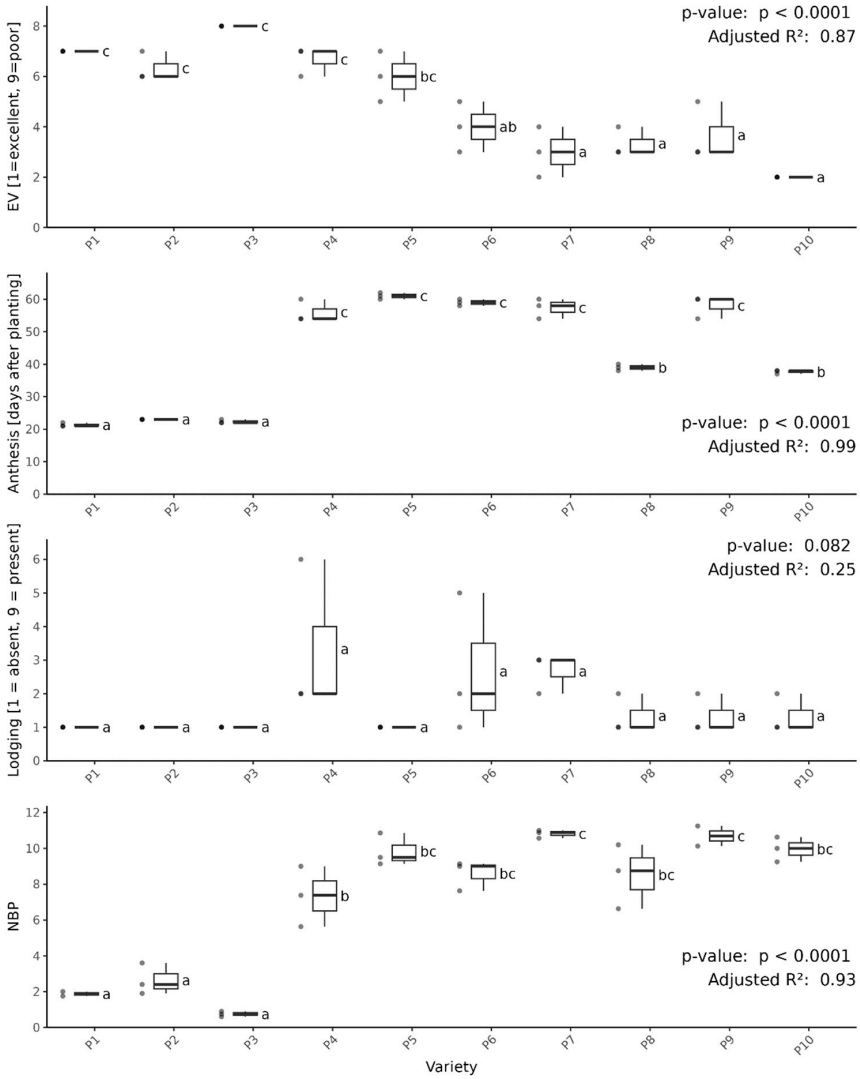

Figure 3. Early vigor (EV) [1 = excellent, 9 = poor], lodging [1–9], anthesis [days after planting] and number of branches per plant (NBP) of ten *Cannabis sativa* L. flower varieties grown at Zurich-Affoltern in 2021. Black dots represent plot data of the variety replicates. Means not sharing any letter are significantly different by the Tukey test at the 5% level of significance. The *p*-values correspond to the global F-test of the differences between varieties for each trait. The adjusted R^2 value indicates the proportion of the trait that can be explained by the variety.

Anthesis was found to be the trait that was most explained by the varieties (adjusted R^2 = 0.99; Figure 3). The varieties could be distributed into three clusters. The early flowering group (P1–P3) showed pistillate flowers about 36 days earlier ($\bar{x}$ = 22 days after planting) than the late flowering group (P4–P7 and P9, $\bar{x}$ = 58 days after planting). Two intermediate varieties (P8, P10) had anthesis about 38 days after planting. Due to considerable differences also in maturity, harvest was carried out at two different dates, and P1–P3 were harvested at 5.4 weeks post-anthesis, whereas P4–P10 were harvested at 7.2 weeks post-anthesis. Compared to the 6–7-week window proposed by Yang et al. [42], these harvests were therefore slightly earlier and slightly later, respectively.

Lodged plants cannot be easily harvested, and the quality of the flowers touching the ground may deteriorate. The varieties with a tendency to lodge were P4, P6 and P7 (Figure 3). However, the variability among replicates was high, so that no significant difference could be detected. An issue might have been the differences in the exposure to wind due to the visual barrier of tall sorghum plants around the trial. This may have had a different effect on replicates, even though the trial design included border plots of at least three meters in size. Moreover, the adjusted R^2 value of 0.25 clearly shows that the proportion of variance explained by lodging was small. This should be taken into account if such a trait is used for VCU evaluation. Nevertheless, further investigation is required to confirm this observation, particularly with a larger dataset encompassing a greater number of years and varieties, as, in 2020, an adjusted R^2 value of 0.73 could be found for lodging.

The mean number of branches of P1, P2 and P3 ($\bar{x}$ = 1.8) was significantly lower compared to all other varieties ($\bar{x}$ = 9.0; Figure 3). This was due to the easily distinguishable morphology before harvest. However, even among the varieties in the range of 7.3 to 10.8 branches per plants, significant distinctions could be made. Variety P7 showed the highest number of ramifications.

In a similar way to the other traits, the height was clearly different for the three early-flowering varieties (Figure A1), showing the lowest values for varieties P1 (18.2 cm), P2 (31.6 cm) and P3 (28.4 cm), while the tallest variety reached 83.4 cm (P10). The unusually cold and rainy month of July 2021 (220 mm), the rainiest of the last 10 years, most likely had an impact on this clearly limited development. The varieties did not reach 1 m in height, so they could be considered small compared to the previous test year. As a result, the "ease of harvest" index was the same for all plants, as there were no problems with harvesting due to inaccessible plant parts.

Various fungal species were observed on the leaves and identified using direct microscopic examination, including powdery mildew (*Erysiphaceae* spp.) and *Septoria* spp. Additionally, culturing on potato dextrose agar (PDA) plates revealed the presence of *Alternaria* spp., *Epicoccum* spp. and *Bipolaris sorokiniana*.

In summary, plant development in 2021 was poor. The potential of the varieties could not be exploited under the growing conditions in 2021; however, the observed traits allowed us to discriminate the selected varieties. The traits describing the plants were significantly different between varieties ($p < 0.0001$) for all traits except "ease of harvest" and lodging. Given the poor plant development and the fact that the plant height plays an important role in the formation of the trait, we suggest that, under more favorable growing conditions, ease of harvest may still be a quick and useful trait to assess. We assume that, for varieties to show differences in their susceptibility to lodging, there need to be more stress events occurring across a wider range of locations and over more years. This would allow genetic variance to be captured, making the differences between varieties apparent.

The differences in growth development among the varieties assessed through the phenological traits are partly reflected in the yield results. Significant differences between varieties could be found for all yield-related traits (Figure 4). Excluding P1–P3, the average

dry flower and leaf yield (FLY) was 0.84 t ha^{-1} or 0.16 kg plant^{-1} (P4–P10), clearly lower than what was achieved by the two flower varieties in the previous year (P4 and P7, $\overline{x}$ = 2.4 t ha^{-1}).

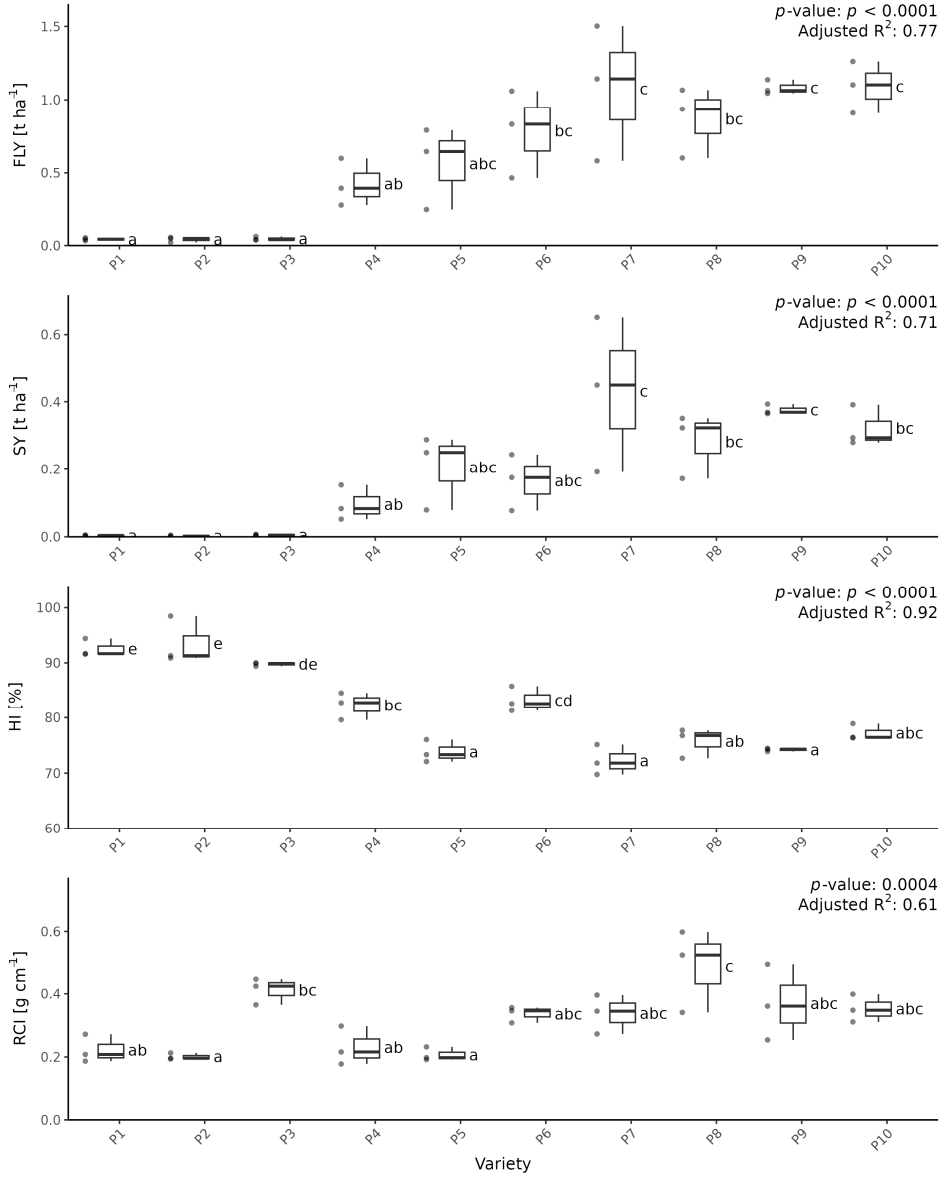

Figure 4. Dry flower and leaf yield (FLY) [t ha^{-1}], dry stem yield (SY) [t ha^{-1}], harvest index (HI) [%] and raceme compactness index (RCI) [g cm^{-1}] of ten *Cannabis sativa* L. flower varieties grown at Zurich-Affoltern in 2021. Black dots represent plot data of the variety replicates. Means not sharing any letter are significantly different by the Tukey test at the 5% level of significance. The *p*-values correspond to the global F-test of the differences between varieties for each trait. The adjusted R^2 value indicates the proportion of the trait that can be explained by the variety.

The three early-flowering varieties (P1–P3) produced the lowest flower and stem yields (Figure 4), yet they displayed the highest harvest index values. The harvest index values of P1, P2 and P3 were recorded with a median value of 91 ± 2.7%. For all other varieties, 76.4 ± 4.3% of the total shoot biomass consisted of flower biomass, including leaves. This was probably due to the compactness of the plants, which allowed small branches to carry numerous racemes. It was observed that taller varieties with more branches appeared to develop more and larger leaves. Therefore, another possible explanation is that less foliage

was harvested for the early-flowering varieties, which may have affected the calculation of the harvest index.

A linear relationship (r = 0.849) between the number of branches (NBP) and FLY was captured (Figure A2). This is not surprising as the more branches that are present, the more flowers can be developed. Plants with a high FLY also showed a high SY (r = 0.979). Bushier shapes with many branches, in addition to an easier harvest, should therefore be beneficial in regard to high yield achievement.

The raceme compactness index (RCI) was not a variety-grouping trait (Figure 4), but significant differences were present, although this yield-related trait was the least explained by the varieties (adjusted R^2 = 0.61). The range varied between 0.18 g cm^{-1} and 0.60 g cm^{-1}. This was the most time-consuming trait to asses because a large number of racemes had to be measured and weighed individually.

3.3. Cannabinoid Content

The amount of CBD in the flowers at harvest in the exploratory trial in 2020 suggested P4 to be the most interesting variety for a high-quality end product (16.8 ± 1.1% *w* w^{-1}; Figures 5 and 6). On the other hand, with a small or no increase over the flowering period and median CBD content of 0.4 ± 0.09% (*w* w^{-1}) at harvest, P11 was clearly not a promising variety for the production of CBD. The CBD content increased until the last harvest event (Figure 5), which, for P4 and P7, was 37 days post-anthesis (5.2 weeks).

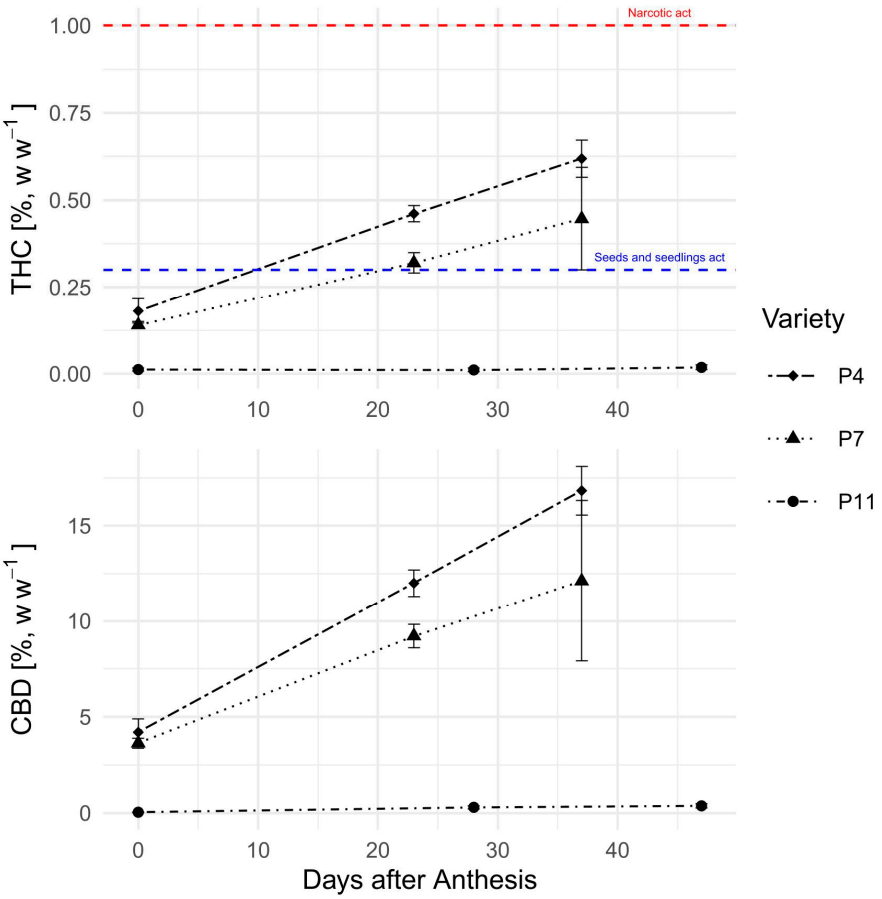

Figure 5. Total CBD (CBD, % *w* w^{-1}) and total THC (THC, % *w* w^{-1}) content in the flowers at three different time points after anthesis (=day 0) for three *Cannabis sativa* L. varieties (P4, P7, and P11) at the site of Zurich-Affoltern in 2020. The standard deviation and the mean of the four replicates for each time point and variety are displayed. Dashed lines indicate the Swiss legal threshold in the Narcotics Act (red dashed line) and the threshold of the Seeds and Seedlings Act (blue dashed line), which is not enforced anymore in Switzerland.

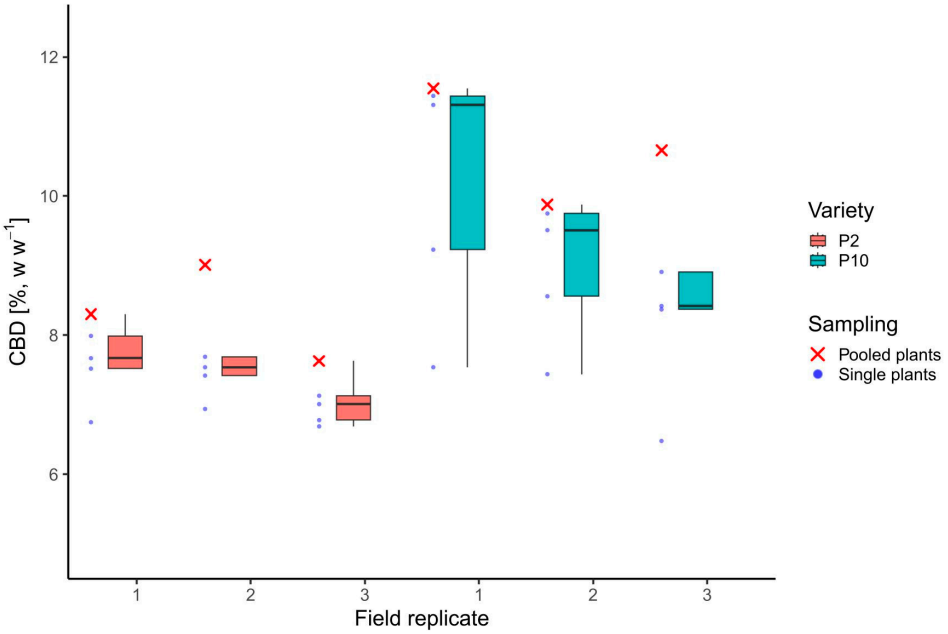

Figure 6. Total CBD content [%, $w\ w^{-1}$] of six plots (replicate 1, 2 and 3) of two *Cannabis sativa* L. flower varieties (P2 and P10) grown at Zurich-Affoltern in 2021. Blue dots represent individual plant data of the variety within a plot. Red crosses represent data of four pooled plants.

The THC content development showed similar behavior to that of the CBD content (Figure 5). The CBD–THC ratio at harvest was 27:1 (P4 and P7) and 20:1 (P11), varying slightly over the flowering period. P4 was again the variety with the highest THC concentration.

In comparison to the exploratory field trial conducted in 2020, P4 and P7 exhibited inferior performance in 2021, with CBD levels approximately 1.4 and 1.9 times lower, respectively. The CBD content ranged from 0.07% (P9) to 11.95% (P4) and was significantly explained by the varieties (Table 6). However, when considering the CBD yield (t ha^{-1}; Figure A1), it was shown that P10, rather than P4, was the most profitable variety for a farmer seeking to optimize the flower yield and CBD content. The varieties with the lowest CBD content were P6 and P9, which were also the varieties with the highest CBG content (3.51% and 2.88%, respectively).

Table 6. Mean cannabinoid content (total CBD, total THC, total CBG, total CBC and CBN) of ten *Cannabis sativa* L. varieties (P1–P10) grown at Zurich-Affoltern in 2021.

Variety	CBD	THC	CBG	CBC	CBN
			[% $w\ w^{-1}$]		
P1	7.03 (0.38) ^{b}	0.23 (0.03) ^{bc}	0.14 (0.02) ^{a}	0.27 (0.04) ^{c}	ND
P2	8.31 (0.56) ^{bc}	0.30 (0.04) ^{bcd}	0.24 (0.03) ^{a}	0.34 (0.01) ^{cd}	ND
P3	6.39 (0.55) ^{b}	0.24 (0.01) ^{bc}	0.35 (0.01) ^{a}	0.22 (0.03) ^{bc}	ND
P4	11.95 (0.80) ^{d}	0.38 (0.04) ^{d}	0.40 (0.03) ^{a}	0.51 (0.03) ^{e}	ND
P5	6.22 (1.71) ^{b}	0.19 (0.04) ^{bc}	0.52 (0.40) ^{a}	0.27 (0.07) ^{c}	ND
P6	0.17 (0.13) ^{a}	ND	3.51 (0.38) ^{b}	0.07 (0.01) ^{a}	ND
P7	6.34 (0.81) ^{b}	0.17 (0.03) ^{b}	0.21 (0.01) ^{a}	0.25 (0.03) ^{c}	ND
P8	7.79 (0.93) ^{b}	0.26 (0.07) ^{bcd}	0.18 (0.06) ^{a}	0.31 (0.06) ^{c}	ND
P9	0.07 (0.02) ^{a}	ND	2.88 (0.82) ^{b}	0.09 (0.01) ^{ab}	ND
P10	10.7 (0.68) ^{cd}	0.31 (0.02) ^{cd}	0.29 (0.03) ^{a}	0.48 (0.03) ^{de}	ND
p-value	<0.0001	<0.0001	<0.0001	<0.0001	
adjusted R^2	0.93	0.88	0.90	0.89	

Note: ND = not detected. Values are from four pooled plants per plot. Values are presented as mean (standard deviation). Means not sharing any letter are significantly different by the Tukey test at the 5% level of significance. The *p*-values correspond to the global F-test of the differences between varieties for each cannabinoid. The adjusted R^2 value indicates the proportion of the trait that can be explained by the variety.

A total of three varieties (P2, P4 and P10) exceeded the 0.3% THC limit permitted in numerous European countries, with P2 being exactly at the 0.3% limit. CBG-dominant types (P6 and P9) exhibit no detectable THC, which, when combined with a high flower yield (Figure 4), renders them highly favorable flower varieties if CBG is targeted.

A detailed examination was conducted on a selected subset of varieties (P2 and P10) to ascertain the inter-plant variability in the cannabinoid content of the same variety within the same plot (Figure 6). Two F-tests were performed: the first included all cannabinoid samples analyzed from a single plot (four individual plants plus the pooling of the other four plants); the second F-test included only the four individual plants (Table 7).

Table 7. Mean cannabinoid content (total CBD and total THC) in weight percent [% $w\ w^{-1}$] of two *Cannabis sativa* L. varieties (P2 and P10) of four plants (single plants) and of eight plants (pooled and single plants) grown at Zurich-Affoltern in 2021 [mean (standard deviation)].

Variety	CBD of Pooled and Single Plants	CBD of Single Plants	THC of Pooled and Single Plants	THC of Single Plants
	[% $w\ w^{-1}$]			
P2	7.47 (0.6) [a]	7.26 (0.41) [a]	0.29 (0.04) [a]	0.29 (0.04) [a]
P10	9.27 (1.48) [b]	8.91 (1.41) [b]	0.29 (0.07) [a]	0.28 (0.08) [a]
p-value	0.0003	0.0012	0.9000	0.8000
adjusted R^2	0.36	0.36	−0.04	−0.04

Note: Means not sharing any letter are significantly different by the Tukey test at the 5% level of significance. The *p*-values correspond to the global F-test of the differences between varieties for each cannabinoid. The adjusted R^2 value indicates the proportion of the trait that can be explained by the variety.

These results are specific to these two varieties and may not be generalized to other varieties in the full dataset. The results indicate significant differences between P2 and P10 within this subset with respect to the CBD content in both F-tests, which was not the case in the overall analysis of only four pooled plants (Table 6), while, for THC, there was no change in significance. The *p*-value was slightly improved with the inclusion of eight plants in the dataset, as opposed to four plants, for both CBD and THC. It is unclear whether this difference justifies full plot sampling, as it represents a minimal improvement. However, the higher number of plants sampled suggests the hypothetical acceptance of varieties on national lists in many European countries. When four pooled plants were tested, the THC levels were 0.30% and 0.31% (Table 6), respectively, but testing eight plants (four pooled and four individual) resulted in THC levels of 0.29%.

Systematically higher values were found in the pooling results compared to the single-plant analysis for CBD (Figure 6). Depending on the replicate, the values from the pooled sample differed more or less from the single-plant samples' values.

4. Discussion

4.1. Insights from Two-Year Field Trials Regarding Variability and Trait Assessment

Several factors influenced the trial outcomes and must be considered when interpreting the results. The environmental conditions differed substantially between the two years: while the plants developed well in the first year, they experienced notable stress in the second year due to prolonged cold and wet conditions. Additionally, the propagation methods varied among varieties, with both seedlings (from seeds) and vegetative cuttings (clones) being used. To enhance the comparability, all transplants—regardless of the propagation method—were introduced to the field at a similar developmental stage. While this approach helped to standardize plant development to some degree, it did not fully eliminate the inherent physiological differences between the propagation methods. This reflects challenges that are typical of real-world VCU testing and may point to the need for clearer guidelines or breeder requirements regarding propagation methods for flower-

type varieties. The less favorable conditions in 2021 led to lower EV, with stressed and smaller plants showing reduced FLY and SY, earlier flowering, increased lodging and a smaller NBP—especially when compared to the potential performance of P4 and P7 under more favorable conditions in 2020 (Table 5, Figure 3). In particular, regarding P1–P3, it is not clear why they flowered so early in 2021. The breeder reported that this behavior had also been observed in other fields. A possible explanation is that it might have been triggered by a stress factor or an issue related to the photoperiod. However, exposure to cold should not trigger flowering in cannabis [48]; high temperatures or drought were not present, but a photoperiod response also seemed unlikely. Short-day conditions can induce flowering in cannabis [49,50], and a 12 h light–12 h dark cycle is normally applied to induce flowering in indoor cultivation [37]. However, other studies have shown that flowering responses induced by longer photoperiods are possible, with ranges of up to 14 h light–10 h dark [51,52]. A waiting time of about 1–2 weeks from the day length switch until flowering has to be considered [51,52]. Taking this parameter into account, we can estimate that, about one week before the flowering of varieties P1–P3, the day length in Zürich-Affoltern was not yet sufficient to induce a flowering response, as the day length was about 15 h–9 h dark. The induction of flowering by photoperiodism seems therefore unlikely, unless these varieties were extremely early-flowering compared to the common varieties used in indoor studies. Nevertheless, this early-flowering behavior is not uncommon. Stack et al. [43] described varieties cultivated in raised beds that flowered extremely quickly after planting, exhibiting a day length similar to ours (~15 h 30 min). They suggested a single locus conferring the early-flowering trait to their cultivar. It is not clear what induced the early flowering in P1–P3, but it is evident that there is a continuum and a wide range of maturity, even within this limited subset of varieties tested.

The first year of the trial showed that the targeted traits in an industrial variety and two flower varieties differed significantly for various agronomic parameters (EV, anthesis, lodging, NBP, NRP, EH, FLY, SY, HI and RCI). These results support the hypothesis that flower-type varieties may outperform industrial varieties when evaluated for their intended use. The variety Eletta Campana, included in the 2020 trial, is considered to be developed for fiber, but it has found use also in producing flowers and biomass for CBD extraction [38]. This variety is registered and was considered a standard in 2023 for the category of vegetative reproduction in greenhouses in the Italian VCU protocol [53]. In our first trial, Eletta Campana performed poorly when applying a protocol conceived for flower varieties. However, with regard to CBD content, it is not yet clear whether some industrial varieties might be as competitive as flower-type varieties. Indeed, another study showed that some varieties of the common catalog of varieties of agricultural plant species (CCA) could achieve satisfactory CBD concentrations. For example, the variety Antal managed to achieve CBD content of around 10% [47], while other varieties exhibited insufficient CBD content. As such, it is conceivable that officially registered industrial varieties included in the common catalog could yield favorable cannabinoid levels in a VCU test designed to assess flower varieties. Even though the flower varieties (P4 and P7) clearly outperformed Eletta Campana, further investigation is required to gain a more comprehensive understanding of the comparative performance of flower and industrial types, as only one year of testing was carried out and only a few varieties were compared.

Following the preliminary trial in 2020, the established traits for flower VCU testing were applied to ten flower varieties in 2021 to further assess the feasibility of the testing protocol. The assessed phenological traits showed significant differences between the varieties ($p < 0.0001$) for all traits except EH and lodging, with significant differences also found in all yield-related traits. In order to obtain more accurate flower yield assessments on a larger testing scale, it would be advisable to use a combine harvester or, at the very

least, a trimming machine. In the present study, leaf biomass was included in the yield assessment due to the considerable workload required to trim each plant. As a result, the FLY was used as a feasible but approximate measure. While this approach provides an initial estimate that may be partially useful for producers, it does not account for varietal differences in leaf proportion, which can affect the accuracy of flower yield assessments. The precise separation of leaves from flowers would enable more accurate and reliable yield measurements. Therefore, while the FLY serves as a pragmatic compromise under field conditions, the development and implementation of mechanical solutions will be essential to ensure accurate, consistent and scalable flower yield measurements in future large-scale VCU trials. This distinction also limits the direct comparability with other studies that have assessed trimmed flower biomass only. For example, Burgel et al. [54] reported dry flower yields ranging from 2.57 to 4.42 t ha^{-1} using industrial cannabis genotypes sown at a high density of 200 seeds m^{-2}—approximately 377 times greater than the planting density used in our trial. Furthermore, their genotypes had CBDA levels below 3% *w* *w*$^{-1}$, resulting in considerably lower CBD yields than observed in our study (Figure A1). Yang et al. [42], assessing the flower biomass per plant in a high-CBD genotype, reported 0.45 kg of flower yield per plant—about 2.8 times higher than the best-performing varieties in our trial. Similarly, conservative indoor production estimates suggest up to 687 g of groomed flowering heads per plant [55], roughly 4.3 times greater than our average yield in 2021. These studies [42,54], however, involved precise leaf trimming to quantify the flower yield, unlike our field-based FLY approach. While the inclusion of leaves in our FLY estimates limits direct comparisons, our lower yield may also reflect the suboptimal field conditions in 2021, including plant stress and poor growth. We suggest that increasing the plant density, as recommended in other studies [31,32], might have mitigated this, as significant gaps remained between plants under reduced growth conditions. EH and lodging, although being informative in the first year, could not be confirmed in the second year of the trial. EH is a trait that can usefully distinguish the morphology of an industrial-type plant from that of a flower-type plant and is intended to be evaluated in the case of manual harvesting. In line with common agricultural practice, this trait could therefore be used optionally. Lodging, also known from other crops to be a complex trait [56,57], is, among other factors, highly dependent on the occurrence and timing of extreme weather events, and it is likely that more trials will be needed to assess this trait consistently across varieties. Although the RCI differed between the varieties, caution is required in the interpretation of the absolute RCI values, as Folina et al. [44] found about ten-times-higher values. In Folina et al. [44], the raceme compactness index was called the "inflorescence compactness index" and was given by the formula = inflorescence dry matter (g)/bud length (cm). It is not clear whether "inflorescence" should be interpreted as a "bud" synonymous with a raceme or a branch full of racemes. In a further study by Folina et al. [58], a bud length of approximately 19 cm was reported, which does not align with our observed raceme length measurements, which averaged 5.4 cm across all varieties. The same observations apply to the study by Kakabouki et al. [45], but, this time, the definition was the "bud compactness index" and it resulted in a maximum bud length of 32 cm. A raceme or "bud" compactness trait, which is stricter (Table 3, Figure 1), could be beneficial in indicating the quality of the raceme to producers and consumers. Therefore, we believe that the definition of racemes shown in Figure 1 is more appropriate to be used in the context of VCU tests.

4.2. Cannabinoid Content and Legal Thresholds

A major issue for a VCU assessment of flower varieties is to describe a cannabinoid content range for CBD that is stable and yearly achievable and a THC content threshold that is not exceeded with common agricultural practices. As CBD and THC are strictly

correlated, because of the underlying biochemical relationship in the production paths [59, 60], varieties with increasing CBD content may also exhibit elevated THC levels. In regard to CBD and THC content, the flower varieties could be differentiated significantly. These varieties were situated precisely within the narrow margins of the legal THC thresholds between the EU and Switzerland. In the context of the threshold of 1% stated by the Swiss Federal Act on Narcotics and Psychotropic Substances [6], it is mandatory that the THC threshold of 1% is not surpassed. All varieties planted were well within the 1% THC limit; P4 showed the highest THC value, confirming in 2021 the results of the previous year. P7 was well within the 0.3% THC threshold in 2021—a notable deviation from the previous year, where a considerably higher concentration of 0.45% was observed. The 1% threshold was also respected at all three evaluation time points during the cannabinoid content development trial conducted in 2020. In a hypothetical official Swiss variety testing system before the legal changes of 2021, the threshold given by the seeds and seedlings ordinance in the Federal Act on Agriculture [6] would have been applied, and varieties P4 and P7 would not have been accepted for marketing in Switzerland, as the 0.3% threshold was an elimination criterion, and it was exceeded from the second harvest onward. However, this threshold no longer applies to recreational cannabis, so all varieties could have been marketed in Switzerland. Instead, only the Narcotics Act applies (effective date: 1 January 2021).

However, in the context of EU legislation, if only the preliminary trial from the first year is considered, variety P7—due to its THC content of 0.45%—would have been excluded from national catalogs. In contrast, it would have been accepted in the second year, with THC content of 0.17%. Moreover, the timing of sampling would also have played a decisive role in the acceptance or rejection of this variety (Figure 5).

We observed that the CBD and THC levels could vary highly according to the time at which the sample was taken. Several studies have shown that the cannabinoid content increases as the plant matures [42,43,61]. How the phenological stage, stress and different environmental conditions affect cannabinoid content is of great importance to ensure that legal thresholds are not exceeded. In their two site trials, Campbell et al. [62] found that about 80% of the CBD and THC variance could be attributed to the genotype, whereas the environmental factor had a limited role. Burgel et al. [54] estimated that the main effects on the cannabinoid content were the genotype and growth stage. Toth et al. [61] also found no effect of stress factors such as flooding, plant growth regulators, powdery mildew or physical wounding on cannabinoid content, with only herbicide treatment being of note, in a one-year and -site field trial. Different nitrogen rates seem to affect the CBD and THC concentrations through a quadratic relationship [63], but the predicted concentration ranges were small, possibly suggesting a small environmental effect.

With a small or zero increase over the flowering period and median CBD content of $0.4 \pm 0.09\%$ ($w\ w^{-1}$) at harvest in 2020, P11 is clearly not a promising variety if used to extract CBD, despite information that claims the variety to achieve 3–10% of CBD content [38]. Even the second-best variety (P7) showed about 30-times-higher CBD content in the end product than P11. Spano et al. [64] harvested the variety Eletta Campana (P11) at three different time points and achieved a maximum of $2.04 \pm 0.01\%$ ($w\ w^{-1}$) of CBD at the second harvesting event, as well as $1.12 \pm 0.02\%$ ($w\ w^{-1}$) of CBDA. Claims of high CBD content for this variety should therefore be taken with caution.

Yang et al. [42] suggested a CBD peak at six weeks post-anthesis. However, because of the decreasing temperature and increased incidence of fungal diseases, the harvest could not be postponed to reach six weeks. Earlier planting may have allowed the two late varieties to mature further. Therefore, even if high CBD content could be detected, it is not clear whether the CBD content could have been increased further or whether a decrease

would have been observed. In the field trial with 30 high-cannabidiol cannabis varieties by Stack et al. [43], it was also found that some CBD accumulation curves leveled off, many continued to increase and others decreased after peaking about 3.5 weeks after terminal flowering. Stabilizing (plateau) or decreasing content of CBD could not be captured in the trial at Zurich-Affoltern, but, based on Yang et al. [42] and Stack et al. [43], both developments might be possible. A plateau in the content would allow a longer harvest window without lessening the profit, as suggested by Yang et al. [42]. A detailed account of the CBD content development of cannabis varieties at the peak of their maturation would be of significant interest to producers, as it would enable them to ascertain the potential consequences of delayed harvesting for the CBD content. Narrowing the qualitative harvest to between five and seven weeks post-anthesis could give a more focused view of the plateau of each variety. Rainy and cold weather conditions, however, limit harvest optimization. Therefore, while the environmental conditions and agricultural practices may have a limited effect on the cannabinoid content, the correct timing of sampling is likely more important to ensure that the full chemical potential of a variety is captured and to prevent the THC content thresholds from being exceeded in future cultivations.

The GEVES protocol [65] states clearly that sampling should be carried out during the period between the twentieth day following the start of flowering and the tenth day following the end of flowering. Therefore, in France, none of the flower varieties (P4 and P7) would have been accepted, given that the THC content exceeded 0.3%. This suggests that these flower varieties, bred for high flower yields and CBD content, are unlikely to be registered unless their THC content is below the regulated thresholds. As shown, the cannabinoid content is highly dependent on the time of harvest. In order to standardize the results, one approach could be to report the cannabinoid content relative to anthesis. Nevertheless, high THC content seems to be well covered by the GEVES protocol, capturing varieties that are out of range.

However, the close proximity of the tested flower varieties to the legal THC threshold (0.3%), along with the year-to-year differences, highlights the need for consistent and high-quality sampling. In addition, methodological issues regarding differences in the propagation method, sample handling and number of plants collected per sample may be of crucial importance in obtaining consistent and comparable results, as we observed differences in inter-plant variability and subsamples of varieties. In order to collect information about the eventual variability in pooled samples to prevent the detection of variety differences, a chemical investigation was performed at the plant level for P2 and P10. It showed that a difference in the variance of P2 and P10 could be observed: P2 had a mean SD among field replicates of 0.41 and P10 had a value of 1.41 for CBD. A plausible explanation is the origin of the plantlets. P2 was propagated through vegetative cutting and was therefore a clone, whereas P10 was propagated through seeds. As suggested by Fischedick et al. [66], clones are more stable in their individual plant variance. Another explanation could be that the higher variance was due to a higher error probability in the laboratory analysis at higher cannabinoid levels, as P10 showed higher concentrations.

Another noteworthy observation is that systematically higher CBD content was found in pooled samples. It is unclear whether the sampling of pooled plants in the field was biased toward higher-quality colas or if the handling of the samples after harvesting differed between the pooled and single plants. It could not be assured that the drying conditions for the bags containing the plants were identical, as the bags were randomly distributed in the Pallox drying system, which could have led to variations in dry matter content and, consequently, variations in the cannabinoid concentrations. Additionally, during the handling of the dried samples, plant debris may have been concentrated at the bottom of the bags, potentially being lost or inadvertently reintroduced to the plants. This is a

critical point for further investigation, as the cannabinoid content could be sensitive to such handling differences. To improve the sampling precision, we would first suggest taking into account the differences in individual plant variance between varieties. It could therefore be proposed that, for varieties propagated via seedlings, a larger number of individual plants should be collected to assess the cannabinoid content in an official VCU trial compared to varieties propagated through cloning. Second, the drying and handling of samples for cannabinoid analysis could also explain the differences between subsamples of the same varieties, highlighting the crucial importance of further defining these steps.

Increasing the number of plants sampled presents a practical challenge due to the low planting density. Varieties P2 and P10 demonstrated that the sampling method significantly influences the eligibility for inclusion on national variety lists; with a higher number of plants sampled per plot, both varieties would have met the 0.3% THC threshold. We tested between four and eight plants sampled per plot. As the plant density is low in agricultural practices aimed at outdoor flower and cannabinoid production, the maximum number of plants sampled is limited. The official GEVES protocol for VCU requires 200 plants to be sampled per plot for THC threshold evaluations [34]. However, with an average planting density of 0.5 plants per m^{-2}, this level of sampling is clearly unfeasible. Increasing the plant density to at least 1 plant m^{-2} and using larger plots could provide flexibility in the number of plants sampled to increase the confidence in the THC stability. In addition, we suggest a higher density per se, as low yields and low field surface utilization were observed. A higher plant density would be beneficial to maximize space utilization and soil coverage. Further research on this matter is needed, as only two varieties were tested for inter-plant variability in our trial. VCU regulations have a mandatory minimal number of testing years and trial sites. For example, French cannabis VCU trials involve testing over at least two years and across different sites [35]. Considering that this type of robust dataset is common in VCU testing, a consistency assessment of cannabinoids still seems feasible.

The above-presented and discussed results show the importance of a clear protocol for plantlet origin and for the harvesting, drying and handling of samples for cannabinoid analysis, as the legal limits can be quickly surpassed. Inter-plant variability, including a broader range of varieties, should be further investigated to draw more comprehensive conclusions, as it is crucial for the development of a reproducible protocol suitable for legislative purposes.

4.3. Toward a VCU System for Flower-Type Cannabis

In order to develop a variety-type targeted VCU testing system, it is necessary to introduce new classification criteria tailored to cannabis flower varieties. The NBP, FLY, HI and RCI, based on the observed differentiation of these traits between varieties, could be considered for inclusion in a future VCU testing system that introduces a new "flower" category alongside industrial cannabis. For the FLY, as previously mentioned, mechanical solutions to separate flowers from leaf material will be necessary to improve the yield accuracy. The use of distinct agronomic and cannabinoid traits may enhance the characterization of varieties selected for their flower yields and cannabinoid concentrations. This will ensure that commercially available varieties are described with greater precision, contributing to stability, consistency and quality, ultimately benefiting the consumer. To our knowledge, variety registration in European catalogs has not been adapted to accommodate flower varieties [27]. As countries become more permissive, these descriptive traits might also be applied to drug-type cannabis, as the targeted end products are similar.

An informative yield trait is the CBD yield. However, as there are 130 cannabinoids extracted and characterized for cannabis [67], focusing on CBD alone would limit the view of the chemical potential of varieties to just one cannabinoid. As shown by the CBG-

dominant chemotypes, which produced very low CBD yields, it is essential to provide a comprehensive account of the cannabinoid variety specificity to ensure that users have access to a wide range of information about the varieties. Naim-Feil et al. [68] propose a minimum focus on CBD, THC, CBG, CBC, THCV and CBDV, as they can maintain steady qualitative traits and variable quantitative traits.

Given that the CBD–THC ratio seems to remain mostly constant [59–61,68], farmers may face a challenge in optimizing the CBD content, as this could also lead to an increase in THC content. Breeders have the challenging task of changing the CBD–THC ratio. A special case is that of the CBG-dominant variety, which did not contain any detectable THC. Consequently, their admission to a national catalog would have only been limited by their performance in trials performed for a purpose other than their intended use. Naturally, the Swiss 1% threshold for THC allows for greater flexibility regarding the variability in THC content when other factors besides genetics play a role, and this appears to be reflected by the flower varieties tested in the present trials, allowing for satisfactory performance in terms of the FLY and CBD content compared to an industrial variety. However, the yearly variation has to be accounted for, as the FLY in 2021 was shown to be below the normal yield potential.

In addition to the cannabinoid content, the flavor and smell of flowers are also of high interest to consumers. These traits were not addressed as they are complex, although they are critical. The GEVES protocol seems to include terpene content as a descriptive trait [35]. Naturally, this is a simplification, but it is unclear how such complex traits should be handled in VCU testing or whether they are beyond its scope.

We estimate that the difficulty in registering cannabis flower varieties, and the lack of a VCU protocol, increases the breeding and production of unreliable varieties. An illicit and deregulated market, as for drug-type cannabis, could lead to a lack of understanding of the genetics and characterization of these varieties. Sawler et al. [20] showed that the genetic identity of a marijuana strain cannot be reliably inferred by its name or by its reported ancestry (*Cannabis indica* or *sativa*). Since flower varieties are not registered in Switzerland, and breeders are not required to use consistent naming, we expect that varieties sold on the Swiss market may face the same issue of being sold under the same name or brand for extended periods, despite the underlying genetics changing over time. In addition, if varieties are not clearly labeled or registered, the violation of THC % thresholds could lead to legal problems for farmers.

4.4. Experimental Limitations and Future Recommendations

Furthermore, there were slight methodological differences between the two years, including variations in the number of plants per plot, number of replicates and number of varieties investigated. However, the low planting density ensured minimal intra-plot competition in both years, allowing for adequate individual plant development. Nevertheless, these differences highlight the need for caution when interpreting the results and limit direct comparability across years. The primary goal of this study was to conduct the first practical assessment of trait measurability for flower-type cannabis under field conditions and to propose these traits as a basis for further refinement and standardization. The experience gained in 2020 was instrumental in preparing and optimizing the trials in the following year. The lack of resources and specialized equipment to process harvested material into fully trimmed racemes represents a limitation of the current experimental setup, particularly from a scientific and practical precision standpoint. The FLY metric used in this study serves as a practical compromise for the evaluation of flower-type hemp varieties. While this approach prioritizes feasibility within the constraints of VCU testing—favoring operational efficiency

over analytical rigor—it may not accurately represent the yield measurement needed for VCU testing.

5. Conclusions

This study represents the first field trial under real conditions for the registration of flower cannabis varieties in Switzerland. Despite the limitations mentioned, we believe that this exploratory work contributes valuable insights toward future VCU testing frameworks for cannabis. Further studies should include more varieties from different breeders and more trial sites. This will enable a more accurate assessment of the stability of the content also within the European legal limits of 0.3% THC. Moreover, it is necessary to investigate whether other agronomic traits (i.e., susceptibility to diseases or pests) should be considered and whether lodging needs to be included in a VCU protocol. While the evaluation of the precise flower yield needs to be evaluated, we suggest discarding the individual counting of racemes per plant due to the excessive workload required for the trait assessment. With a focus on the cannabinoid composition, further investigation into the differing behaviors of flower varieties in the evolution of their cannabinoid content after anthesis is recommended. This is essential to ensure that the VCU protocol accurately assesses cannabinoid levels and allows for fair and consistent registration in national catalogs. The observed differences in inter-plant variability and among subsamples highlight the need for a carefully designed methodological approach to sample harvesting, handling and drying—potentially even including considerations related to plantlet propagation methods—in order to achieve consistent and comparable results.

We advise reflecting on the possibility of introducing a specific flower category in the VCU testing system for cannabis, focusing on key traits such as the number of branches per plant (NBP), harvest index (HI), raceme compactness index (RCI) and cannabinoid content. Additionally, the flower and leaf yield (FLY) should be distinguished from the pure flower yield, where technical feasibility allows. These traits would enable a high-quality and objective assessment of the potential and comparability of the varieties proposed by breeders. The ability to register flower-type cannabis varieties would expand the range of traits available in commercial cultivars. Implementing a registration system that includes DUS and VCU testing would ensure that producers have access to genetically stable, uniform and high-performing varieties over time.

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Appendix A

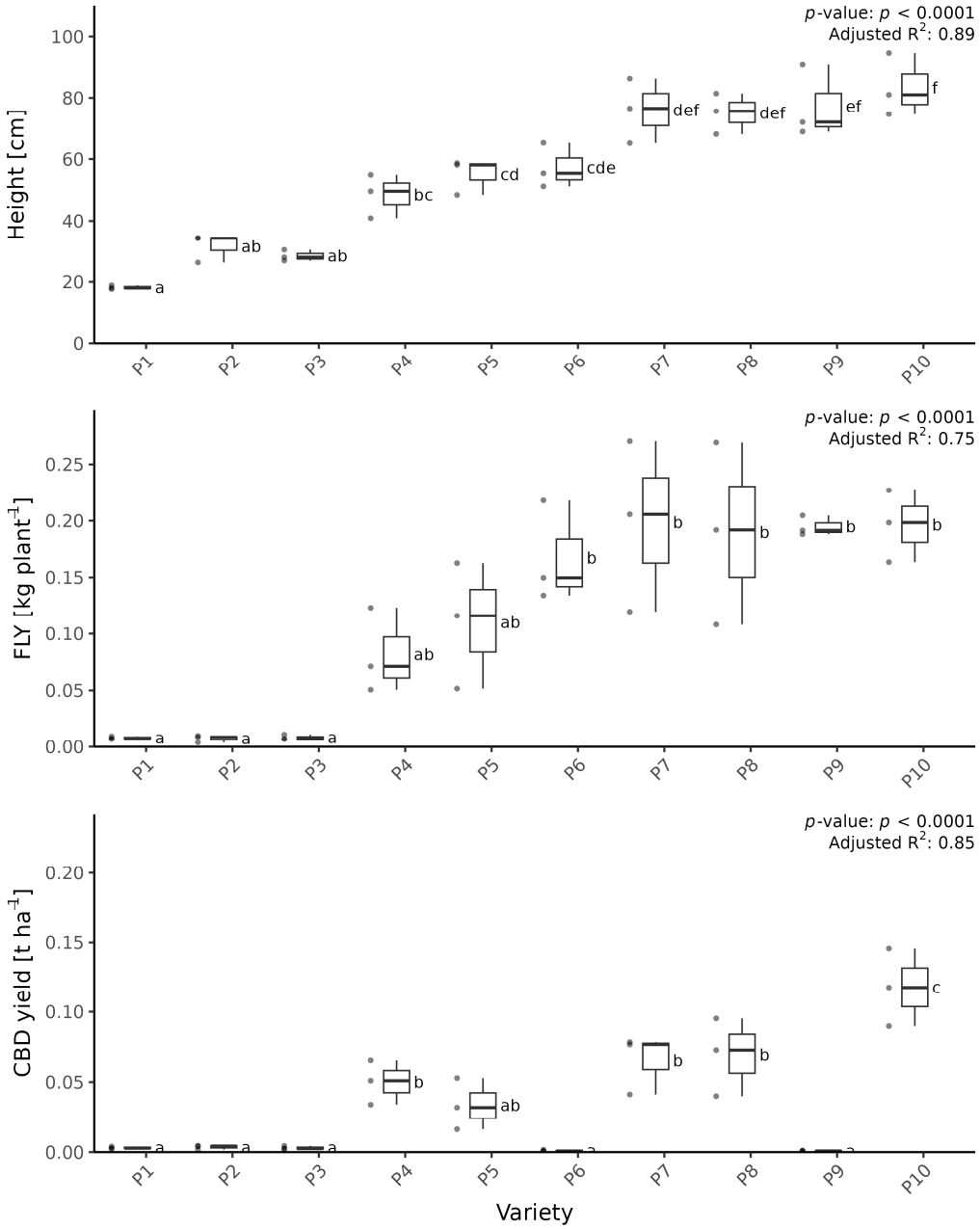

Figure A1. Height [cm], dry flower and leaf yield (FLY) [kg plant^{-1}] and CBD yield [t ha^{-1}] of ten *Cannabis sativa* L. flower varieties grown at Zurich-Affoltern in 2021. Black dots represent raw data of the three replicates. Means not sharing any letter are significantly different by the Tukey test at the 5% level of significance. The *p*-values correspond to the global F-test of the differences between varieties for each trait. The adjusted R^2 value indicates the proportion of the trait that can be explained by the variety.

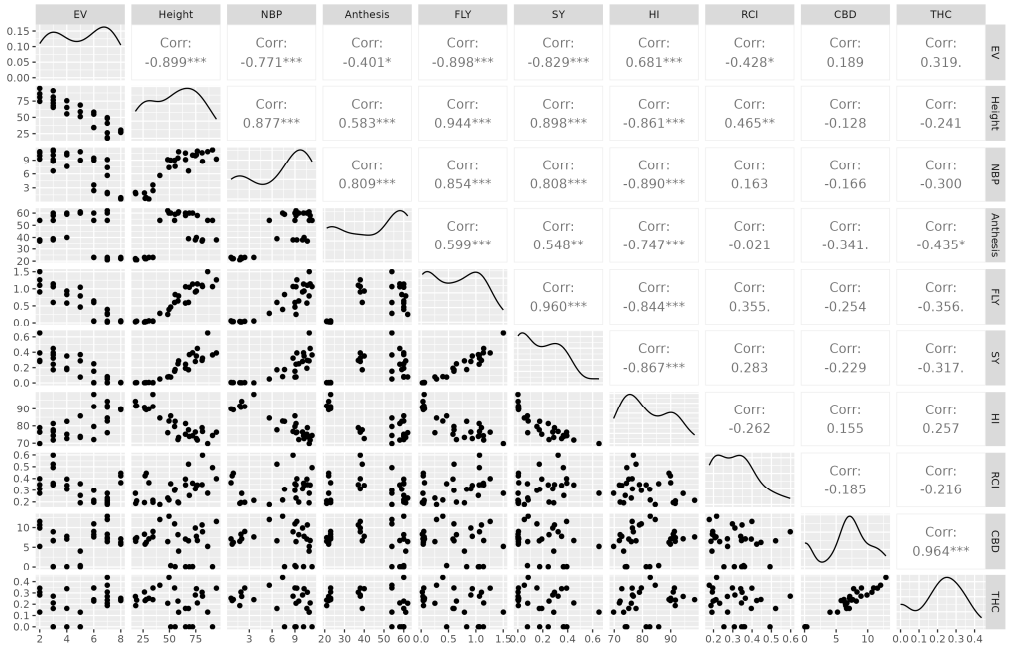

Figure A2. Pearson's correlation coefficients (r) of early vigor (EV) [1 = excellent, 9 = poor], height [cm], number of branches (NBP), anthesis [days after planting], dry flower and leaf yield (FLY) [t ha^{-1}], harvest index (HI) [%], raceme compactness index (RCI) [g cm^{-1}], total CBD (CBD, % *w* *w*$^{-1}$) and total THC (THC, % *w* *w*$^{-1}$) of ten *Cannabis sativa* L. flower varieties grown at Zurich-Affoltern in 2021. Significance levels of Pearson correlation coefficients are shown: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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