Evaluation of 30 High-Cannabinoid Hemp (*Cannabis sativa* L.) Cultivars in New York State

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Abstract

Given the annual tripling of licensed high-cannabinoid hemp growers and acreage in the US between 2015 and 2019, there is an increasingly urgent need to characterize commercially available cultivars and develop best management practices to support hemp farmers. This material already exists for grain and fiber hemp, but equivalent science-based information for high-cannabinoid hemp growers is not available. In this trial, we sought to 1) evaluate yield, agronomic performance, and pest and disease resistance for 30 commercially available high-CBD (cannabidiol) hemp cultivars, and 2) conduct a detailed study of cannabinoid accumulation over the course of floral maturation for all of the cultivars in the trial. We describe significant variation in flowering time, growth rate, powdery mildew susceptibility, biomass production, cannabinoid accumulation, and cannabinoid yield.

Introduction

Hemp (*Cannabis sativa* L.) has long been cultivated by humans for food, fiber, and medicinal purposes (Russo, 2007). Hemp is primarily cultivated for three post-harvest uses: fiber, grain, and cannabinoids. In recent years, hemp has emerged as a promising crop due to its multitude of uses and suitability for cultivation in diverse climates. With the passing of the 2018 Farm Bill and subsequent interim rule on hemp production, the regulatory environment for growing hemp in the United States (US) has shifted (McConnell et al., 2018). The removal of hemp from the list of controlled substances has loosened the restrictions on growing hemp in many areas, though it changed the legal threshold distinguishing hemp from high-THC (tetrahydrocannabinol) *Cannabis sativa*. Prior to the new legislation, only Δ^9-THC, the psychoactive compound, was considered for the 0.3% threshold. As legislation phases in across the US for the 2021 growing season, the threshold will likely consider total potential THC, including all of the Δ^9-THC, plus a calculated percentage of the concentration of the acidic form of THC (tetrahydrocannabinolic acid, THCA).

In order to stay compliant with this new threshold, growers need information on how cannabinoids accumulate in plants they are growing. Previous studies have used time course sampling to study the accumulation of cannabinoids as plants matured, however they conducted all of their studies in greenhouses, with relatively few high-CBD plants that reached a maximum concentration <10% CBD (Pacifico et al., 2008; Aizpurura-Olaizola et al., 2016).

Both of the aforementioned studies recognized the integral role that chemotype plays in determining a plant's cannabinoid profile. Previous analyses have confirmed that the allelic status of two loci, *B* and *O*, determine plant chemotype (Madolino et al., 2003; de Meijer et al., 2003, de Meijer et al., 2005; de Meijer et al., 2009). Despite recent genetic studies that have characterized the structure of the CBDAS (cannabidiolic acid synthase) and THCAS (tetrahydrocannabinolic acid synthase) at the *B* locus (Grassa et al. 2018; Laverty et al., 2019), they can largely be modeled as a single allelic locus as the THCAS and CBDAS genes are tightly linked in repulsion. Thus, we can summarize the five chemotypes as in Table 1.

Table 1. The five Cannabis sativa chemotypes described by their cannabinoid profile and allelic status.

Chemotype	Profile	*B* locus	*O* locus
I	Mostly THC	(B_T/B_T or B_T/B_0)	(*O*/*O* or *O*/*o*)
II	~1.6:1 CBD:THC	(B_T/B_D)	(*O*/*O* or *O*/*o*)
III	Mostly CBD	(B_D/B_D or B_D/B_0)	(*O*/*O* or *O*/*o*)
IV	Mostly CBG	(B_0/B_0)	(*O*/*O* or *O*/*o*)
V	Cannabinoid-free	Any	(*o*/*o*)

Flowering time also plays an integral role in the accumulation of cannabinoids over the growing season. Several studies have confirmed that many hemp cultivars behave as short-day plants switching from a vegetative phase to a reproductive phase at a certain inductive photoperiod; for hemp, between 14 and 15 h of daylight/9-10 h of darkness (Lisson, Mendham, and Carberry, 2000; Consentio et al., 2012). They also report the effect of temperature on induction of flowering. Some available cultivars, often deemed "auto-flowering" or day neutral, are photoperiod insensitive (Potter, 2014). Day neutral cultivars are generally much smaller in stature and are appealing to growers as they mature in a fraction of the time of photoperiod sensitive cultivars, independent of the ambient daylength.

As hemp becomes a more widely cultivated crop, growers will need to consider pest and pathogen management to be successful. Hemp powdery mildew is caused by the obligate biotrophic fungal pathogen *Golovinomyces spadiceus* (Braun, 1987). The pathogen is common during transplant

production and in controlled environment facilities around the US as well as in fields with favorable environmental conditions. There is no described resistance to hemp powdery mildew in hemp.

Materials and Methods

Plant Material

We started plants of 30 cultivars in a soilless media in a greenhouse in early May 2019. Plants were propagated from dioecious (mixed sex) seed, 'feminized' (all female) seed, or cloned via vegetative cuttings (see Table 2). Cuttings were rooted using Clonex rooting hormone (Hydrodynamics International). Seedlings and cuttings were maintained in the greenhouse at 18L:6D until transplant in the first week of June. Prior to transplanting in the field, dioecious cultivars were screened at the seedling stage with a Y-specific molecular marker in order to cull males (XY) (Toth et al., 2020).

Table 2. The 30 high-cannabinoid cultivars from 12 sources included in the experiment. Cultivars were started from seed (dioecious or feminized) or from vegetative cuttings (clones).

Cultivar	Propagation (Clone/seed)	Source
A2R4	Seed (dioecious)	Winterfox Farm
AC/DC	Seed (dioecious)	Winterfox Farm
Brilliance	Seed (dioecious)	Green Lynx Farms
Cherry 5	Seed (dioecious)	HempLogic
Cherry 307	Seed (dioecious)	HempLogic
Cherry 308	Seed (dioecious)	HempLogic
Deschutes	Seed (feminized)	Industrial Seed Innovations
First Light 49 (FL 49)	Clone	Sunrise Genetics
First Light 58 (FL 58)	Clone	Sunrise Genetics
First Light 70 (FL 70)	Clone	Sunrise Genetics
First Light 71 (FL 71)	Clone	Sunrise Genetics
First Light 80 (FL 80)	Clone	Sunrise Genetics
FL 70x70	Seed (feminized)	Sunrise Genetics
FL 71x71	Seed (feminized)	Sunrise Genetics
GVA-H-19-1039	Seed (dioecious)	Cornell Hemp Project
GVA-H-19-1097	Seed (dioecious)	Cornell Hemp Project
KG9201	Seed (feminized)	Kayagene
KG9202	Seed (feminized)	Kayagene
Late Sue	Clone	NY Hemp Source
NY Cherry	Seed (dioecious)	Genesis Hemp Alliance
Otto II	Seed (dioecious)	Winterfox Farm
RN13a	Seed (dioecious)	Go Farm Hemp
RN16	Seed (dioecious)	Go Farm Hemp
RN17	Seed (dioecious)	Go Farm Hemp
RN19	Seed (dioecious)	Go Farm Hemp
Rogue	Seed (feminized)	Industrial Seed Innovations
T2	Seed (feminized)	Boring Hemp
Tangerine	Clone	NY Hemp Source
TJ's CBD	Clone	Stem Holdings Agri
Umpqua	Seed (feminized)	Industrial Seed Innovations

Field Preparation and Maintenance

We planted trials at two Cornell University field sites, one in Geneva, NY (McCarthy Farm: 42.895426, -77.005467) and one in Ithaca, NY (Bluegrass Lane Turf and Ornamental Farm: 42.461478, -76.462679). See table 3 for the soil analysis at the two field sites.

Table 3. Soil analysis for the two trial sites.

	McCarthy Farm (Geneva, NY)	Bluegrass Lane Farm (Ithaca, NY)
Soil Type	Dunkirk fine sandy loam	Arkport fine sandy loam
Phosphorous (lbs A^{-1})	6	9
Potassium (lbs A^{-1})	79	170
Calcium (lbs A^{-1})	3,300	2,117
Magnesium (lbs A^{-1})	370	222
Manganese (lbs A^{-1})	27	41.8
Aluminum (lbs A^{-1})	6	85.5
Iron (lbs A^{-1})	1	25
Zinc (lbs A^{-1})	0	0.9
Soil pH	7.3	5.4
Organic Matter (%)	3.3	3.3

Each site was cultivated and 75 lb N A^{-1} was applied as 19-19-19 N-P-K during field preparation. Raised beds with drip irrigation and black plastic mulch were prepared every 6 feet on center in the fields. Additional 19-19-19 fertilizer, equivalent to 65 lb N A^{-1}, was spread under the plastic mulch. We used landscape fabric (McCarthy Farm) and regular-interval mowing (Bluegrass Lane Farm) to control weeds in the alleys.

The McCarthy trial was planted with five-plant plots in a randomized complete block design with four complete blocks including all 30 cultivars. The Bluegrass Lane trial was planted in the same design, except the following cultivars were not included in all four replicates due to a shortage of planting stock: RN13a (3 reps), Cherry 308 (2 reps), Cherry 5 (2 reps), NY Cherry (1 rep). This incomplete replication was due to deviation from expected sex ratio (one seed lot was strongly male biased) or poor germination. In addition, the material obtained for 'Late Sue', one of the clonal cultivars, was not uniform. This deviation was due to the intermixing of cuttings from a mother plant (probably 'Tangerine') that was not 'Late Sue', which was validated via genotyping of off-type plants. Only the true to type 'Late Sue' individuals were considered for the analysis.

We transplanted the seedlings and rooted cuttings into the raised beds on June 5, 2019 (McCarthy Farm) and June 7, 2019 (Bluegrass Lane Farm). Plants were spaced every 4 feet in the rows. After transplanting, the plots were irrigated using the in-bed drip irrigation as needed throughout the season. HOBOnet 10HS soil moisture sensors (Onset) were installed in the 'AC/DC' plots at the McCarthy site and used to asses when irrigation was necessary. Fertilizer (Jack's 12-4-16 Hydro FeED RO, 25 lbs. per treatment) was injected through the irrigation as needed. The Bluegrass Lane Farm trial was watered when the soil under the plastic appeared dry. Fertilizer (Peters Excel 15-5-15 Cal-Mag, 20.25 lbs. per treatment) was injected through the irrigation twice during the growing season. Due to plant lodging, we added wooden stakes and metal T-posts throughout the field to trellis the plants. Plants were trellised with twine using the 'Florida weave' method. This helped to support many of the plants until harvest, but many others lost lower branches in wind storms towards the end of the growing season.

Measuring Height and Growth Rate
Height was measured weekly for the first 11 weeks post-transplant on the middle three plants of each five plant plot. Growth rate was calculated by taking the difference between sequential week measurements and dividing by the number of days between measurements.

We modeled height and growth rate using a local linear regression model. The maximum growth date was determined by solving for the date that maximized the value of the growth rate model. After modeling, we sampled data points from all of the models and used *k*-means clustering to group similar models. We used the Hartigan and Wong algorithm (1979) to assign the clusters and the elbow method to select the optimal number of clusters.

Flowering Surveys
We monitored all of the plants weekly for evidence of flowering. For each plant we assessed whether there were any female flowers presenting pistils, and whether the plant had initiated terminal flowering. Plants were marked as terminally flowering when a cluster of female flowers was observed at shoot apices. Terminal flowering is distinct from sparse single flowers developing in the axils of the leaves. The few male or monoecious plants that produce staminate flowers were immediately removed from the field to keep the other plants unpollinated.

Five cultivars did not flower uniformly. For many of the analyses we considered the early flowering individuals distinct from the later flowering individuals. Early flowering is indicated with the addition of "(E)" to the end of a cultivar name.

An unbalanced, one-way ANOVA was used to determine if there was a significant effect of cultivar, site, or the interaction of cultivar and site on flowering date. We also conducted a post-hoc Tukey's HSD analysis to test pairwise differences between cultivars when the effect of cultivar was found to be significant.

Powdery Mildew Susceptibility
At the end of the growing season, all of the plants at both sites were visually rated for severity of powdery mildew on a continuous scale of 0-100% leaf area diseased. Some early flowering cultivars had already been harvested prior to disease rating and were not included.

Cannabinoid Time Series
We sampled 10 cm shoot tips from every plot starting the week of flowering and re-sampling weekly until harvest. In each of the five plant plots the following sampling scheme was implemented: 1st sample = 1st plant, 2nd sample = 5th plant, 3rd sample = 2nd plant, 4th sample = 4th plant. The cycle was repeated until harvest. If all of the plants in a plot were not flowering uniformly, we rotated between sampling the first and forth plants one week and the second and fifth plants the following week. We did not sample the middle (third) plant in each plot, which was instead preserved intact for destructive biomass sampling at harvest. In accordance with regulatory standard in New York State, the top 10 cm of the shoot tips were sampled for the time series. Shoot

tip samples were dried in a closed room with a dehumidifier, then milled to a fine powder in a Nutri Ninja Pro blender.

After milling, samples were stored in a coldroom (4°C) until high pressure liquid chromatography (HPLC) analysis. To prepare the samples for HPLC analysis, 50 mg of dried, milled tissue was mixed with 1.5 mL ethanol by high-speed shaking at room temperature with a Tissuelyser (Qiagen), and filtered through a SINGLE StEP PTFE Filter Vial (Thomson). The resultant liquid was directly subjected to HPLC analysis (Dionex UltiMate 3000; Thermo Fisher) with biphenyl-4-carboxylic acid (BPCA) as an internal standard, using a Phenomenex Kinetex 2.6 µm Polar 100 Å column 150 × 4.6 mm heated at 35°C. Samples were injected and eluted at 1.2 mL min^{-1} over a 6 min gradient, from 65% acetonitrile, 0.1% formic acid, to 80% acetonitrile, 0.1% formic acid, followed by a 4 min isocratic step. Absorbance was measured at 214 nm. The following standards were used to generate a calibration curve for each run: tetrahydrocannabinolic acid (THCA), Δ^9-tetrahydrocannabinol (THC), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabichromenic acid (CBCA), cannabichromene (CBC), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabinol (CBN), tetrahydrocannabivarin(THCV), tetrahydrocannabivarinic acid (THCVA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), and Δ^8-tetrahydrocannabinol (Δ^8 THC) (Sigma Aldrich).

We attempted to keep samples below 45°C to avoid decarboxylation of acid-form cannabinoids. To control for potential variation in decarboxylation of acid-form cannabinoids, we analyzed the total cannabinoids, using the following formulas to combine the acidic and neutral forms to an estimated total amount of the neutral cannabinoid:

Total CBD % = CBD % + (CBDA % * 0.877)
Total THC % = THC % + (THCA % * 0.877)
Total CBG % = CBG % + (CBGA % * 0.878)
Total CBC % = CBC % + (CBCA % * 0.877)
Total CBDV % = CBDV % + (CBDVA % * 0.867)
Total THCV % = THCV % + (THCVA % * 0.867)

We modeled total CBD and total THC accumulation for all cultivars in the trial using third degree polynomial generalized linear models, with the interaction of location and replicate included as a random effect. The estimated date when the cultivar exceeds 0.3% total THC was based on when the modeled total THC accumulation curve crossed the 0.3% total THC threshold.

Cultivars were categorized into accumulation rate groups based on the number of weeks between terminal flowering and the modeled THC accumulation curve crossing the 0.3% total THC threshold.

End of Season Biomass
When a cultivar was ready for harvest, we cut the stems of the plants at soil level and measured the wet biomass for all plants in a plot. We hung the middle plant in every five-plant plot in a barn to air-dry using industrial fans. After the plant was dry, we measured total dry biomass and

subsequently stripped the floral material off the plant to measure dry floral biomass. Cannabinoids were quantified using a subsample of the stripped biomass, which followed the same protocol outlined for the time series samples.

To report the estimated biomass measurements while controlling for site and rep effects, we modeled the biomass measurements using a mixed model with 'Cultivar' as a fixed effect and the interaction between 'Site' and 'Rep' as a random effect. The following calculations were then derived from the estimates:

% Dry matter = total dry biomass / total wet biomass * 100 %
% floral tissue = dry floral biomass / total dry biomass * 100 %
CBD yield per plant = dry floral biomass * % CBD in biomass subsample
Biomass/Shoot Ratio = % CBD in biomass sample / % CBD in last shoot sample
CBD:THC Ratio = Total CBD % / (Total THC % + CBN %)

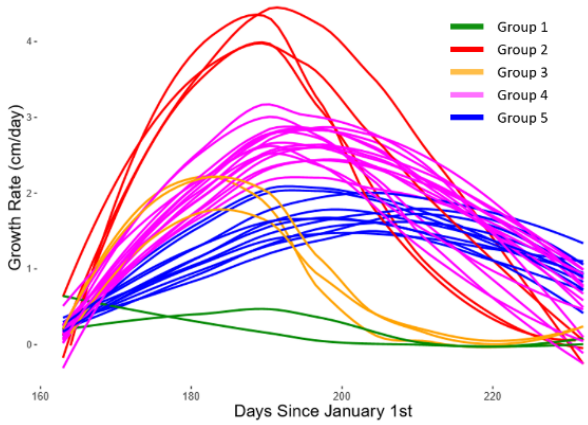
Plant Form and Apical Dominance
We categorized plant architecture based on a set of descriptors defined for tree and shrub morphology (United States Forest Service, 1980). Cultivars were assigned relative apical dominance groups based on where they fell on a spectrum from strong apical dominance (one dominant shoot/columnar form) to weak apical dominance (no dominant shoots/round form).

Results & Discussion

Height and growth rate
Based on the clustering analysis, the height and growth rate measurements separate the cultivars into five general groups (Figure 1C & 1D, Table 4). Group 1, including KG9201 and KG9202, consisted of only day neutral cultivars. These cultivars averaged growth less than ~0.8 cm day^{-1} after transplant and never reached heights more than 50 cm. Group 2, A2R4 and GVA-H-19-1097, was the fastest growing, averaging >3 cm day^{-1} throughout July. Though these cultivars were segregating for flowering time, the growth curves were similar for the early and late flowering individuals. This group had the tallest plants with some reaching over 2 m in height, though they were very narrow and yielded little biomass. Group 3 included the early flowering individuals of Umpqua, Rogue, and Deschutes. These were distinct from the later flowering individuals within their respective cultivars, with growth rates peaking in early July around the time that they began to terminally flower. Group 4 consisted of the cultivars reaching maximum growth rates between 2 and 3 cm day^{-1} in mid- to late-July. Group 5 mostly consisting of the First Light cultivars, had slightly lower maximum growth rates (between 1 and 2 cm day^{-1}) that occurred at the end of July, slightly later than the other cultivars. Changes in height and growth rate during the growing season could be easy to measure indicators of plant development. Flowering time and, in turn, cannabinoid accumulation varies according to latitude.

Table 4. K-means clusters of cultivars based on growth rate curves.

Group	1	2	3	4	5
Characters	Avg. Growth: *<0.8* cm day^{-1} Max Height: *50 cm*	Max Growth: *>3.5* cm day^{-1} Max Height: *>2 m*	Max Growth: *Early July* Max Height: *~1 m*	Max Growth: *2-3* cm day^{-1} *Mid-July*	Max Growth: *1-2* cm day^{-1} *Late July*
Cultivars	KG9201 KG9202	A2R4 GVA-H-19-1097	Umpqua (E) Rogue (E) Deschutes (E)	RN13a RN16 RN17 RN19 GVA-H-19-1039 Brilliance AC/DC Umpqua Rogue Deschutes TJ's CBD Cherry 308 NY Cherry Otto II Late Sue	FL 49 FL 58 FL 70 FL 71 FL 80 FL 70x70 FL 71x71 T2 Tangerine Cherry 307 Cherry 5



Cannabinoid accumulation

Because of the strong effect of flowering time on cannabinoid accumulation, we standardized the time series measurements by flowering date prior to fitting the curves. After standardization, it is clear that the majority of the cultivars follow the same pattern of rapid cannabinoid accumulation 0.5 to 2 weeks after terminal flowering (Figure 1A & 1B). However, there were a few notable exceptions to this pattern. A2R4 and GVA-H-19-1097 both showed much slower accumulation across the growing season and appeared to flower in a more 'indeterminate' fashion, continuing to grow and develop flowers throughout July, August, and September. Their inflorescences were much looser, which may be a factor in the low cannabinoid levels of shoot tips. The other cultivars that did not follow the trend were the day neutral Kayagene cultivars and Late Sue. These both presented challenges for sampling because the Kayagene cultivars flowered so early that collection of shoot tip samples would have destroyed a large proportion of plant biomass, and Late Sue did not flower until October, which reduced the number of weeks that we could sample before harvest.

Of the cultivars that accumulated greater than ~5% CBD, some accumulation curves level-off, while others continue to increase or decrease after peaking ~4.5 weeks after terminal flowering. It is difficult to conclude whether these trends are representative of the cultivar's late-season cannabinoid accumulation because our time series sampling was interrupted by the need to harvest. While hemp plants cannot accumulate cannabinoids indefinitely, it is unclear what the potential physiological maximum percent of cannabinoids is in a shoot tip sample. After reaching this maximum, it seems likely that the amount of cannabinoids would start to decrease over time as the plant begins to senesce, after which flowers and trichomes fall off, and necrotrophic pathogens start colonizing the dying plant.

Upon overlaying the CBD and THC accumulation curves, one of the clearest observations is that CBD and THC accumulation are strongly correlated in Chemotype III plants. We observed CBD:THC ratios from ~1:20 to ca. 1:30 depending on cultivar and timepoint. The very high ratios were often from samples with very high levels of total CBD, which may have caused an overestimation of concentration through the HPLC-based analysis. Ratios ca. 1:23 are consistent with the *in vitro* ratio observed for CBDAS by Zirpel, Kayser, and Stehle (2018). This supports the idea that, in chemotype III plants, THC is produced primarily through the activity of CBDAS, rather than THCAS or CBCAS (cannabichromenic acid synthase). Due to the promiscuous synthesis of THCA by CBDAS, accumulation of > 7-8% total CBD leads to coupled accumulation of total THC exceeding the federal legal threshold of 0.3%. In order to stay compliant, regulatory testing must be completed very soon after terminal flowering for cultivars expected to exceed 8% CBD.

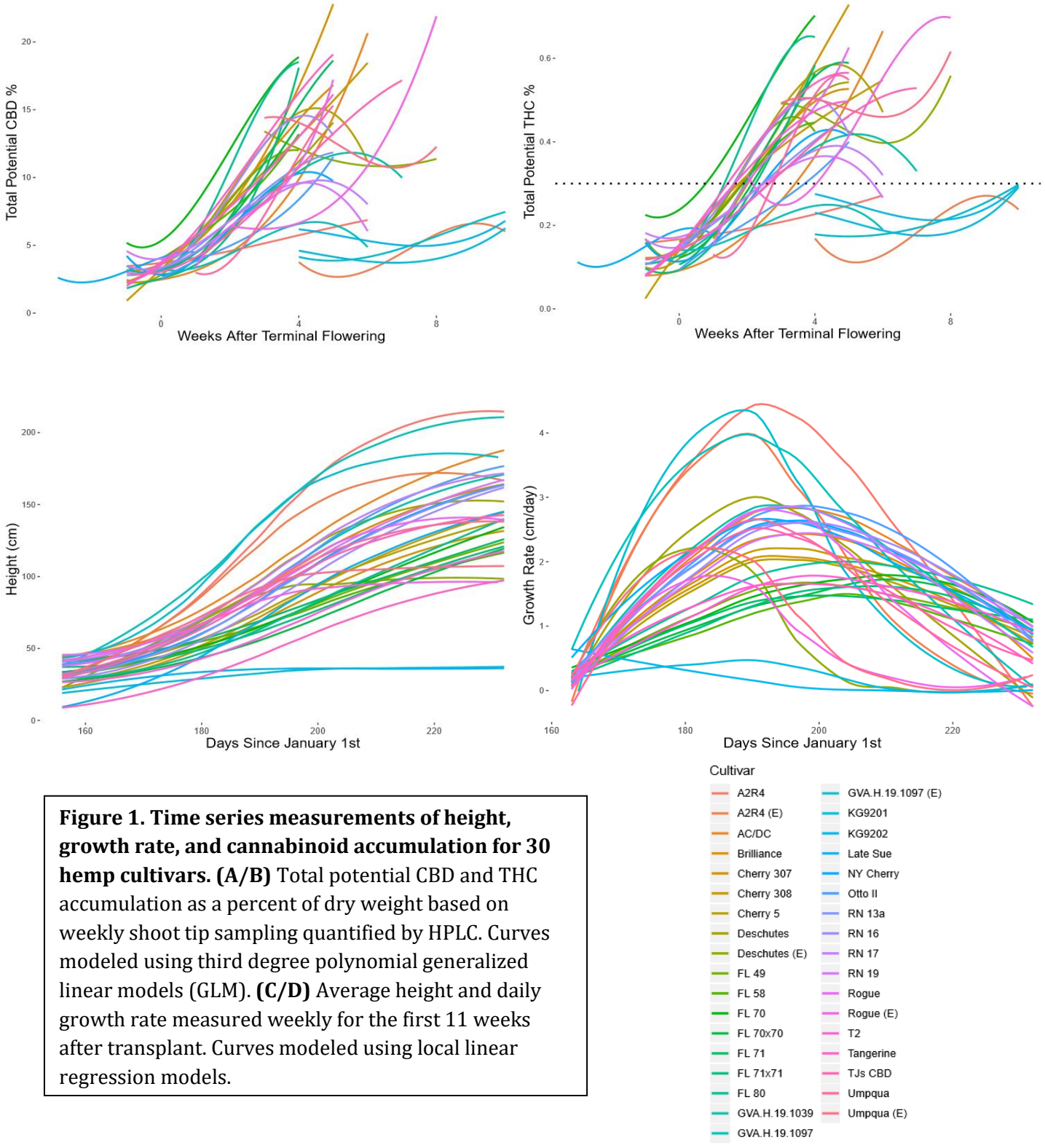


Figure 1. Time series measurements of height, growth rate, and cannabinoid accumulation for 30 hemp cultivars. (A/B) Total potential CBD and THC accumulation as a percent of dry weight based on weekly shoot tip sampling quantified by HPLC. Curves modeled using third degree polynomial generalized linear models (GLM). **(C/D)** Average height and daily growth rate measured weekly for the first 11 weeks after transplant. Curves modeled using local linear regression models.

Season-Long Timeline

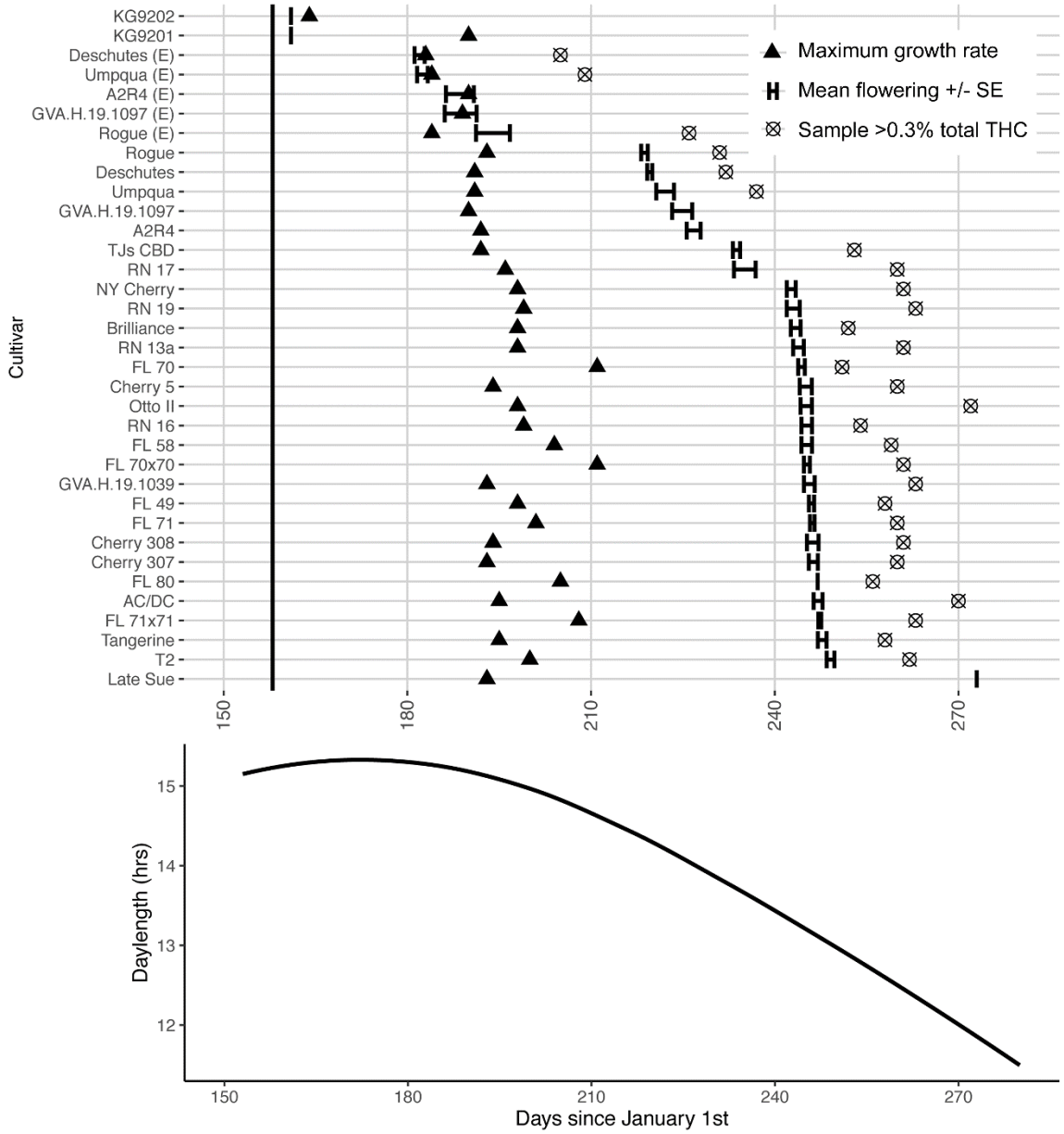


Figure 2. Timeline of growing season milestones for 30 hemp cultivars. Cultivars were ordered from earliest flowering (top) to latest flowering (bottom). The vertical black line indicates transplant date (June 5th). Triangles indicate the estimated day of maximum growth rate, error bars indicate terminal flowering date +/- SE of the mean, and crossed out circles indicate the estimated date when shoot-tip sampled chemotype III individuals of the cultivar will exceed 0.3% total THC. The bottom panel plots daylength in Geneva, NY from planting until harvest.

Flowering time

There was a significant effect of cultivar on flowering date (p<0.001, F=889.522, df=34). There was not a significant effect of site on flowering time (p>0.05, F=0.812, df=1), however there was a significant interaction between site and cultivar (p<0.001, F=2.526, df=34).

The cultivars in the trial could be broadly separated into five groups based on flowering time (Figure 2). The day neutral Kayagene cultivars clearly flowered before all other cultivars. The next to flower were the early flowering individuals of A2R4, GVA-H-19-1097, Umpqua, Rogue, and Deschutes, which flowered in early- to mid-July. The later flowering individuals of the five aforementioned cultivars flowered about one month later in mid- to late-August. We observed a 1:1 ratio of early- to late-flowering individuals in Umpqua and Deschutes and a 1:3 ratio for Rogue. These cultivars were marketed as F_1 hybrids, derived from crossing two inbred clones. With limited pedigree information, the phenotypes we observed in the field suggests a single gene segregating in the populations conferring early flowering. The majority of the cultivars flowered in early-September, which is in line with the previously documented 14 h critical photoperiod for *Cannabis sativa* (Lisson, Mendham, and Carberry, 2000). The latest cultivar to flower, aptly named Late Sue, did not initiate flowering until the first week in October.

Terminal flowering time, reported here, was distinct from the presence of female flowers in the axils of the leaves. There was not a strong correlation between the emergence of axillary female flowers and the initiation of terminal flowering. On one extreme, the First Light cultivars did not produce any axillary flowers until they started terminally flowering. In contrast, Late Sue produced axillary flowers throughout the growing season starting in June, and did not terminally flower until October.

Chemotype

As is widely documented in the literature, the chemotype of a plant is the primary indicator of the relative proportions of endogenous cannabinoids (Figure 3). Several of the cultivars in the trial were segregating at the *B* locus and produced the expected ratios of CBD:THC. Chemotypes were assigned to three groups, based on CBD:THC ratios. Chemotype I, II, and III plants of the same cultivar produced similar concentrations of total cannabinoids. While chemotype does not determine the total concentration of cannabinoids produced, all chemotype I and II plants in our trials consistently exceeded the federal threshold of 0.3% total THC after flowering.

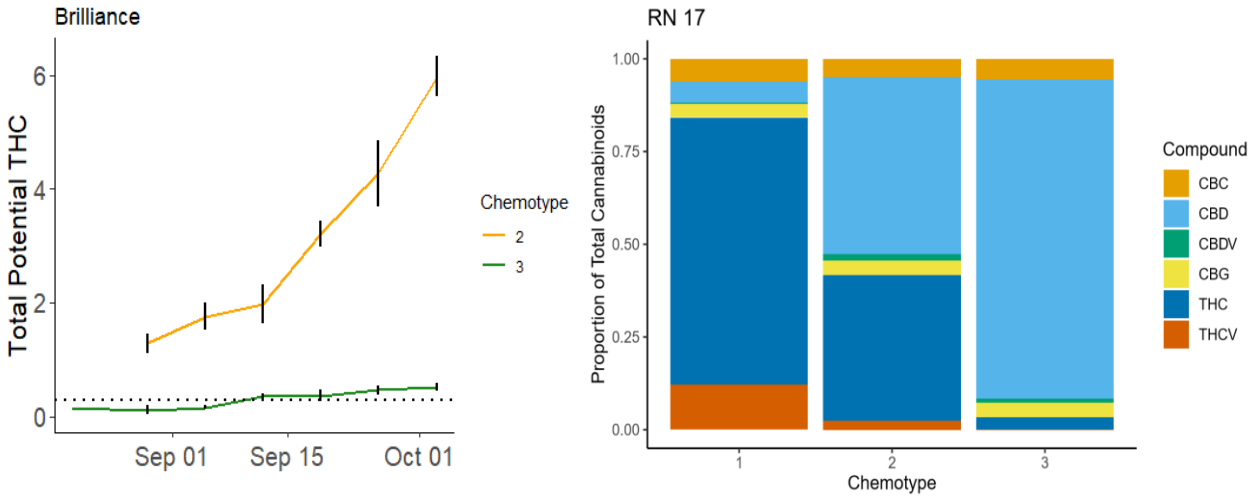


Figure 3. Chemotype segregation for two of hemp cultivars. (L) Accumulation of THC over the course of floral maturation of cultivar 'Brilliance.' Chemotype III plants accumulated only low levels of THC. The dotted line indicates the federal legal threshold of 0.3% total THC. In contrast, the chemotype II plants consistently exceeded the legal threshold and accumulated much greater levels of THC. **(R)** Relative proportions of six cannabinoids (combined acidic and neutral forms) measured by HPLC. Cultivar 'RN17' had individuals of chemotypes I, II, and III.

Powdery mildew

There was a wide distribution in the severity of powdery mildew at the two sites, although there was generally more disease in the McCarthy Farm trial than the Bluegrass Lane Farm trial (Figure 4). Because we did not inoculate with the pathogen in the field, some of the variability within and between sites is likely due to the variability in natural pathogen inoculum levels. For some cultivars, disease severity by cultivar varied by site. NY Cherry had no powdery mildew in the Bluegrass Lane Farm trial, but had near 20% leaf area with powdery mildew in the McCarthy Farm trial. Despite the abundance of powdery mildew disease in both trials, especially in the McCarthy Farm trial, no powdery mildew was observed on any of the First Light 58 plants.

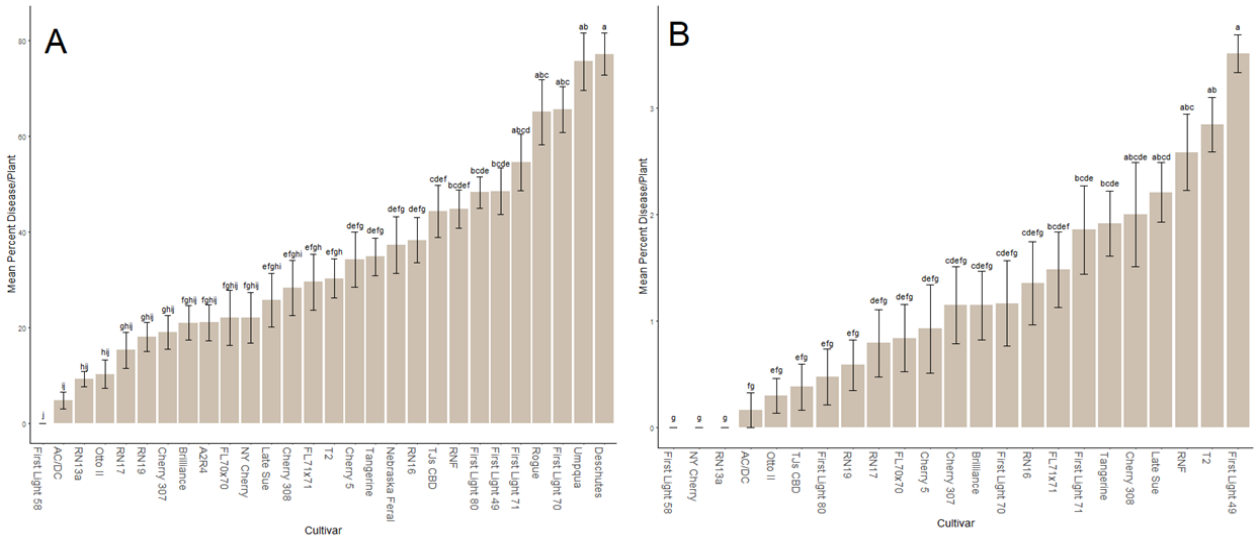


Figure 4. Powdery mildew severity. Visual ratings of percent leaf area with powdery mildew at the end of September. Overall disease severity was significantly different at the two field sites: McCarthy Farm/Geneva, NY **(A)** and Bluegrass Lane Farm/Ithaca, NY **(B)**. Data were log transformed for the Bluegrass Lane Farm trial.

Cultivar	CBD Yield (kg)	Stripped Biomass (kg)	Dry Biomass (kg)	Wet Biomass (kg)	% Dry	% Stripped	% Total CBD	% Total THC	% Total CBC	% Total CBG	% Total CBDV	% Total THCV	% Total CBN	CBD:THC Ratio
Umpqua	0.133	0.98	1.46	3.01	48.5%	67.4%	13.48	0.54	0.57	0.30	0.07	0.07	0.04	23.41
Rogue	0.129	0.89	1.42	4.21	33.6%	63.1%	14.48	0.52	0.59	0.43	0.09	0.06	0.01	27.39
Deschutes	0.114	0.93	1.51	2.95	51.1%	61.7%	12.24	0.47	0.48	0.31	0.18	0.14	0.05	23.61
Cherry 5	0.113	0.89	1.95	5.17	37.7%	45.7%	12.72	0.49	1.14	0.40	0.10	0.17	0.03	24.41
TJ's CBD	0.112	0.95	1.76	6.00	29.4%	53.8%	11.80	0.44	0.50	0.22	0.09	0.05	0.02	25.81
FL 70	0.109	0.73	1.54	4.79	32.2%	47.4%	14.93	0.53	0.66	0.47	0.08	0.10	0.01	27.68
RN16	0.095	0.87	1.76	6.30	28.0%	49.3%	10.96	0.42	0.83	0.32	0.04	0.04	0.01	25.54
Late Sue	0.088	1.40	4.52	8.32	54.3%	31.0%	6.31	0.25	0.48	0.23	0.03	0.02	0.01	24.05
Cherry 308	0.084	0.93	2.02	6.89	29.4%	45.9%	9.03	0.36	0.71	0.29	0.06	0.06	0.02	23.64
Brilliance	0.084	0.79	1.73	5.47	31.7%	45.5%	10.61	0.38	1.04	0.19	0.06	0.04	0.01	27.15
FL 80	0.082	0.61	1.29	4.41	29.2%	47.3%	13.44	0.48	0.67	0.40	0.08	0.07	0.01	27.38
FL 49	0.081	0.70	1.42	4.99	28.4%	49.3%	11.54	0.45	0.57	0.34	0.04	0.11	0.01	25.33
NY Cherry	0.076	0.89	1.72	6.28	27.3%	51.9%	8.55	0.33	1.00	0.28	0.06	0.04	0.03	24.06
Cherry 307	0.076	0.71	1.41	5.95	23.7%	50.1%	10.69	0.41	0.71	0.28	0.05	0.04	0.03	24.45
FL 71	0.074	0.72	1.56	5.55	28.1%	46.5%	10.19	0.41	0.57	0.35	0.05	0.05	0.01	24.14
Tangerine	0.069	0.61	1.17	4.17	28.0%	52.5%	11.20	0.45	0.59	0.29	0.04	0.09	0.03	23.30
RN17	0.063	0.69	1.35	6.15	21.9%	50.8%	9.13	0.35	0.83	0.28	0.09	0.05	0.02	24.87
GVA.H.19.1039	0.062	0.78	1.96	6.65	29.5%	39.8%	7.90	0.30	0.82	0.23	0.05	0.06	0.01	25.21
RN13a	0.060	0.77	1.71	6.17	27.8%	44.7%	7.87	0.31	0.83	0.23	0.06	0.03	0.03	23.22
T2	0.056	0.70	1.26	4.06	31.1%	55.5%	7.95	0.33	0.43	0.20	0.02	0.03	0.02	22.79
AC/DC	0.056	0.74	2.00	7.23	27.7%	37.0%	7.49	0.28	0.85	0.21	0.05	0.06	0.06	22.06
FL 58	0.055	0.53	1.04	4.59	22.7%	50.9%	10.29	0.40	0.55	0.33	0.04	0.06	0.01	25.31
RN19	0.054	0.65	1.50	5.69	26.3%	43.4%	8.33	0.32	1.11	0.27	0.07	0.08	0.01	25.55
Otto II	0.050	0.67	1.84	5.77	31.9%	36.3%	7.47	0.28	0.60	0.24	0.08	0.05	0.01	26.12
GVA.H.19.1097	0.045	0.71	1.19	4.38	27.2%	59.9%	6.27	0.26	0.29	0.14	0.06	0.04	0.02	22.34
FL 71x71	0.036	0.43	0.87	3.61	24.1%	49.7%	8.31	0.34	0.48	0.28	0.04	0.07	0.01	24.03
A2R4	0.026	0.49	1.02	5.28	19.3%	48.0%	5.40	0.23	0.25	0.12	0.05	0.02	0.02	21.92
FL 70x70	0.019	0.22	0.39	3.16	12.4%	56.7%	8.48	0.34	0.47	0.30	0.04	0.05	0.01	23.99
KG9202	0.007	0.10	0.12	0.39	30.4%	87.6%	6.84	0.21	0.42	0.09	0.05	0.01	0.06	25.41
KG9201	0.006	0.10	0.11	0.25	44.0%	87.9%	6.38	0.20	0.30	0.09	0.04	0.02	0.05	25.26

Table 5. Biomass and yield measurements. Per plant averages for biomass measurements (wet biomass, stripped biomass, dry biomass, % dry biomass, % stripped biomass), cannabinoid analysis on chemotype III stripped biomass samples (combined acidic and neutral forms of CBD, THC, CBC, CBG, CBDV. THCV), and CBD yield estimates based on stripped biomass and % total CBD. CBD:THC ratio included CBN as a portion of total THC.

Biomass

Biomass yield varied significantly by cultivar (Table 5). When we consider the proportion of the biomass that was dry matter, a few of the cultivars stood out. The Kayagene cultivars, as well as Umpqua, Rogue, and Deschutes, had relatively high percent dry matter. This could be due to the time they sat in the field after reaching maturity, which may have dried the plants down some before the wet weight measurement. Late Sue also had a very high percent dry matter, though it was certainly not from drying down in the field, as the plants were still actively growing up until harvest. The Late Sue plants did have very large stems and many branches, evidenced by the low proportion of stripped floral biomass, which could have contributed to the larger proportion dry matter. In regard to proportion floral biomass, the Kayagene cultivars were also higher than the rest. This was because they were less than 0.5 m tall, had few leaves, and short and narrow stems. As previously mentioned, Late Sue had a very low proportion of floral biomass due to late initiation of terminal flowering and production of many large stems.

Cannabinoid content in the stripped floral biomass was similar in composition (relative proportion of each cannabinoid) to the shoot tip samples. There is more CBN in the biomass samples that were stored longer prior to cannabinoid extraction and HPLC analysis. In the Kayagene plants that were in storage the longest, it appears that as much as one quarter of the THC was converted to CBN post-harvest. We included the CBN as a portion of THC in the CBD:THC ratio in order not to skew the ratios in the cultivars that were stored longer.

The ratio of shoot tip percent cannabinoids to biomass percent cannabinoids was inconsistent. Some cultivars had a ratio near 1.00, indicating a shoot tip measurement consistent with the concentration of cannabinoid in the biomass. Others, however, had a ratio as low as 0.44 indicating the concentration of cannabinoid in the biomass was less than half of that in the shoot tip samples. There was a negative correlation between the concentration of cannabinoids in the biomass and the biomass/shoot cannabinoid ratio.

CBD Yield

The two contributors to the yield calculation were the amount of stripped biomass produced and the concentration of CBD in stripped biomass (Table 6). Some cultivars had very high biomass that contributed to their above average yield, like Late Sue. Other cultivars, like First Light 80, had a high concentration of CBD but produced little biomass. The data do not support a resource sink tradeoff between the amount of floral biomass produced and the concentration of cannabinoids in the biomass; cultivars with the highest concentration of cannabinoids also produced some of the highest amounts of floral biomass.

It is important to note that the yield estimates are on a per plant basis, and not corrected to reflect optimal planting density. The Kayagene cultivars, as well as Umpqua, Rogue, and Deschutes, could be planted at a higher density than most of the other cultivars in the trial, which would provide a significant increase in yield when considered on a per cultivated area basis. They were also much easier to harvest than the plants that exceeded 10kg in wet biomass. Additionally, the yield estimate

does not consider that growers could potentially grow more than one cycle of hemp per year (or grow another crop) if they plant one of the early maturing cultivars.

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Cultivar	Chemotypes	Flowering	Accumulation Rate	Powdery Mildew	Plant Architecture	Apical Dominance	CBD Yield (kg)	Biomass/Shoot Ratio
Umpqua	All Chemotype III	Very Early/Early*	Fast	Very Susceptible	Pyramidal	Very Dominant	0.133	0.87
Rogue	All Chemotype III	Very Early/Early*	Fast	Very Susceptible	Pyramidal	Very Dominant	0.129	0.84
Deschutes	All Chemotype III	Very Early/Early*	Fast	Very Susceptible	Pyramidal	Very Dominant	0.114	0.76
Cherry 5	All Chemotype III	Middle	Moderate	Susceptible	Round	Not Dominant	0.113	0.88
TJ's CBD	All Chemotype III	Early	Moderate	Susceptible	Irregular	Some Dominance	0.112	0.64
FL 70	All Chemotype III	Middle	Very Fast	Very Susceptible	Round	Not Dominant	0.109	0.81
RN16	All Chemotype III	Middle	Very Fast	Susceptible	Round	Not Dominant	0.095	0.63
Late Sue	All Chemotype III	Late	Very Slow***	Susceptible	Round (Large)	Not Dominant	0.088	1.17
Cherry 308	All Chemotype III	Middle	Fast	Susceptible	Round	Not Dominant	0.084	0.96
Brilliance	Many Chemotype II	Middle	Very Fast	Mod. Resistant	Round	Not Dominant	0.084	0.94
FL 80	All Chemotype III	Middle	Very Fast	Mod. Resistant	Round	Not Dominant	0.082	0.44
FL 49	All Chemotype III	Middle	Fast	Susceptible	Round	Not Dominant	0.081	1.00
NY Cherry	All Chemotype III	Middle	Moderate	Mod. Resistant	Round	Not Dominant	0.076	0.68
Cherry 307	All Chemotype III	Middle	Fast	Mod. Resistant	Round	Not Dominant	0.076	0.52
FL 71	All Chemotype III	Middle	Fast	Mod. Resistant	Round	Not Dominant	0.074	0.67
Tangerine	All Chemotype III	Middle	Very Fast	Susceptible	Round (Compact)	Not Dominant	0.069	0.59
RN17	Some Chemotype I and II	Early	Slow	Mod. Resistant	Goblet	Some Dominance	0.063	1.07
GVA.H.19.1039	All Chemotype III	Middle	Moderate	Susceptible	Round	Not Dominant	0.062	0.65
RN13a	Few Chemotype II	Middle	Moderate	Very Resistant	Round	Not Dominant	0.060	0.51
T2	All Chemotype III	Middle	Fast	Susceptible	Vase-Like	Some Dominance	0.056	0.47
AC/DC	Some Chemotype II	Middle	Slow	Very Resistant	Round (Large)	Not Dominant	0.056	1.02
FL 58	All Chemotype III	Middle	Fast	Very Reisistant**	Round	Not Dominant	0.055	0.68
RN19	Some Chemotype II	Middle	Slow	Mod. Resistant	Vase-Like	Some Dominance	0.054	0.79
Otto II	Some Chemotype II	Middle	Slow	Very Resistant	Round (Large)	Not Dominant	0.050	0.66
GVA.H.19.1097	Few Chemotype II	Very Early/Early*	Very Slow	Susceptible	Columnar	Very Dominant	0.045	1.15
FL 71x71	All Chemotype III	Middle	Moderate	Susceptible	Round	Not Dominant	0.036	0.48
A2R4	Few Chemotype II	Very Early/Early*	Very Slow	Mod. Resistant	Columnar	Very Dominant	0.026	0.87
FL 70x70	All Chemotype III	Middle	Moderate	Mod. Resistant	Round	Not Dominant	0.019	0.71
KG9202	All Chemotype III	Autoflower	Very Slow	Very Susceptible	Dwarf	Some Dominance	0.007	1.05
KG9201	All Chemotype III	Autoflower	Very Slow	Very Susceptible	Dwarf	Some Dominance	0.006	1.07

*Cultivars segregating for two distinct flowering times

** No powdery mildew observed on FL 58

*** Late flowering confounded with slow cannabinoid accumulation

Table 6. Summary of traits by cultivar. Measured traits summarized into categorical groups based on observed distribution of traits in the trials.

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