Season-long characterization of high-cannabinoid hemp (Cannabis sativa L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance

George M. Stack | Jacob A. Toth | Craig H. Carlson | Ali R. Cala
Mariana I. Marrero-González | Rebecca L. Wilk | Deanna R. Gentner
Jamie L. Crawford | Glenn Philippe | Jocelyn K. C. Rose | Donald R. Viands
Christine D. Smart | Lawrence B. Smart

Abstract
Given the dramatic rise in high-cannabinoid hemp (Cannabis sativa L.) production in the last decade, there is an increasingly urgent need to characterize available germplasm and develop knowledge to accelerate the breeding of uniform and stable cultivars. Despite persistent cultivation of hemp cultivars for grain and or fiber around the world, the diversity and genetic underpinning of key traits for breeding and cultivation are poorly understood. For 30 high-cannabinoid hemp cultivars replicated on two field sites, we sought to evaluate yield, agronomic performance, and disease resistance while also conducting a detailed study of cannabinoid accumulation over the course of floral maturation. We observed significant variation in both within and among cultivars. During the growing season, the plants clustered into five groups by growth rate and varied in flowering time from photoperiod insensitive to photoperiod sensitive with very short critical photoperiods. Based on the observed ratio of total potential cannabidiol (CBD) to total potential tetrahydrocannabinol (THC), there was segregation for cannabinoid chemotype in some seeded cultivar populations. Analysis of cannabichromene (CBC) production revealed that some cultivars had a discretely lower CBD:CBC ratio than the others. There was a continuous range of powdery mildew severity by cultivar, with one that had little to no observed powdery mildew suggesting it might have genetic resistance. Biomass production at harvest was strongly influenced by location and cultivar, and there was variation by cultivar in the relative cannabinoid production in shoot tip samples compared with whole plant samples. While our results provide preliminary guidance regarding relative performance of current cultivars, our analyses indicate a need for additional hemp breeding to provide stable, uniform, and legally compliant cultivars with improved disease resistance and flowering times optimized for the latitudes of different growing locations.
1 INTRODUCTION

Hemp (Cannabis sativa L.) has long been cultivated by humans for food, fiber, and medicinal purposes (Russo, 2007), and is primarily grown for three post-harvest market classes: fiber, grain, and cannabinoid compounds, notably cannabidiol (CBD). The regulatory environment surrounding hemp is currently complex and rapidly evolving. In legal terms, hemp plants are generally considered to be those with concentrations of the restricted psychoactive compound Δ9-tetrahydrocannabinol (THC) below a certain legal threshold. This threshold varies depending on jurisdiction, but is typically 0.2% or 0.3% of the dry weight. To stay compliant with the legal THC threshold, growers need information related to the cannabinoid accumulation characteristics of high-cannabinoid hemp cultivars. Cannabinoids are primarily produced in the stalked capitulate glandular trichomes found most abundantly in the female inflorescences (Livingston et al., 2020). Prior to 2019, several studies used time course sampling to study the accumulation of cannabinoids as plants matured (Aizpurua-Olaizola et al., 2016; Pacifico et al., 2008). While these studies provide valuable insight into cannabinoid accumulation in hemp, both were conducted in greenhouse settings with plants that reached a maximum concentration <10% CBD. De Backer et al. (2012) conducted a similar study with plants that accumulated high levels of THC (>15%), observing a plateau in cannabinoid concentration after flowering. Richins et al. (2018) also studied cannabinoid accumulation in high-THC Cannabis but, importantly, inflorescences were sampled from two locations on the plant as well as vegetative “fan” leaves (separate from the inflorescence). The authors found significant differences in cannabinoid concentration between tissue types and inflorescence locations. More recently, Yang et al. (2020) used time course sampling assess the cannabinoid accumulation patterns of five high-CBD (maximum shoot tip total CBD levels of 5%–15%) cultivars in the field. They reported that the maximum concentration of cannabinoids occurred between 6- and 8-week post-anthesis, where anthesis is defined as “50% of plants within a plot show[ing] the first distinguishable pistillate flowers.”

All of the aforementioned studies recognized the fundamental role of genotype in determining a plant’s chemotype and cannabinoid profile. Previous analyses have confirmed that the allelic status of two loci, B and O, are the primary determinants of C. sativa cannabinoid chemotype (de Meijer et al., 2003; de Meijer & Hammond, 2005; de Meijer, Hammond, & Sutton, 2009; Mandolino et al., 2003). Despite recent genetic studies that have characterized the complex arrangement of the CBDAS (cannabidiolic acid synthase) and THCAS (tetrahydrocannabinolic acid synthase) genes at the B locus (Grassa et al., 2018; Laverty et al., 2019), they can largely be modeled as a single allelic locus because the THCAS and CBDAS genes are tightly linked in repulsion. The classification scheme for the five common chemotypes (Table 1) does not incorporate variation in abundance of propyl cannabinoids, CBC (cannabinichromene), or less common alleles at the B locus (de Meijer, Hammond, & Micheler, 2009; Onofri et al., 2015; Welling et al., 2019). de Meijer and Hammond (2016) proposed a more complete model of inheritance, including the postulated polygenic loci A1–n, controlling abundance of propyl cannabinoids, and PIC1–n, controlling a set of morphological factors conferring a “prolonged juvenile chemotype” with a greater proportion of CBC.

Flowering time also has a major effect on the accumulation of cannabinoids. Several studies have shown 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 15 and 13 h of daylight (9–11 h of darkness; Cosentino et al., 2012; Lisson et al., 2000). These studies have also reported an effect of temperature on induction of flowering. Some cultivars, colloquially deemed “auto-flowering,” or day neutral, are photoperiod insensitive. 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 day length.

| Chemoype | Profile | B locus | O locus |
|----------|---------|---------|---------|
| I        | Mostly THC(A) | $B_T (B_T \ or \ B_T (B_O))$ | $O/O \ or \ O/O$ |
| II       | ~1.5:1 CBD(A):THC(A) | $B_T (B_D)$ | $O/O \ or \ O/O$ |
| III      | Mostly CBD(A) | $B_T (B_T) \ or \ B_T (B_O)$ | $O/O \ or \ O/O$ |
| IV       | Mostly CBG(A) | $B_T (B_O)$ | $O/O \ or \ O/O$ |
| V        | Cannabinoid-free | Any | $(O/O)$ |
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; Weldon et al., 2020). This pathogen is common during transplant production and in controlled environment facilities, as well as in fields with favorable environmental conditions. To date, there is no documented resistance to powdery mildew in the hemp literature.

To replicate and extend work in the existing literature, the objectives of the replicated field trials involving 30 high-CBD cultivars in this experiment were to:

1. Assess variation in cultivar height and growth rate throughout the growing season;
2. Evaluate genetic segregation for chemotype in cultivars;
3. Quantify variation in flowering time within and between cultivars;
4. Model cannabinoid accumulation by cultivar using a time series of shoot tip samples;
5. Rate cultivars for susceptibility to powdery mildew; and
6. Quantify yield components by cultivar including biomass yield and cannabinoid content in stripped biomass.

## MATERIALS AND METHODS

### 2.1 Plant material

In all, 30 hemp cultivars from 12 commercial sources and the Cornell hemp breeding program were established in a peat-based soilless media (Lambert LM111) in early May 2019. We use the term “cultivar” as defined by Liberty Hyde Bailey in 1923: “a race [or variety] subordinate to species, that has originated and persisted under cultivation” (Bailey, 1923). However, at the time of this publication, none of the described cultivars were recognized by the Association of Official Seed Certifying Agencies (https://www.aosca.org/hemp). In accordance with techniques employed by commercial cultivators, plants were propagated from dioecious (male and female) seed, “feminized” (all female) seed (see Lubell & Brand, 2018; Ram & Sett, 1982), or via vegetative cuttings (Table 2). Cuttings were rooted using Clonex® rooting hormone (Hydrodynamics International). Seedlings and cuttings were maintained in the greenhouse at 18 h light:6 h dark until transplant in the first week of June. Dioecious cultivars were screened at the seedling stage with the CSP-1 Y chromosome-specific molecular marker to select females for transplant to the field (Toth et al., 2020). These propagation methods enable the exclusion of male plants from the field, and important step in the widespread practice of maintaining unpollinated female inflorescences for cannabinoid production.

### 2.2 Field preparation and maintenance

Trials were planted at two Cornell University field sites: Geneva, NY (McCarthy Farm: 42.895426, −77.005467) and Ithaca, NY (Bluegrass Lane Turf and Ornamental Farm: 42.461478, −76.462679). See Table S1 for soil analysis at the two field sites and Figure S1 for weather data.

Each site was cultivated and 84.1 kg N ha⁻¹ was applied as 19-19-19 N-P-K (Phelps Supply Inc.) during field preparation. Raised beds with drip irrigation and black plastic mulch were prepared every 1.82 m on center in the fields. Additional 19-19-19 fertilizer, equivalent to 72.9 kg N ha⁻¹, was spread under the plastic mulch. Landscape fabric (Geneva) and regular-interval mowing (Ithaca) were used to control weeds in the alleys.

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

Seedlings and rooted cuttings were transplanted into raised beds on June 5, 2019 (Geneva) and June 7, 2019 (Ithaca). Plants were spaced 1.22 m apart within rows. After transplanting, the plots were irrigated using in-bed drip irrigation as needed throughout the season. At the Geneva site, HOBOnet 10HS soil moisture sensors (Onset) were installed and used to assess when irrigation was necessary. Fertilizer (Jack’s 12-4-16 Hydro FeED RO, 11.3 kg per treatment) was included in the irrigation as needed. The Ithaca trial was watered when the soil under the plastic appeared dry. Fertilizer (Peters Excel 15-5-15 Cal-Mag, 9.2 kg per treatment) was applied twice during the growing season. To prevent plant lodging, wooden stakes and metal T-posts were added throughout the field and plants were trellised with twine using the “Florida weave” method. This helped support many of the plants until harvest, but many others lost lower branches in windstorms toward the end of the growing season.

### 2.3 Measuring height and growth rate

The heights of the middle three plants of each five-plant plot were measured weekly for the first 11 weeks post-transplant.
Growth rate was calculated using the formula in Table 3. Height and growth rate were modeled using local polynomial regression. All statistical analyses and modeling were conducted using R statistical software version 3.6.1 (R Core Team, 2019). The maximum growth date was determined based on the date that maximized the value of the growth rate model. After modeling, data points were sampled from all of the models and used to conduct a \( k \)-means clustering analysis to group similar models. The Hartigan and Wong algorithm was used to assign the clusters and the elbow method (Figure S2) to select the optimal number of clusters (Hartigan & Wong, 1979).

### TABLE 2: Descriptions and germination rates of the 30 high-cannabinoid cultivars from 12 commercial sources and the Cornell hemp breeding program included in the trial.

| Cultivar/ID | Propagation (cutting/seed) | Source | Germination |
|-------------|----------------------------|--------|-------------|
| A2R4        | Seed (dioecious)           | Winterfox Farm | 68%         |
| AC/DC       | Seed (dioecious)           | Winterfox Farm | 97%         |
| Brilliance  | Seed (feminized)           | Green Lynx Farms | 90%         |
| Cherry 5    | Seed (dioecious)           | HempLogic | 83%         |
| Cherry 307  | Seed (dioecious)           | HempLogic | 45%         |
| Cherry 308  | Seed (dioecious)           | HempLogic | 88%         |
| Deschutes   | Seed (feminized)           | Industrial Seed Innovations | 96% |
| FL 49       | Cutting                    | Sunrise Genetics | —           |
| FL 58       | Cutting                    | Sunrise Genetics | —           |
| FL 70       | Cutting                    | Sunrise Genetics | —           |
| FL 71       | Cutting                    | Sunrise Genetics | —           |
| FL 80       | Cutting                    | Sunrise Genetics | —           |
| FL 70x70    | Seed (feminized)           | Sunrise Genetics | 62%         |
| FL 71x71    | Seed (feminized)           | Sunrise Genetics | 96%         |
| GVA-H-19-1039 | Seed (dioecious)         | Cornell Hemp Program | 85% |
| GVA-H-19-1097 | Seed (dioecious)        | Cornell Hemp Program | 46% |
| KG9201      | Seed (feminized)           | Kayagene      | 95%         |
| KG9202      | Seed (feminized)           | Kayagene      | 95%         |
| Late Sue    | Cutting                    | NY Hemp Source | —           |
| NY Cherry   | Seed (dioecious)           | Genesis Hemp Alliance | 69% |
| Otto II     | Seed (dioecious)           | Winterfox Farm | 93%         |
| RN13a       | Seed (dioecious)           | Go Farm Hemp  | 73%         |
| RN16        | Seed (dioecious)           | Go Farm Hemp  | 98%         |
| RN17        | Seed (dioecious)           | Go Farm Hemp  | 97%         |
| RN19        | Seed (dioecious)           | Go Farm Hemp  | 97%         |
| Rogue       | Seed (feminized)           | Industrial Seed Innovations | 100% |
| T2          | Seed (feminized)           | Boring Hemp   | 100%        |
| Tangerine   | Cutting                    | NY Hemp Source | —           |
| TJ’s CBD    | Cutting                    | Stem Holdings Agri | —           |
| Umpqua      | Seed (feminized)           | Industrial Seed Innovations | 96% |

2.4 | Flowering surveys

All plants were rated weekly for evidence of flowering. Each plant was assessed for the presence of female flowers presenting pistils and whether the plant had initiated terminal flowering. Plants were marked as “terminally flowering” when clusters of female flowers were observed at shoot apices. Terminal flowering is distinct from sparse, solitary flowers developing in the axils of the leaves (Spitzer-Rimon et al., 2019). Any plants that produced staminate flowers were immediately removed from the field to avoid cross-pollination. Five cultivars
did not flower uniformly. For some of the analyses, the early flowering individuals were considered distinct from the later flowering individuals. Early flowering is indicated here by the addition of “(E)” to the end of a cultivar name. An unbalanced, one-way ANOVA test was used to determine whether there was a significant effect of cultivar, site, or the interaction of cultivar and site on flowering date.

2.5 | 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 infection based on a continuous scale of 0%–100% leaf area showing disease symptoms. Some early flowering cultivars had already been harvested prior to the disease rating and so were not rated. For each field site, ratings for the plants within each plot were averaged, and an unbalanced, one-way ANOVA test was used to determine whether there was a significant effect of cultivar on percent coverage in powdery mildew. The data for the Ithaca site was log (% + 1) transformed to improve the normality of the residuals. When the effect of cultivar was found to be significant, a post-hoc Tukey’s HSD analysis was used to test pairwise differences between cultivars.

2.6 | Cannabinoid time series

Shoot tips were sampled from every plot starting the week of flowering and re-sampling weekly until harvest. The sampling scheme outlined in Figure S3 was used with each of the five plant plots, although his scheme was imperfect for cultivars that flowered very early or very late in the season. Cultivars ‘KG9201’ and ‘KG9202’ accumulated so little biomass before flowering that collection of shoot tip samples prior to week 4 would have destroyed a large proportion of the plant. ‘Late Sue’ did not flower until October, which reduced the number of sampling weeks before the onset of frost conditions necessitated harvest. In accordance with current regulatory standard in New York State, the top 10 cm of the shoot tips was 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 Ninja® Pro blender (SharkNinja). Milled samples were stored at 4°C prior to high pressure liquid chromatography (HPLC) analysis (Toth et al., 2020) with the following modification: samples were injected and eluted at 1.2 ml min⁻¹ over a 10 min gradient, from 80% acetonitrile, 0.1% formic acid, to 90% acetonitrile, 0.1% formic acid, followed by a 2 min isocratic step. The following cannabinoids were quantified for each sample: tetrahydrocannabinolic acid (THCA), Δ⁹-tetrahydrocannabinol (THC), cannabidiolic acid (CBD A), 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 Δ⁸-tetrahydrocannabinol (Δ⁸-THC). Whenever possible, samples were kept below 40°C to avoid decarboxylation of acid-form cannabinoids. To control for potential variation in decarboxylation of acid-form cannabinoids, the analysis was conducted based on the total potential cannabinoid percentages. The formulas in Table 3 were used to estimate the total percentage of the neutral cannabinoid compounds after combining the acidic and neutral forms.

| Value                        | Formula                                                                 |
|------------------------------|------------------------------------------------------------------------|
| Growth rate                  | (Weekₙ₀ height – Weekₙ₋₁ height)/# days between measurements            |
| Total cannabinoid %          | CBDA % + CBD % + THCA % + Δ9-THC % + CBGA % + CBG % + CBCA % + CBC % + THCVA % + THCV % + CBDVA % + CBDV % + CBN% |
| Total CBD %                  | CBD % + (CBDA %*0.877)                                                 |
| Total THC %                  | Δ²-THC % + (THCA %*0.877)                                               |
| Total CBG %                  | CBG % + (CBGA %*0.878)                                                 |
| Total CBC %                  | CBC % + (CBCA %*0.877)                                                 |
| Total THCV %                 | THCV % + (THCVA %*0.867)                                                |
| Total CBDV %                 | CBDV % + (CBDVA %*0.867)                                                |
| % 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 % + 1.013*CBN %)                                 |

**TABLE 3** Formulas used in analysis of height, cannabinoid content, and biomass data
Total CBD, CBC, and THC accumulation was modeled 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 early flowering 'Umpqua' (E), 'Rogue' (E), and 'Deschutes' (E) were not modeled because there were too few data points ($n < 20$).

To distinguish samples with a lower CBD:CBC ratio, a $k$-means analysis was conducted using the same methods as described above for the growth curve data, yielding four clusters. The data points from the three higher CBD:CBC ratio clusters were used to model the relationship between total CBD concentration and total CBC concentration with a second-degree polynomial generalized linear model. The model was then used to construct a prediction interval ($\alpha = 0.001$) around the fitted curve with the ciTools package for R statistical software (Haman & Avery, 2019). Samples beyond the upper bound of the prediction interval were considered to have a lower than expected CBD:CBC ratio.

### 2.7 | End of season biomass

At harvest, the stems were cut at soil level and the total wet biomass of each plant in a plot was measured. The middle plant in each five-plant plot was hung and air-dried with industrial fans, then total dry biomass (whole plant) and dry floral biomass (floral tissue stripped from the plant) were measured. Cannabinoids were quantified by HPLC, as above, using a subsample of the stripped biomass. Biomass measurements were modeled using a mixed linear model with “Cultivar” as a fixed effect and the interaction between “Site” and “Block” as a random effect. Models were generated using the lmerTest package for R statistical software (Kuznetsova et al., 2017). The estimates were used in the biomass formulas in Table 3. CBN was included in the calculation of total THC in the CBD:THC ratio to account for the total enzymatic production of THC to avoid skewing the ratios of cultivars that remained longer in the field, resulting in degradation of THC to CBN. A two-way ANOVA test was used to determine whether a cultivar or site had a significant effect on biomass. In the cases where the interaction term was not significant, the interaction was dropped from the model and the $p$ values of the main effects are reported.

### 3 | RESULTS

#### 3.1 | Height and growth rate

Clustering analysis of the height and growth rate measurements separated the cultivars into five groups (Figure 1; Table 4; Figure S3). Group 1 consisted of only the day-neutral Kayagene cultivars. Group 2 was the fastest growing and had the tallest plants, with some reaching $>2$ m in height. Group 3 included the early flowering individuals of 'Umpqua,' 'Rogue,' and 'Deschutes,' which were distinct from the later flowering individuals within their respective cultivars. Group 4 was the largest, consisting 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 lower maximum growth rates that occurred slightly later than the other cultivars.

#### 3.2 | Chemotype and cannabinoid ratios

Several of the cultivars in the trial were segregating at the $B$ locus and produced the expected CBD:THC ratios (Table 5; Table S2). Chemotypes were assigned based on the trimodal distribution of CBD:THC ratios, corresponding to the first three chemotypes (Figure 2). All chemotype I and II plants consistently exceeded a threshold of 0.3% total THC after

---

**FIGURE 1** Time-series measurements of height (a and c) and growth rate (b and d) for 30 hemp cultivars. Average height and daily growth rate measured weekly for the first 11 weeks after transplant. Curves were modeled using local polynomial regression. Groups assigned in panel (c) were based on $k$-means clustering.
flowering. Chemotype III plants had CBD:THC ratios ranging from ~20:1 to ~30:1, depending on cultivar and time point. The very high CBD:THC ratios were often from samples with very high levels of total CBD (>15%). For samples <15% total CBD, the mean CBD:THC ratio was 24:1 (n = 1,158). In addition to the strong correlation between total CBD and total THC levels in chemotype III plants, there was also a strong correlation between the concentrations of total CBD and total CBC (Figure 3). Most of the samples were clustered around a 19:1 ratio of CBD:CBC; however, several had much lower CBD:CBC ratios (<14:1) and fell outside the prediction interval for the 19:1 cluster (Figure 3A). This deviation occurred more frequently in some cultivars, including 'RN19,' 'NY Cherry,' 'AC/DC,' 'RN13a,' GVA-H-19-1039, and 'Brilliance.'

### 3.3 | Cannabinoid accumulation

Given the strong effect of flowering time on cannabinoid accumulation, time-series measurements were standardized by flowering date prior to fitting the curves. After standardization, most of the cultivars were seen to follow the same pattern, with rapid cannabinoid accumulation from 0.5 to 3 weeks after terminal flowering (Figure 4). However, there were a few notable exceptions to this pattern; for example, 'A2R4' and GVA-H-19-1097 both showed much slower accumulation across the growing season. It is unclear whether 'Late Sue' and the day-neutral cultivars followed similar trends to the other cultivars as a result of the sampling scheme. Individual graphs of CBD, THC, and total cannabinoid accumulation by cultivar and chemotype can be found online at www.github.com/Willowpedia/Stack_et_al_Cannabinoid_Data.

### 3.4 | Terminal flowering

We found a significant effect of cultivar on terminal flowering date (p < 0.001, F = 889.522, df = 34) and a significant interaction between site and cultivar (p < 0.001, F = 2.526, df = 34), but no significant effect of site on flowering date (p > 0.05, F = 0.812, df = 1). For cultivars that exceeded 7% total CBD, there was a 1- to 4-week window between terminal flowering and exceeding a 0.3% total THC threshold (Figures 4 and 5). The date of maximum growth rate was a poor indicator of when cultivars would terminally flower (Figure 5).

The cultivars in the trial could be broadly separated into five groups based on flowering time, although there was significant intracultivar variation, particularly for seeded cultivars (Figures 5 and 6). The day-neutral Kayagene cultivars flowered before all the other cultivars and 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 (Figure 6). The later flowering individuals of the five aforementioned cultivars flowered approximately 1 month later in mid- to late-August. There was a ~1:1 ratio of early- to late-flowering individuals in 'Umpqua' and 'Deschutes' and a ~1:3 ratio...
for 'Rogue' (Figure 6). All of the remaining cultivars flowered in late-August or early-September, except 'Late Sue,' which did not flower until early 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; for example, the First Light™ cultivars did not produce any axillary flowers until they started terminally flowering while 'Late Sue' produced axillary flowers throughout the growing season starting in June and did not terminally flower until October.

TABLE 5  Summary of key traits by cultivar. Chemotype was assigned based on CBD:THC ratio. Flowering date was assigned when clusters of pistillate flowers were observed at shoot tips. CBD yield was calculated as the product of dry stripped biomass and total CBD % in biomass. Biomass/shoot ratio is the ratio between the average total CBD % in the biomass samples and the average total CBD % in the last set of shoot tip samples. See Table S2 for further accounting of chemotypes by cultivar.

| Cultivar/ID | Chemotype | Monoecy | Flowering | CBD yield (kg) | Biomass:Shoot Ratio |
|-------------|-----------|---------|-----------|---------------|-------------------|
| Umpqua      | All III   | None    | Very early/early | 0.133 | 0.87 |
| Rogue       | All III   | None    | Very early/early | 0.129 | 0.84 |
| Deschutes   | All III   | None    | Very early/early | 0.114 | 0.76 |
| Cherry 5    | All III   | None    | Middle    | 0.113 | 0.88 |
| Tj's CBD    | All III   | None    | Early     | 0.112 | 0.64 |
| FL 70       | All III   | None    | Middle    | 0.109 | 0.81 |
| FL 71       | All III   | None    | Middle    | 0.097 | 0.67 |
| RN16        | All III   | None    | Middle    | 0.09  | 0.63 |
| Cherry 308  | All III   | None    | Middle    | 0.084 | 0.96 |
| FL 49       | All III   | None    | Middle    | 0.081 | 1.00 |
| NY Cherry   | All III   | None    | Middle    | 0.076 | 0.68 |
| Cherry 307  | All III   | None    | Middle    | 0.076 | 0.52 |
| Late Sue    | All III   | None    | Late      | 0.074 | 1.17 |
| Tangerine   | All III   | None    | Middle    | 0.068 | 0.59 |
| Brilliance  | Many II   | Some    | Middle    | 0.067 | 0.94 |
| GVA-H-19-1039 | All III | None | Middle  | 0.062 | 0.65 |
| RN13a       | Few II    | None    | Middle    | 0.058 | 0.51 |
| T2          | All III   | None    | Middle    | 0.056 | 0.47 |
| AC/DC       | Some II   | None    | Middle    | 0.056 | 1.02 |
| FL 58       | All III   | None    | Middle    | 0.055 | 0.68 |
| RN19        | Some II   | None    | Middle    | 0.054 | 0.79 |
| FL 80       | All III   | None    | Middle    | 0.051 | 0.44 |
| GVA-H-19-1097 | All III | Some | Very early/early | 0.045 | 1.15 |
| RN17        | Some I and II | None | Early | 0.044 | 1.07 |
| Otto II     | Some II   | None    | Middle    | 0.043 | 0.66 |
| FL 71x71    | All III   | None    | Middle    | 0.037 | 0.48 |
| A2R4        | Few II    | Some    | Very early/early | 0.026 | 0.87 |
| FL 70x70    | All III   | None    | Middle    | 0.023 | 0.71 |
| KG9202      | All III   | None    | Day neutral | 0.007 | 1.05 |
| KG9201      | All III   | None    | Day neutral | 0.006 | 1.07 |

aVery early/early was July/August; Early was August; Middle was September; Late was October.

3.5  | Powdery mildew

There was a wide distribution in the severity of infection by powdery mildew by cultivar at the two sites, although there was more disease in the Geneva trial than the Ithaca trial (Figure 7). For some cultivars, disease severity by cultivar varied by site: 'NY Cherry' had no signs of powdery mildew in the Ithaca trial, but nearly 20% mean leaf area with powdery mildew in the Geneva trial. Despite the abundance of powdery mildew disease in both trials, especially in the Geneva trial, no powdery mildew was observed on any of the 'FL 58' plants.
3.6 | Biomass and yield

We observed a significant effect of cultivar and site on the amount of stripped biomass, dry biomass, wet biomass, percent dry matter, and percent floral biomass (Table 6). The stripped biomass samples averaged from 6% to >18% total cannabinoids (Table 7). Cultivar and site had a significant effect on the total cannabinoid content and on some of the individual cannabinoids. The only cannabinoid that had a significant cultivar by site interaction was THCV. The relative proportions of each cannabinoid in the stripped floral biomass were similar to those in the shoot tip samples. Notably, there was more CBN in the biomass samples that were left in the field longer and stored longer prior to cannabinoid extraction and HPLC analysis. Cultivar, but not site, had a significant effect on the CBD:THC ratio.

Estimated CBD yield per plant varied by cultivar (Table 5). Some cultivars, such as ‘Late Sue,’ had very high biomass...
that contributed to their above average yield (Table 6) while other cultivars (e.g., ‘FL 80’) had a high concentration of CBD but produced little biomass (Table 7). The ratio of shoot tip percent cannabinoids to biomass percent cannabinoids was inconsistent. Some cultivars had a ratio ~1.00, indicating a shoot tip measurement consistent with the concentration of cannabinoid in the biomass while others had a ratio as low as 0.44.

4 | DISCUSSION

The high-cannabinoid hemp industry has been scaling up rapidly in the last 5 years and lacks many of the regulatory and quality standards for cultivar descriptions and seed certification that growers are accustomed to for most other crops. While breeding of hemp grain and fiber cultivars has a long history in Europe and Asia, most of the cultivars for production of CBD have been bred in only the last decade. The recent market demand has pushed release of cultivars in a much shorter timeline than would be typical for most other crops. If breeders did not devote sufficient time to execute cycles of field evaluation, selection for uniformity, and proper removal of alleles that contribute to high THC production, the result is significant variation for key traits within cultivar populations, as we observed. The lack of uniformity includes populations containing chemotype I and II individuals that can contribute to fields that are non-compliant in regulatory testing.

4.1 | Growth rate

The observed clustering of cultivar growth rate curves suggests that there is heritable variation among high-CBD cultivars. This variation is promising for the potential development of new cultivars that are optimized for production in low-density horticultural, as well as high-density broad-acre management. Additional sources of variation that were present, but not quantified, were the ratio of lateral growth to vertical growth, internode length, branch angle, and degree of secondary branching. The genetic basis for variation in hemp plant architecture is poorly understood and likely varies with different environments and planting densities. The growth rate data presented here highlight the need for additional characterization and classification of hemp plant architecture. We observed that architecture, and notably the accumulation of biomass, is strongly affected by flowering time, and that the onset of flowering is central to maximizing yield. This factor is likely to be critical for commercial cultivation, particularly in operations that utilize mechanical harvesting and must time that with optimal maturity across an entire field.

4.2 | Flowering time

Plants use several types of receptors, including phytochromes, cryptochromes, and phototropins, to monitor their light
environment (Chaves et al., 2011; Li & Mathews, 2016; Petroutsos et al., 2016; Rensing et al., 2016). Signals from these photoperiod receptors, as well as inputs from other pathways, are integrated by a common set of evolutionarily conserved genes that determine flowering time (Khan et al., 2014; Putterill et al., 2004; Serrano-Bueno et al., 2017). In rice, another short-day flowering plant, a small number of major effect loci that modulate flowering time have been identified, and several hundred additional loci have been detected in diverse segregating populations (Hori et al., 2016; Yonemaru et al., 2010). Early flowering in rice is thought to be a result of non-functional alleles of flowering time genes (Shrestha et al., 2014; Zheng et al., 2016). The variation observed in our study, both among and within cultivars, suggests that a suite of major effect loci contribute to flowering time in hemp. The flowering time segregation ratios observed in 'Umpqua,' 'Rogue,' and 'Deschutes' suggest a single locus conferring earlier flowering in those cultivars. Over half of the plants in our trial initiated flowering in late-August, which is consistent with the previously documented 14 h critical photoperiod for *C. sativa* (Cosentino et al., 2012; Lisson et al., 2000). Our results indicate that many cultivars require further selection for uniform flowering time to improve yields and avoid non-compliant test results if early flowering plants are selected from a non-uniform field.

### 4.3 Chemotype and cannabinoid ratios

As is widely documented in the literature, the cannabinoid chemotype of a hemp plant is determined primarily by alleles at only a few loci with major effects (de Meijer et al., 2003; de Meijer & Hammond, 2005; de Meijer, Hammond, & Sutton, 2009; Mandolino et al., 2003). CBD:THC ratios of \(~21:1\) are consistent with the in vitro ratio reported by Zirpel et al. (2018) for CBDAS. The similarity between the in vitro and in planta ratios supports the hypothesis that in chemotype III plants, THCA is produced primarily through the activity of CBDAS, rather than THCAS or CBCAS (cannabichromenic acid synthase) (Toth et al., 2020). Similar to the CBD:THC ratio, the in planta CBD:CBC ratio for most samples was near the in vitro ratio of 17:1 observed by Zirpel et al. (2018). This suggests that in many chemotype III plants, the production of CBCA is primarily through side-product synthesis by CBDAS, rather than the activity of CBCAS. However, this alone does not account for the chemotype III individuals with low CBD:CBC ratios, which may carry the active *PJC* \(^{1-n}\) alleles postulated by de Meijer, Hammond, and Micheler (2009). Allelic variation in the cannabinoid synthases and genetically controlled variation in trichome morphology or chemistry that results in variation in enzymatic activity are potential sources of heritable differences in cannabinoid ratios.

Additional studies are needed to resolve changes in cannabinoid ratios over time. Yang et al. (2020) noted that the CBD:THC ratio decreased as flowers matured for photo-period sensitive cultivars and increased in the senescing flowers of day-neutral plants. This increase in day-neutral plants may be attributable to non-enzymatic conversion of \(\Delta^9\)-THC to CBN during senescence. In the two day-neutral cultivars included in this trial, which matured in the field after terminal flowering for more weeks than any other cultivar, as much as one quarter of the \(\Delta^9\)-THC appears to have been converted to CBN. As the conversion of THC to CBN is non-enzymatic, inclusion of CBN as a portion of THC in the CBD:THC ratio likely provides a better measure of the ratio resulting from the action of CBDAS. Since CBN is not considered in regulatory testing, the breakdown of \(\Delta^9\)-THC to CBN prior to regulatory sampling...
could bring plants that originally produced excessive THC into compliance.

### 4.4 Cannabinoid accumulation

Due to the side-product synthesis of THCA by CBDAS in chemotype III plants, accumulation of CBD leads to concomitant accumulation of THC exceeding most regulatory thresholds prior to the time that the plant accumulates 10% total CBD. To remain legally compliant when growing chemotype III plants, our results suggest that regulatory sampling should be completed very soon after terminal flowering for cultivars that are expected to accumulate high levels of CBD. Of the cultivars that accumulated >5% total CBD, some accumulation curves leveled-off, many continued to increase, and others decreased after peaking ~3.5 weeks after terminal flowering. It is not clear whether these trends are representative of the late-season cannabinoid accumulation in these cultivars as time-series sampling was interrupted by harvesting. Both De Backer et al. (2012) and Yang et al. (2020) observed a plateau in cannabinoid accumulation in

| Cultivar/ID | Stripped biomass (kg) | Dry biomass (kg) | Wet biomass (kg) | % Dry | % Stripped |
|-------------|-----------------------|-----------------|-----------------|-------|-----------|
| Late Sue    | 1.17                  | 2.65            | 8.04            | 33.0  | 44.2      |
| Umpqua      | 0.98                  | 1.46            | 3.01            | 48.5  | 67.4      |
| TJ’s CBD    | 0.95                  | 1.76            | 6.00            | 29.4  | 53.8      |
| Cherry 308  | 0.93                  | 2.02            | 6.89            | 29.4  | 45.9      |
| Deschutes   | 0.93                  | 1.51            | 2.95            | 51.1  | 61.7      |
| Rogue       | 0.89                  | 1.42            | 4.21            | 33.6  | 63.1      |
| NY Cherry   | 0.89                  | 1.72            | 6.28            | 27.3  | 51.9      |
| Cherry 5    | 0.89                  | 1.95            | 5.17            | 37.7  | 45.7      |
| RN16        | 0.87                  | 1.76            | 6.3             | 28.0  | 49.3      |
| Brilliance  | 0.79                  | 1.73            | 5.47            | 31.7  | 45.5      |
| 19-1039     | 0.78                  | 1.96            | 6.65            | 29.5  | 39.8      |
| RN13a       | 0.77                  | 1.71            | 5.17            | 27.3  | 44.7      |
| AC/DC       | 0.74                  | 2.00            | 7.23            | 27.7  | 37.0      |
| FL 70       | 0.73                  | 1.54            | 4.79            | 32.2  | 47.4      |
| FL 71       | 0.72                  | 1.56            | 5.55            | 28.1  | 46.5      |
| 19-1097     | 0.71                  | 1.19            | 4.38            | 27.2  | 59.9      |
| Cherry 307  | 0.71                  | 1.41            | 5.95            | 23.7  | 50.1      |
| T2          | 0.70                  | 1.26            | 4.06            | 31.1  | 55.5      |
| FL 49       | 0.70                  | 1.42            | 4.99            | 28.4  | 49.3      |
| RN17        | 0.69                  | 1.35            | 6.15            | 21.9  | 50.8      |
| Otto II     | 0.67                  | 1.84            | 5.77            | 31.9  | 36.3      |
| RN19        | 0.65                  | 1.50            | 5.69            | 26.3  | 43.4      |
| Tangerine   | 0.61                  | 1.17            | 4.17            | 28.0  | 52.5      |
| FL 80       | 0.61                  | 1.29            | 4.41            | 29.2  | 47.3      |
| FL 58       | 0.53                  | 1.04            | 4.59            | 22.7  | 50.9      |
| A2R4        | 0.49                  | 1.58            | 5.28            | 29.9  | 31.0      |
| FL 71 × 71  | 0.43                  | 0.87            | 3.61            | 24.1  | 49.7      |
| FL 70 × 70  | 0.28                  | 0.71            | 3.16            | 22.4  | 39.4      |
| KG9202      | 0.10                  | 0.12            | 0.39            | 30.4  | 87.6      |
| KG9201      | 0.10                  | 0.11            | 0.25            | 44.0  | 87.9      |
| Cultivar    | ***                   | ***             | ***             | **    | ***       |
| Site        | ***                   | ***             | ***             | *     | ***       |
| Cultivar × Site | n.s. | n.s. | *** | n.s. | n.s. |

Abbreviation: n.s., not significant.

*p < 0.05; **p < 0.01; ***p < 0.001.
the weeks following the induction of flowering; however, more studies are needed to better characterize variation in the timing and duration of the maximum cannabinoid concentration. Importantly, the maximum concentration of cannabinoids that may be achieved in the shoot tip, based on critical physiological or cellular constraints, is not known.

### TABLE 7
Cannabinoid analysis of stripped biomass samples (combined acidic and neutral forms of CBD, THC, CBC, CBG, CBDV, THCV). CBD:THC ratio included 1.013*CBN % as a portion of total THC. Percentages and ratio are estimates from mixed linear models with “Cultivar” as a fixed effect and the interaction between “Site” and “Block” as a random effect.

| Cultivar/ID   | Total cannabinoids (%) | Total CBD (%) | Total THC (%) | Total CBC (%) | Total CBG (%) | Total CBDV (%) | Total THCV (%) | Total CBN (%) | CBD:THC ratio |
|---------------|------------------------|---------------|---------------|---------------|---------------|----------------|----------------|---------------|---------------|
| FL 70         | 18.92                  | 14.93         | 0.53          | 0.66          | 0.47          | 0.08           | 0.10           | 0.01          | 27.45         |
| Rogue         | 18.24                  | 14.48         | 0.52          | 0.59          | 0.43          | 0.09           | 0.06           | 0.01          | 27.20         |
| FL 71         | 17.07                  | 13.43         | 0.48          | 0.67          | 0.40          | 0.08           | 0.07           | 0.01          | 27.18         |
| Cherry 5      | 16.92                  | 12.72         | 0.49          | 1.14          | 0.40          | 0.1            | 0.17           | 0.02          | 25.27         |
| Umpqua        | 16.36                  | 13.48         | 0.54          | 0.57          | 0.30          | 0.07           | 0.07           | 0.05          | 22.83         |
| Deschutes     | 15.39                  | 12.24         | 0.47          | 0.48          | 0.31          | 0.18           | 0.14           | 0.04          | 23.70         |
| TJ’s CBD      | 14.85                  | 11.8          | 0.44          | 0.50          | 0.22          | 0.09           | 0.05           | 0.02          | 25.43         |
| FL 49         | 14.65                  | 11.54         | 0.45          | 0.57          | 0.34          | 0.04           | 0.11           | 0.01          | 25.40         |
| RN16a         | 14.27                  | 10.32         | 1.17          | 0.79          | 0.30          | 0.04           | 0.04           | 0.01          | 22.15         |
| Tangerine     | 14.25                  | 11.18         | 0.45          | 0.59          | 0.29          | 0.04           | 0.09           | 0.03          | 23.42         |
| Brilliancea   | 14.21                  | 8.54          | 2.96          | 0.81          | 0.21          | 0.05           | 0.06           | 0.02          | 13.63         |
| Cherry 307    | 13.73                  | 10.69         | 0.41          | 0.71          | 0.28          | 0.05           | 0.04           | 0.02          | 24.87         |
| FL 58         | 13.12                  | 10.29         | 0.40          | 0.55          | 0.33          | 0.04           | 0.06           | 0.01          | 25.39         |
| FL 70 x 70    | 13.09                  | 10.19         | 0.41          | 0.57          | 0.35          | 0.05           | 0.05           | 0.01          | 24.41         |
| Cherry 308    | 11.84                  | 9.02          | 0.35          | 0.71          | 0.29          | 0.06           | 0.06           | 0.02          | 23.77         |
| NY Cherry     | 11.53                  | 8.52          | 0.31          | 1.00          | 0.28          | 0.06           | 0.04           | 0.03          | 24.33         |
| RN19          | 11.46                  | 8.33          | 0.32          | 1.11          | 0.27          | 0.07           | 0.08           | 0.02          | 24.81         |
| RN13a         | 11.42                  | 7.63          | 1.34          | 0.79          | 0.26          | 0.07           | 0.03           | 0.03          | 20.65         |
| FL 71 x 71    | 10.9                   | 8.48          | 0.34          | 0.47          | 0.30          | 0.04           | 0.05           | 0.01          | 24.23         |
| FL 80         | 10.69                  | 8.31          | 0.34          | 0.48          | 0.28          | 0.04           | 0.07           | 0.01          | 24.25         |
| 19-1039       | 10.57                  | 7.90          | 0.30          | 0.82          | 0.23          | 0.05           | 0.06           | 0.02          | 24.34         |
| AC/DC         | 10.11                  | 7.49          | 0.28          | 0.85          | 0.21          | 0.05           | 0.06           | 0.01          | 25.65         |
| T2            | 10.08                  | 7.95          | 0.33          | 0.43          | 0.20          | 0.02           | 0.03           | 0.02          | 22.74         |
| Otto IIa      | 10.03                  | 6.41          | 1.46          | 0.68          | 0.23          | 0.06           | 0.05           | 0.02          | 19.55         |
| RN17a         | 9.91                   | 6.44          | 1.38          | 0.65          | 0.22          | 0.08           | 0.04           | 0.02          | 15.38         |
| Late Sue      | 8.26                   | 6.31          | 0.25          | 0.48          | 0.23          | 0.03           | 0.02           | 0.01          | 23.95         |
| 19-1097       | 7.99                   | 6.27          | 0.26          | 0.29          | 0.14          | 0.06           | 0.04           | 0.02          | 22.09         |
| KG9202        | 7.82                   | 6.84          | 0.21          | 0.42          | 0.09          | 0.05           | 0.01           | 0.06          | 25.62         |
| KG9201        | 7.19                   | 6.38          | 0.20          | 0.30          | 0.09          | 0.04           | 0.02           | 0.05          | 24.84         |
| A2R4          | 6.85                   | 5.40          | 0.23          | 0.25          | 0.12          | 0.05           | 0.02           | 0.02          | 21.69         |

**Cultivar*** | *** | *** | *** | *** | n.s. | ** | *** | n.s. | n.s. |
| **Site**      | *** | ** | n.s. | n.s. | n.s. | ** | *** | n.s. | n.s. |
| **Cultivar x Site** | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | n.s. | n.s. |

Abbreviation: n.s., not significant.

aModels included samples of multiple chemotypes.

*p < 0.05; **p < 0.01; ***p < 0.001.

#### 4.5 Powdery mildew

Genetic resistance is the main tool used in agriculture for disease management, so the documentation of resistant genotypes is a key to improving yields through the development of disease-resistant cultivars. The variation in susceptibility
to hemp powdery mildew that we observed at the two field sites is promising for identifying loci conferring resistance and susceptibility. Based on this data, potential sources of resistance include 'FL 58,' which powdery mildew was never observed on, as well as 'RN13a,' 'Otto II,' and 'AC/DC,' which had very low levels of disease at both sites. Since disease was initiated naturally, rather than by controlled inoculation, some of the variability within and between sites is likely due to differences in abundance of pathogen inoculum and environment for initiation of disease. It is not known whether the powdery mildew at both sites resulted from infection by the same fungal genotype but, regardless, an important next step will be to test cultivars against multiple diverse isolates of the pathogen to understand the robustness of the resistance. Given that disease ratings were at a based on a single time point in the growing season, the impact of the genotype of the cultivar on incidence of powdery mildew is confounded by the differences in plant maturity. Additional studies are needed to dissect the relationship between the onset of flowering and susceptibility to powdery mildew.

4.6 Biomass yield

To optimize yield when producing biomass, growers must maximize both the amount of biomass produced and the cannabinoid concentration. The data generated in this study 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 greatest concentration of cannabinoids also produced some of the greatest yields of floral biomass. The biomass:shoot ratio has significant implications for yield as growers are often paid based on cannabinoid levels in the biomass, but are regulated based on the cannabinoid levels sampled from the shoot tips. Since THC and CBD productions are coupled, a greater biomass:shoot ratio is desirable to maximize yield and minimize the chance of exceeding the THC threshold. We note that the reported yield estimates are on a per plant basis, and not corrected to reflect optimal planting density. Day-neutral cultivars, such as 'KG9201' and 'KG9202,' as well as early maturing cultivars, such as 'Umpqua,' 'Rogue,' and 'Deschutes,' could be planted at a higher density than later flowering, day length sensitive cultivars, which would provide a significant increase in yield when considered on a per cultivated area basis. Additionally, the yield estimate does not consider the potential of double cropping with hemp or another crop if an early maturing cultivar is selected.

It is noted that this study evaluated only a small fraction of the hemp germplasm that is known to exist. Even so, we observed considerable variation in cannabinoid accumulation, flowering time, biomass production, growth rate, and disease resistance. There was significant phenotypic variation within many of the seeded and clonal cultivars, and additional variation may have been captured with larger plot size and/or additional replicates. Future studies to better characterize the diversity and genetic architecture of these complex traits will inform and accelerate breeding programs in developing elite cultivars, as well as guide the optimization of sustainable hemp cultivation.

ACKNOWLEDGEMENTS

We are grateful to the L. Smart, C. Smart, Viands, and Rose research teams for their support, especially Teagan Zingg, Savanna Shelnutt, Allison DeSario, Stephen Snyder, Julie Hansen, Lauren Carlson, Ben DeMoras, Sara Wright, Reagan Reed, Emily McFadden, Niko Kanaris, Diana Ciechorska, and Andy Park. In addition, we thank Sunrise Genetics, Go Farm Hemp, Green Lynx Farms, NY Hemp Source, Genesis Hemp Alliance, and Stem Holdings Agri for providing cultivars. This study was partially supported by New York State Department of Agriculture and Markets through grants (AC477 and AC483) from Empire State Development and by a sponsored research trial agreement with Pyxus International. George Stack was supported by a graduate fellowship from Cornell University.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jacob A. Toth https://orcid.org/0000-0002-0454-1619
Lawrence B. Smart https://orcid.org/0000-0002-7812-7736

REFERENCES

Aizpurua-Olaizola, O., Soydaner, U., Öztürk, E., Schibano, D., Simsir, Y., Navarro, P., Etxebarria, N., & Usobiaga, A. (2016). Evolution of the cannabinoid and terpene content during the growth of Cannabis sativa plants from different chemotypes. Journal of Natural Products, 79(2), 324–331. https://doi.org/10.1021/acs.jnatprod.5b00949

Bailey, L. H. (1923). Various cultigens, and transfers in nomenclature. Gentes Herb, 1, 1–125.

Braun, U. (1987). A monograph of the Erysiphales (powdery mildews). Beihete Zur Nova Hedwigia, 89.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L. O., van der Horst, G. T., Batschauer, A., & Ahmad, M. (2011). The cryptochromes: Blue light photoreceptors in plants and animals. Annual Review of Plant Biology, 62, 335–364.

Cosentino, S. L., Testa, G., Scordia, D., & Copani, V. (2012). Sowing time and prediction of flowering of different hemp (Cannabis sativa L.) genotypes in southern Europe. Industrial Crops and Products, 37(1), 20–33. https://doi.org/10.1016/j.indcrop.2011.11.017

De Backer, B., Maebe, K., Verstraete, A. G., & Charlier, C. (2012). Evolution of the content of THC and other major cannabinoids in drug-type cannabis cuttings and seedlings during growth of
plants. Journal of Forensic Sciences, 57(4), 918–922. https://doi.org/10.1111/1556-4029.2012.02068.x

de Meijer, E. P. M., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V. M. C., Ranalli, P., & Mandolino, G. (2003). The inheritance of chemical phenotype in Cannabis sativa L. Genetics, 163(1), 335–346.

de Meijer, E. P. M., & Hammond, K. M. (2005). The inheritance of chemical phenotype in Cannabis sativa L. (II): Cannabigerol predominant plants. Euphytica, 1–2(145), 189–198. https://doi.org/10.1007/s10681-005-1164-8

de Meijer, E. P. M., & Hammond, K. M. (2016). The inheritance of chemical phenotype in Cannabis sativa L. (V): Regulation of the propyl-/pentyl cannabinoid ratio, completion of a genetic model. Euphytica, 210(2), 291–307. https://doi.org/10.1007/s10681-016-1721-3

de Meijer, E. P. M., Hammond, K. M., & Micheler, M. (2009). The inheritance of chemical phenotype in Cannabis sativa L. (III): Variation in cannabichromene proportion. Euphytica, 165(2), 293–311. https://doi.org/10.1007/s10681-008-9787-1.

de Meijer, E. P. M., Hammond, K. M., & Sutton, A. (2009). The inheritance of chemical phenotype in Cannabis sativa L. (IV): Cannabinoid-free plants. Euphytica, 168(1), 95–112. https://doi.org/10.1007/s10681-009-9894-7

Grassa, C. J., Wenger, J. P., Dabney, C., & Poplawski, S. G. (2018). A complete Cannabis chromosome assembly and adaptive admixture for elevated cannabidiol (CBD) content. BioRxiv. https://doi.org/10.1101/458083

Haman, J., & Avery, M. (2019). ciTools: Confidence or prediction intervals, quantiles and probabilities for statistical models. R package version 0.5.1.

Hartigan, J. A., & Wong, M. A. (1979). Algorithm AS 136: A K-means clustering algorithm. Journal of the Royal Statistical Society. Series C Applied Statistics, 28(1), 100–108. https://doi.org/10.2307/2346830.

Hori, K., Matsubara, K., & Yano, M. (2016). Genetic control of flowering time in rice: Integration of Mendelian genetics and genomics. Theoretical and Applied Genetics., 129(12), 2241–2252.

Khan, M. R. G., Ai, X.-Y., & Zhang, J.-Z. (2014). Genetic regulation of flowering time in annual and perennial plants. Wiley Interdisciplinary Reviews, 5(3), 347–359.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. Journal of Statistical Software, 82(13), 1–26.

Lavery, K. U., Stout, J. M., Sullivan, M. J., Shah, H., Gill, N., Holbrook, L., Deikus, G., Sebra, R., Hughes, T. R., Page, J. E., & van Bakel, H. (2019). A physical and genetic map of Cannabis sativa identifies extensive rearrangements at the THC/CBD acid synthase loci. Genome Research, 29(1), 146–156. https://doi.org/10.1101/gr.242594.118

Li, F.-W., & Mathews, S. (2016). Evolutionary aspects of plant photoreceptors. Journal of Plant Research, 129(2), 115–122. https://doi.org/10.1007/s10265-016-0785-4

Lisson, S. N., Mendham, N. J., & Carberry, P. S. (2000). Development of a hemp (Cannabis sativa L.) simulation model 2. The flowering response of two hemp cultivars to photoperiod. Australian Journal of Experimental Agriculture, 40(3), 413–417. https://doi.org/10.1071/ea99059

Livingston, S. J., Quilichini, T. D., Booth, J. K., Wong, D. C. J., Rensing, K. H., Laflamme-Yonkman, J., Castellarin, S. D., Bohlmann, J., Page, J. E., & Samuels, A. L. (2020). Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. The Plant Journal, 101(1), 37–56.

Lubell, J. D., & Brand, M. H. (2018). Foliar sprays of silver thiosulfate produce male flowers on female hemp plants. Hortotechnology, 28(6), 743–747. https://doi.org/10.21277/HORTTECH04188-18

Mandolino, G., Bagatta, M., Carboni, A., Ranalli, P., & de Meijer, E. (2003). Qualitative and quantitative aspects of the inheritance of chemical phenotype in Cannabis. Journal of Industrial Hemp, 8(2), 51–72. https://doi.org/10.1300/J237v08n02_04

Mandolino, G., & Carboni, A. (2004). Potential of marker-assisted selection in hemp genetic improvement. Euphytica, 140(1), 107–120. https://doi.org/10.1007/s10681-004-4759-6

Onofri, C., de Meijer, E. P. M., & Mandolino, G. (2015). Sequence heterogeneity of cannabidiolic- and tetrahydrocannabinolic acid-synthase in Cannabis sativa L. and its relationship with chemical phenotype. Phytochemistry, 116, 57–68. https://doi.org/10.1016/j.phytoc hem.2015.03.006

Pacifico, D., Miselli, F., Carboni, A., Moschella, A., & Mandolino, G. (2008). Time course of cannabinoid accumulation and chemotype development during the growth of Cannabis sativa L. Euphytica, 160(2), 231–240.

Petrotudos, D., Tokutsu, R., Maruyama, S., Flori, S., Greiner, A., Magneschi, L., Cusant, L., Kotke, T., Mittag, M., Hegemann, P., & Minagawa, J. (2016). A blue-light photoreceptor mediates the feedback regulation of photosynthesis. Nature, 537(7621), 563–566.

Putterill, J., Laurie, R., & Macknight, R. (2004). It’s time to flower: The genetic control of flowering time. BioEssays, 26(4), 363–373.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org/

Ram, H. Y. M., & Sett, R. (1982). Induction of fertile male flowers in genetically female Cannabis sativa plants by silver nitrate and silver thiosulphate anionic complex. Theoretical and Applied Genetics, 62(4), 369–375.

Rensing, S. A., Sheerin, D. J., & Hilbrunner, A. (2016). Phytochromes: More than meets the eye. Trends in Plant Science, 21(7), 543–546.

Richins, R. D., Rodriguez-Uribe, L., Lowe, K., Ferral, R., & O’Connell, M. A. (2018). Accumulation of bioactive metabolites in cultivated medical Cannabis. PLoS One, 13(7), e0201119. https://doi.org/10.1371/journal.pone.0201119

Russo, E. B. (2007). History of cannabis and its preparations in saga, science, and sobriquet. Chemistry & Biodiversity, 4(8), 1614–1648. https://doi.org/10.1002/cbdv.200790144

Serrano-Bueno, G., Romero-Campero, F. J., Lucas-Reina, E., Romero, J. M., & Valverde, F. (2017). Evolution of photoperiod sensing in plants and algae. Current Opinion in Plant Biology, 37, 10–17.

Shrestha, R., Gómez-Arizia, J., Brambilla, V., & Fornara, F. (2014). Molecular control of seasonal flowering in rice, arabidopsis and temperate cereals. Annals of Botany, 114(7), 1445–1458. https://doi.org/10.1093/aob/mcu032

Spitzer-Rimon, B., Duchin, S., Bernstein, N., & Kamenecksky, R. (2019). Architecture and florogenesis in female cannabis sativa plants. Frontiers in Plant Science, 10, 350.

Toth, J. A., Stack, G. M., Cala, A. R., Carlson, C. H., Wilk, R. L., Crawford, J. L., Viands, D. R., Philippe, G., Smart, C. D., Rose, J. K. C., & Smart, L. B. (2020). Development and validation of genetic markers for sex and cannabinoid chemotype in Cannabis
sativa L. GCB Bioenergy, 12(3), 213–222. https://doi.org/10.1111/gcbb.12667
Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., & Gadoury, D. M. (2020). Cross infectivity of powdery mildew isolates originating from hemp (Cannabis sativa) and Japanese Hop (Humulus japonicus) in New York. Plant Health Progress, 21, 47–53. https://doi.org/10.1094/PHP-09-19-0067-RS
Welling, M. T., Liu, L., Raymond, C. A., Kretzschmar, T., Ansari, O., & King, G. J. (2019). Complex patterns of cannabinoid alkyl side-chain inheritance in Cannabis. Scientific Reports, 9(1), 11421. https://doi.org/10.1038/s41598-019-47812-2
Yang, R., Berthold, E., McCurdy, C. R., da Silva Benevenute, S., Brym, Z. T., & Freeman, J. H. (2020). Development of cannabinoids in flowers of industrial hemp (Cannabis sativa L.) – A pilot study. Journal of Agricultural and Food Chemistry, https://doi.org/10.1021/acs.jafc.0c01211
Yonemaru, J.-I., Yamamoto, T., Fukuoka, S., Uga, Y., Hori, K., & Yano, M. (2010). Q-TARO: QTL annotation rice online database. Rice, 3(2), 194–203.
Zheng, X.-M., Feng, L., Wang, J., Qiao, W., Zhang, L., Cheng, Y., & Yang, Q. (2016). Nonfunctional alleles of long-day suppressor genes independently regulate flowering time. Journal of Integrative Plant Biology, 58(6), 540–548.
Zirpel, B., Kayser, O., & Stehle, F. (2018). Elucidation of structure-function relationship of THCA and CBDA synthase from Cannabis sativa L. Journal of Biotechnology, 284, 17–26. https://doi.org/10.1016/j.jbiot ec.2018.07.031

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Stack GM, Toth JA, Carlson CH, et al. Season-long characterization of high-cannabinoid hemp (Cannabis sativa L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. GCB Bioenergy. 2021;13:546–561. https://doi.org/10.1111/gcbb.12793