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ORIGINAL RESEARCH

Limited effect of environmental stress on cannabinoid profiles in high-cannabidiol hemp (*Cannabis sativa* L.)

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Abstract

Hemp (*Cannabis sativa*) is a burgeoning crop, but research-based information about genetic and environmental effects of cannabinoid production is limited and will be essential for expanded cultivation. There are limited data available about the effect of environmental stressors on cannabinoid content, particularly for tetrahydrocannabinol (THC) in high-cannabidiol (CBD) hemp. To address this, five stress treatments were applied in a replicated field trial with three high-CBD hemp cultivars and cannabinoid content was assayed over a 3-week time-course spanning floral maturation. Cannabinoid production in terminal inflorescence shoot tip samples of three cultivars was measured under stress imposed by flooding, ethephon, powdery mildew, herbicide, and physical wounding in a split plot design. The treatments had limited effects on cannabinoid levels, with the exception of herbicide treatment which resulted in decreased cannabinoid content. Notably, there was no evidence that any of these stresses caused THC concentration or the ratio of THC to CBD to increase at harvest.

KEYWORDS

cannabidiol, *Cannabis sativa*, flooding, hemp, herbicide, powdery mildew, stress, tetrahydrocannabinol, wounding

1 | INTRODUCTION

Hemp (*Cannabis sativa* L.) has many potential uses, including grain, fiber, and cannabinoid production. Fundamental knowledge of the genetic and environmental influences on important traits is critical for the breeding of improved, stable, and uniform cultivars that are compliant with regulations of tetrahydrocannabinol (THC) concentration. In many countries, there is a regulatory threshold of THC concentration in dry floral tissue that defines *C. sativa* as hemp. This threshold varies between countries, with a value of 0.2% in most of Europe (Salentijn et al., 2015), 0.3% in the United States (Adesso et al., 2019), and 1% in Australia (Davidson et al., 2018). It has been suggested that various environmental stresses increase the abundance of cannabinoids in hemp, especially THC (Nir, 2019); however, there are limited published data to address this idea.

Previous work has determined that the suite of major cannabinoids produced (THC, CBD, and cannabigerol, CBG), also referred to as the cannabinoid chemotype, is a simple genetic trait, but that variation in cannabinoid content is genetically complex and potentially affected by environment (Campbell et al., 2019; Mandolino et al., 2003; de Meijer et al., 2003). Cannabinoid chemotype can be predicted by the allelic state of the *B* locus, with production of mostly THC (chemotype I) characteristic of homozygous B_T individuals,

production of about equal THC and CBD (chemotype II) typical of heterozygous B_T/B_D individuals, and production of mostly CBD (chemotype III) typifying homozygous B_D individuals (de Meijer et al., 2003). Many commercially available cultivar populations, including high-cannabinoid hemp as well as grain and fiber types, are segregating at the *B* locus (Toth et al., 2020). Breeding for homozygous B_D individuals will be essential to stabilize hemp cultivars for THC compliance, but the degree to which other factors, such as environmental stressors, affect cannabinoid production is not well established.

The major acidic cannabinoids, cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), and cannabichromenic acid (CBCA), are synthesized from CBGA by CBDA, THCA, or CBCA synthases. These acidic cannabinoids decarboxylate under certain conditions (Perrotin-Brunel et al., 2011), forming CBG, CBD, THC, and cannabichromene (CBC). It is conceivable that stress alters conditions relevant to decarboxylation, such as production of antioxidants (Singh et al., 2020). CBDA and THCA synthases exhibit product promiscuity when heterologously expressed in yeast, meaning they make small amounts of the other cannabinoids in addition to their major products when incubated with CBGA precursor (Zirpel et al., 2018). Notably, CBDA synthase has been reported to synthesize approximately 5% THCA and 5% CBCA normalized to 100% CBDA (Zirpel et al., 2018). This is likely to be the primary source of the THCA and CBCA that have been detected in chemotype III hemp plants that do not express active THCA synthase (Stack et al., 2021; Toth et al., 2020). It is conceivable that allelic variation among CBDA synthases or expression of other cannabinoid synthases could lead to altered CBD:THC ratios. While there was good agreement between *in planta* data and *in vitro* data for this ratio in a previous report (Toth et al., 2020), further testing is required to determine if there is variation of this ratio within chemotype III plants and to determine the environmental effect, if any.

Most studies to date on the effect of stresses on cannabinoid production have focused on cannabinoid chemotype I and II plants grown under controlled environment conditions. For example, in a small study of greenhouse-grown chemotype II plants, drought stress was associated with increased levels of cannabinoids (THC and CBD) (Caplan et al., 2019). Another study found an increase in THC upon UVB exposure (Lydon et al., 1987) in drug-type (chemotype I) plants, but no increase in any cannabinoids in fiber (chemotype III) plants. Other work linked abscisic acid (ABA) with changes in THC concentration (Mansouri & Asrar, 2012; Mansouri et al., 2009), although the direction of this effect was not consistent.

The effect of stress on field-grown high-cannabinoid chemotype III hemp plants is not well understood and of great potential importance for production systems. If stresses resulted in increased cannabinoid content or variation in CBD:THC ratio, management of stress (induction or avoidance) would play a critical role in production systems. This current study examines the effect of stresses on the accumulation of cannabinoids in three high-cannabinoid CBD cultivars using exclusively female chemotype III plants in a split-plot design in a single outdoor location. Here we examined five stresses, as well as an unstressed control. The five stresses were as follows:

1. Ethephon: Ethephon (2-chloroethyl phosphoric acid) is a plant growth regulator that is converted *in planta* to ethylene, a plant hormone involved in aspects of plant development. Previous work has found an effect of ethephon on cannabinoids (Mansouri et al., 2013, 2016), but its effect on field-grown high-CBD plants has yet to be investigated. Ethephon has also been used to induce genetically male plants to produce female flowers (Ram & Jaiswal, 1970). It is possible that ethephon treatment, by inducing female-associated gene expression, could lead to increased trichome numbers on female inflorescences and accordingly increased cannabinoid concentration.
2. Flooding: Flooding is an abiotic stress that can occur following high rainfall, especially in poorly drained soils. Flooding can lead to hypoxia in the roots, leading to reduced nutrient uptake and the production of stress hormones (Colmer & Voesenek, 2009). However, previous work found limited difference in cannabinoid content between a naturally flooded field and one without this stress (Toth et al., 2020), or between an irrigated and non-irrigated field (Campbell et al., 2019).
3. Herbicide: As hemp acreage grows, it will be important to consider how hemp responds to commonly applied chemicals such as herbicides. While the general effect of herbicides on hemp has been not been rigorously studied, herbicide drift is a relatively common phenomenon that has been found to injure susceptible plants and interfere with secondary metabolism (Ding et al., 2011). For example, the herbicide glyphosate interferes with the shikimate pathway in plants (Duke & Powles, 2008). While the shikimate pathway is not directly involved in cannabinoid biosynthesis, glyphosate-induced stress might alter cannabinoid levels through general stress responses or result in reduced vigor.
4. Powdery Mildew: Powdery mildew, caused by the fungal pathogen *Golovinomyces spadiceus*, is a biotic stress which is common in greenhouses and fields with favorable environmental conditions (Szarka et al., 2019; Weldon et al., 2020). Powdery mildew has the potential to reduce yield, especially in greenhouse conditions (Lyu et al., 2019), but can also be severe in outdoor field settings. The effect of powdery mildew on cannabinoid production is largely unknown, but cannabinoids may have evolved to

deter pests and pathogens (Gorelick & Bernstein, 2017) and so such a relationship would not be surprising.

5. Wounding: Mechanical damage can be caused by natural sources, such as hail or herbivory, or result from cultivation and mechanical weed removal. It has been suggested that wounding that mimics insect damage might increase cannabinoid levels, and that the resistance of *Cannabis* to insects might be substantially affected by cannabinoids (Gorelick & Bernstein, 2017). In general, wounding has the potential to cause a systemic response, inducing the systemic production of hormones such as jasmonic acid and abscisic acid (Savatin et al., 2014), which have been linked to changes in cannabinoid abundance (Mansouri et al., 2009; Salari & Mansori, 2013).

2 | MATERIALS AND METHODS

Three cultivars of high-cannabinoid hemp were used for this study: 'TJ's CBD' (Stem Holdings Agri, Eugene, OR; clonal), 'T2' (Boring Hemp, Boring, OR; feminized seeds), and Cornell breeding line GVA-H-19–1039 (dioecious seeds). All cultivars were started at a similar time from either cuttings or seeds, and GVA-H-19–1039 was screened using the molecular marker CSP-1 (Toth et al., 2020) to remove male plants. All selected plants were entirely phenotypically female and no pollination in the field was noted. A split-plot design was used, with the three cultivars randomized within treatment plots, which were randomized in a complete block design with four replicate blocks (Figure 1). Each treatment plot contained three plants of each cultivar. Seedlings and cuttings were established in a greenhouse in potting mix (Lambert's LM111) in 50-cell deep trays. These were transplanted into raised beds with black plastic mulch and drip irrigation on July 28, 2019 at the Cornell AgriTech McCarthy Farm (42.896300, −77.008062) in a field with well-drained Ontario loam soil with more than 2 m depth to a restrictive feature. Conventional fertilizer (19-19-19 N-P-K, Phelps Supply Inc.,) was applied at a rate of 157 kg N ha^{-1} during bed formation. No additional fertilizer was added after transplanting. Soil moisture was monitored in the control and flooded plots using an Onset HOBO RXW-SMD-10HS sensor installed to a depth of 10 cm in the middle of each plot and wirelessly linked to a HOBO RX3000 remote monitoring station. Adequate soil moisture was applied through trickle irrigation during periods with insufficient rainfall to maintain soil volumetric water content >0.27 m^3 m^{-3}. Temperature and rainfall data for this site are reported in Stack et al., (2021).

Stress treatments were initially applied on September 14 and 15, 2019 when the plants had initiated terminal flowering. For the flooding stress, irrigation was applied through trickle irrigation only on flood treatment plots sufficient to raise soil volumetric water content to field capacity (0.35–0.4 m^3 m^{-3}) and was repeated throughout the sampling period to maintain soil volumetric water content >0.32 m^3 m^{-3}, typically two or three times per week. Ethephon (0.5% Ethephon 2, Nufarm, Alsip, IL, 1% active ingredient, 75 mM) was applied as a spray to the entire plant until leaves were fully wet. Ethephon was applied twice, once on September 14 and again on September 22, 2019. Powdery mildew inoculation was accomplished by transferring dry conidia from diseased leaves to shoot tips of treatment plants using a paint brush. Leaves infected with *G. spadiceus* were taken from naturally infected plants cultivar 'TJ's CBD' growing in a variety trial in Geneva, NY. Four shoots of each plant in the powdery mildew treatment plots were marked with flagging tape for subsequent shoot tip sampling, and the terminal five leaves of each shoot were painted with dry conidia. Glyphosate (0.5% Roundup Pro, Monsanto) was applied one time as a spray to the entire plant until leaves were wet. The wounding treatment was accomplished by partially damaging the lower and middle foliage with a grass and weed trimmer (Model FS70R, Stihl Inc) in such a way as to remove or wound a

Rep1		Rep1		Rep2		Rep2		Rep3		Rep3		Rep4		Rep4	
Powdery mildew	TJ's CBD	Control	T2	Herbicide	T2	Flooding	T2	Powdery mildew	GVA-H-19-1039	Flooding	T2	Flooding	GVA-H-19-1039	Ethephon	GVA-H-19-1039
	GVA-H-19-1039		TJ's CBD		TJ's CBD		GVA-H-19-1039		T2		GVA-H-19-1039		TJ's CBD		TJ's CBD
	T2		GVA-H-19-1039		GVA-H-19-1039		TJ's CBD		TJ's CBD		TJ's CBD		T2		T2
Ethephon	T2	Herbicide	GVA-H-19-1039	Wounding	T2	Ethephon	T2	Wounding	TJ's CBD	Herbicide	TJ's CBD	Herbicide	T2	Control	GVA-H-19-1039
	GVA-H-19-1039		T2		GVA-H-19-1039		GVA-H-19-1039		T2		GVA-H-19-1039		TJ's CBD		T2
	TJ's CBD		TJ's CBD		TJ's CBD		TJ's CBD		GVA-H-19-1039		T2		GVA-H-19-1039		TJ's CBD
Wounding	GVA-H-19-1039	Flooding	T2	Control	TJ's CBD	Powdery mildew	GVA-H-19-1039	Ethephon	TJ's CBD	Control	GVA-H-19-1039	Wounding	TJ's CBD	Powdery mildew	T2
	TJ's CBD		GVA-H-19-1039		GVA-H-19-1039		T2		T2		TJ's CBD		T2		GVA-H-19-1039
	T2		TJ's CBD		T2		TJ's CBD		GVA-H-19-1039		T2		GVA-H-19-1039		TJ's CBD

FIGURE 1 Experimental plot layout. Three plants per cultivar were planted in each subplot

majority of the foliage on the outer portion of the plant below the inflorescence. The percentage of damage was not precisely quantified, but since the inner portions of each stem were not affected, the damage was approximately 40%–50% of foliage wounded below the inflorescence. The damage was implemented to remove and damage the leaves, but not to break or prune stems. The wounding treatment was applied on September 14 and repeated immediately after the week two sampling on September 29, 2019.

Shoot tips were sampled for cannabinoid extraction and analysis immediately prior to application of the stress treatments and again in 1-week intervals for 3 weeks (September 14, 22, 29, October 6, 2019) for a total of four sampling times. The third week after initial stress application was designated as the presumptive harvest date. The plants that received the herbicide treatment began to exhibit necrosis and browning by the week two sampling period, so sampling targeted the healthiest looking shoot tips remaining by week 3. One shoot tip sample was collected by harvesting the top 10 cm of an upper canopy shoot from each of the three plants in a plot, and those three shoot tips were combined in a paper bag, air-dried at room temperature, and milled in a Nutri Ninja Pro food blender (SharkNinja Operating LLC). Cannabinoids were extracted and quantified by high-pressure liquid chromatography (HPLC) using a previously established method (Stack et al., 2021). Total cannabinoids were calculated by summing the neutral form with the acidic form multiplied by a factor (0.877 for THCA, CBDA, and CBCA; 0.878 for CBGA) to account for decarboxylation.

Statistical analysis was done in R version 3.5.1 (R Core Team, 2018). The library agricolae version 1.4.0 (de Mendiburu & Yaseen, 2020) was used for Tukey mean separation and significance tests. Split-plot ANOVA was modeled with "Treatment" as the main-plot factor and "Cultivar" as the sub-plot factor using the Satterthwaite approximation for calculating degrees of freedom with the following equation:

$$\text{Trait}_{ijk} = \mu + \text{Rep}_i + \text{Treatment}_k + \eta_{ik} + \text{Cultivar}_j + (\text{Cultivar} \times \text{Treatment})_{ij} + \varepsilon_{ijk}.$$

Neutral:total cannabinoid ratio was calculated as the average of the concentration of neutral forms (CBD, THC, CBC, and CBG) divided by the corresponding total as calculated above.

3 | RESULTS

3.1 | Cannabinoid accumulation over time in three *C. sativa* cultivars grown in unstressed conditions

Total potential CBD, THC, CBC, and CBG increased over time in the unstressed control plots, achieving maximum concentrations of 7.5%–12% total CBD by week 3 (Figure 2). GVA-H-19-1039 had greater total CBC concentration at week 3 than 'T2' (Figure 2c, Tukey $\alpha = 0.05$). Total CBG levels followed a pattern of accumulation from the other cannabinoids, reaching a maximum in week 2 for GVA-H-19–1039 and 'TJ's CBD' and then declining slightly, while in 'T2' total CBG concentration continued to increase through week 3 (Figure 2d).

3.2 | Cannabinoid ratios over time in three *C. sativa* cultivars grown in unstressed conditions

The ratio of total potential CBD to THC is of great importance to hemp growers as high CBD and low THC is desired to maintain regulatory compliance and maximize yield. The range of mean values of the CBD:THC ratio by cultivar in the unstressed control treatment was 23.3–28.2 (Figure 3a). There was a significant effect of cultivar (percent variation explained, PVE = 14%, $p < 0.01$) and week (PVE = 34%, $p < 0.01$) on CBD:THC ratio, but no interaction effect ($p = 0.19$) (Table 1).

The ratios of CBD to CBC were significantly different between cultivars, reflecting differences in total CBC abundance, and had a range of 10.2–28.2 (Figure 2c, Figure 3b, ANOVA $\alpha = 0.05$). GVA-H-19–1039 had a substantially lower CBD:CBC ratio at harvest, averaging 10.2 vs 22.4 for 'T2' and 23.5 for 'TJ's CBD.' The CBD to CBG ratios across all weeks were significantly different by cultivar (PVE = 36%, $p < 0.01$, Figure 3c), and the range of mean CBD to CBG ratios was 15.6–45.0. The ratio of neutral:total cannabinoids decreased substantially after week 0, and the range of mean ratios across all time points was 12%–52% (Figure 3d).

3.3 | Genotype-by-environment interaction of cultivar and stress treatment

Using a split-plot mixed linear model in ANOVA, there was no significant ($\alpha = 0.01$) cultivar × treatment effect at any sampling points for total CBD, total THC, total CBC, total CBG; cannabinoid ratios of CBD:THC, CBD:CBC, CBD:CBG; and neutral:total cannabinoid ratio. Due to the lack of cultivar-specific response, cultivars were combined to examine stress effects.

3.4 | Cannabinoid accumulation in response to stress treatments

When considering all data across all weeks by stress treatment, herbicide application was the only treatment that led

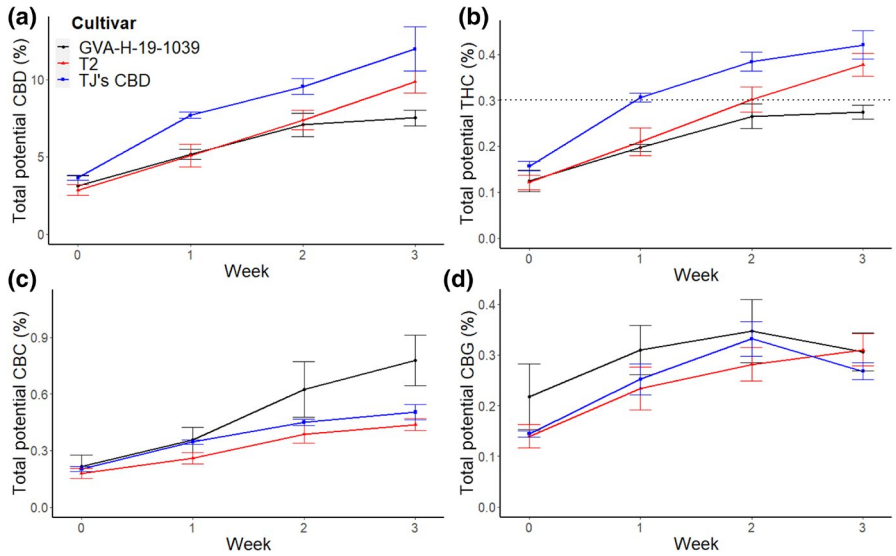

FIGURE 2 Cannabinoid accumulation by cultivar over 3 weeks in the unstressed control treatment. Week 0 refers to samples harvested immediately prior to stress application in the other treatment blocks. (a) Total potential CBD (%), (b) total potential THC (%), dotted line is 0.3%, (c) total potential CBC (%), and (d) total potential CBG (%). Error bars are standard error (n = 4)

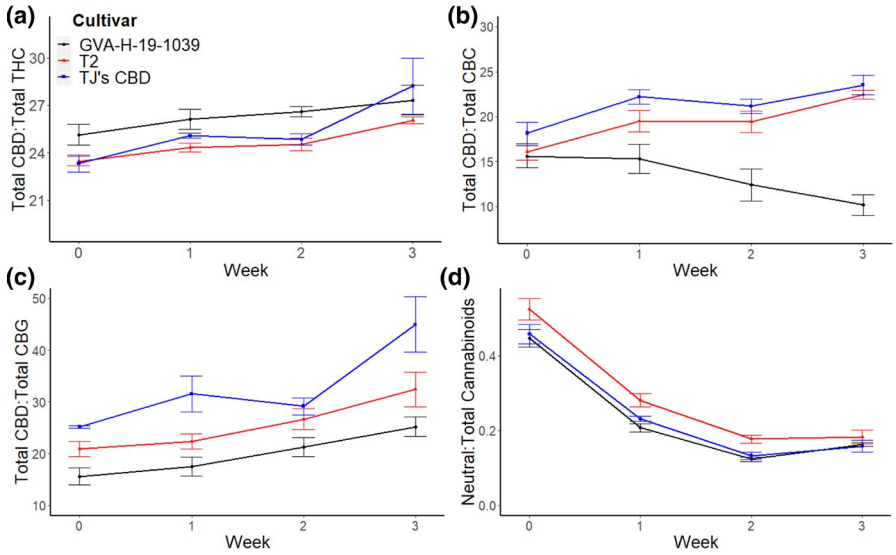

FIGURE 3 Cannabinoid ratios over 3 weeks with respect to cultivar in the unstressed control treatment. Week 0 refers to samples harvested immediately prior to stress application in the other treatment blocks. (a) Total CBD:total THC, (b) total CBD:total CBC, (c) total CBD:total CBG, and (d) neutral:total cannabinoids. Error bars are standard error (n = 4)

TABLE 1 ANOVA of CBD:THC ratio in the unstressed control treatment

Source of variation	Degrees of freedom	Sum of squares	PVE (%)	F value	Pr(>F)
Cultivar	2	23	14	6.1	<0.001
Week	1	58	35	30	<0.001
Cultivar: Week	2	6.9	3.9	1.7	0.19

to a statistically significant reduction in total potential CBD (Figure 4a). Similar reductions in the herbicide-treated group were seen for THC ($p < 0.01$), and CBG ($p < 0.01$) compared to the unstressed control, but no significant reduction was found for CBC. The concentration of cannabinoids in each week was similar for each treatment with the exception of herbicide treatment, which was consistently lower (Figure 4). Mean cannabinoid concentrations for plants in the wounding treatment were often greater than other treatment blocks but this effect was not statistically significant at any time point ($p > 0.05$), except against herbicide-treated blocks.

3.5 | The effects of stress treatments on cannabinoid ratios

The range of mean CBD:THC ratios in stress-treated plants over the course of the trial was similar to the range of mean ratios in the cultivars grown in the control treatment: 24.0–28.2 (Figure 5a). There was an unexplained treatment block effect on CBD:THC ratio before treatment was applied (week 0, ANOVA $p < 0.01$), largely due to a high CBD:THC ratio in the group that was intended to be flooded compared to the other treatment blocks (Tukey $\alpha = 0.05$). There was

also a treatment block effect on CBD:CBC ratio at week 0 and harvest only (Figure 5b, Table 2). There was no effect of treatment on CBD:CBG ratio at any time point except week 1, where herbicide treatment was significantly higher than wounding treatment (Tukey $\alpha = 0.05$), although neither was significantly different than the control treatment. The neutral:total cannabinoid ratio was unaffected by the stress treatments (Figure 4d, $p > 0.05$) at any time point.

3.6 | Cannabinoid profiles at harvest

At the prospective harvest date, the only significant difference in any comparisons of THC levels were significantly lower levels in the herbicide-treated plants (Figure 6a, Table 2). At this time point, there was no significant difference in CBD:THC ratios between plants exposed to any stress treatment (Figure 6b, Table 2). Stress treatment at harvest affected other measured cannabinoids (due to lowered production in the herbicide treated plants) but not ratios of CBD:CBG or neutral:total cannabinoids (Figure 4, Figure 5, Table 2).

4 | DISCUSSION

4.1 | Cannabinoid accumulation and ratios

CBD, THC, and CBC accumulated during maturation of the inflorescence over the course the trial, as expected (Stack et al., 2021). Most plots sampled at week 3 (except herbicide-treated plots) had a total THC concentration >0.3%. Considering all data in this study, there is a strong linear relationship between total CBD and total THC (Pearson's $r = 0.98$). Given this linear relationship, samples with >8% total CBD would be expected to have >0.3% total THC. This level is slightly greater than the 6% CBD critical value

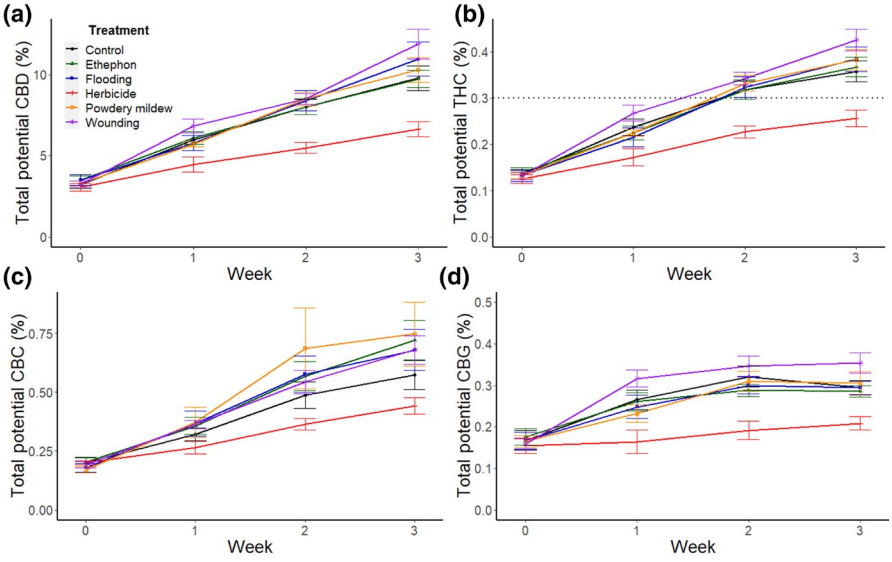

FIGURE 4 Cannabinoid accumulation in response to stress treatments over 3 weeks. Week 0 refers to samples harvested immediately prior to initial stress application. (a) Total potential CBD (%), (b) total potential THC (%), dotted line is 0.3%, (c) total potential CBC (%), and (d) total potential CBG (%). Means represent all cultivars combined. Error bars are standard error (n = 12)

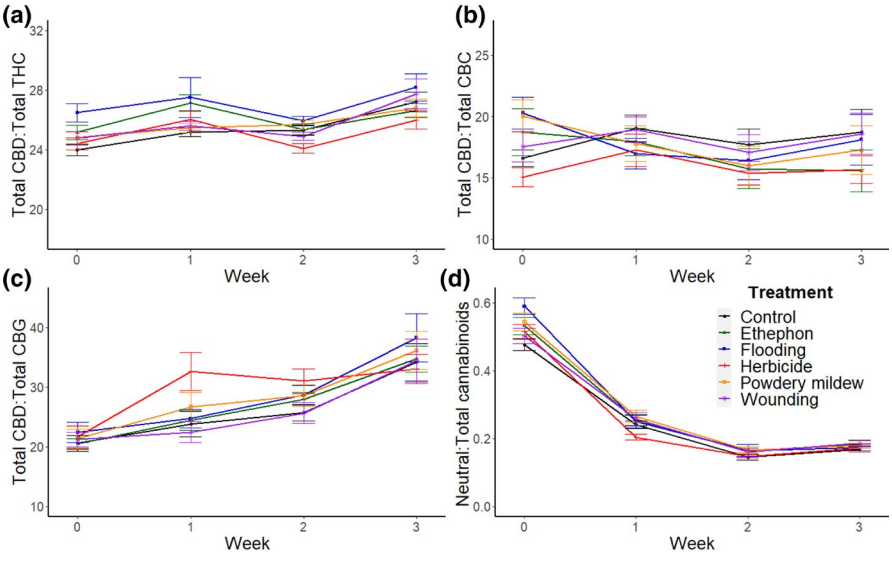

FIGURE 5 Cannabinoid ratios in response to stress treatment over 3 weeks. (a) Total CBD:total THC, (b) total CBD:total CBC, (c) total CBD:total CBG, and (d) neutral:total cannabinoids. Means represent all cultivars combined. Error bars are standard error (n = 12)

	Treatment	Cultivar	Cultivar × Treatment	Rep
Total potential CBD (%)	<0.001	0.0041	0.45	0.57
Total potential THC (%)	<0.001	<0.001	0.12	0.45
Total potential CBC (%)	0.025	<0.001	0.078	0.68
Total potential CBG (%)	<0.001	<0.001	0.088	0.41
Total CBD:Total THC	0.44	0.29	0.74	0.94
Total CBD:Total CBC	0.023	<0.001	0.15	0.48
Total CBD:Total CBG	0.72	<0.001	0.94	0.43
Neutral:Total cannabinoids	0.58	<0.001	0.029	0.22

TABLE 2 P-values from split-plot ANOVA results of traits at harvest

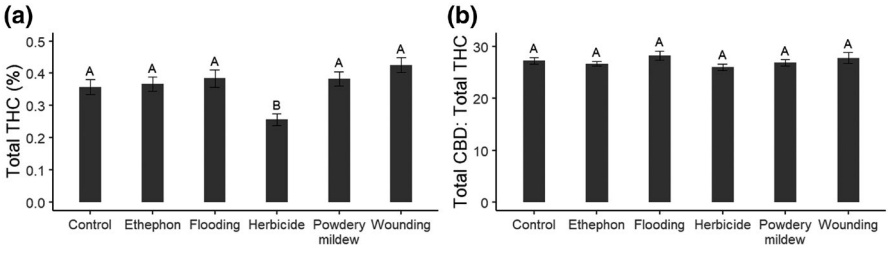

FIGURE 6 Bar chart of key measures at harvest. (a) Total potential THC % and (b) total potential CBD:THC ratio. Letters are Tukey HSD post-hoc levels ($p < 0.05$)

reported in our previous study (Toth et al., 2020), and may be due to differences in CBD:THC ratio in the cultivars tested or improvements in sample handling that resulted in reduced cannabinoid degradation. The critical value found here is in close agreement with Stack et al., (2021), which involved multiple cultivars in two different locations.

There was no effect of stress treatment on the total CBD to total THC ratio at harvest, supporting the hypothesis that this ratio is genetic and not strongly influenced by environmental stress. The variation in CBC in 'TJ's CBD' and 'T2' was also largely explained as a function of CBD, at a rate of about 19:1 CBD:CBC (Pearson's r = 0.94). This corroborates data from heterologous expression of CBD synthase in yeast, where CBC was produced at a rate of about 5% of CBD (Zirpel et al., 2018). However, at harvest, GVA-H-19-1039 had significantly greater total CBC than would be expected from this mechanism considering the CBD concentration. It is possible that GVA-H-19-1039 expresses an additional cannabinoid synthase enzyme, as other hemp plants have been noted to express additional cannabinoid synthase enzymes including a dedicated CBCA synthase (Kojoma et al., 2006; Laverty et al., 2019; Weiblen et al., 2015). It is unclear whether this high CBC phenotype is the same "prolonged juvenile chemotype" leading to high CBC noted by de Meijer et al., (2009). In contrast to the high proportion of CBC observed at the beginning of flowering by de Meijer et al., (2009), GVA-H-19-1039 had a much lower proportion of CBC at the equivalent early time point. Furthermore, de Meijer et al., (2009) reported a decrease in the proportion of CBC over time, whereas the proportion of CBC in GVA-H-19–1039 increased in successive weeks in this study. The minor effect of stress treatment noted in Table 2 on total potential CBC and CBD:CBC ratio may be due to altered regulation of CBDAS and CBCAS.

While Yang et al., (2020) found that the CBD:THC ratio decreased throughout floral development with autoflowering cultivars experiencing a secondary increase, our data suggest a stable or slight increase in total CBD:total THC ratio over the course of floral development. This discrepancy may have been due to differences in cultivar or testing, or yet unidentified environmental effects. Results from other field trials suggest there is a stable CBD:THC ratio throughout the life of the plant (De Backer et al., 2012; Pacifico et al., 2008; Stack et al., 2021).

4.2 | Decarboxylation

There are limited data on cannabinoid decarboxylation (neutral:total cannabinoids) *in planta* (Toth et al., 2020; Yang et al., 2020). Decarboxylation is largely thought to be non-enzymatic, and suggested to be promoted by age, heat, light, and small molecule catalysts such as formic acid and methanol, but repressed by antioxidants (Perrotin-Brunel et al., 2011; Singh et al., 2020). The broad trend of high percentage decarboxylation (mostly neutral forms) early in flowering followed by a rapid drop is consistent with a previous study (Yang et al., 2020). The high initial decarboxylation percentage in young flowers may be a result of different chemical environments promoting decarboxylation in young inflorescence tissue.

4.3 | Further studies

There were several limitations to this study. First, only regulatory-type shoot tip cannabinoid testing was undertaken, and it is possible that these stresses affected total yield, but not shoot tip cannabinoid concentration. Second,

three cultivars were chosen including seeded and clonal cultivars, but there is certainly a wide range of hemp genetic diversity that has yet to be studied. Third, the stress treatments examined here were chosen to be representative of growing conditions in a wet northeast US climate but did not include stresses that are typical of other growing areas, such as drought, extreme heat, or high salinity. The stresses were also only applied at a single intensity, which may have been insufficient to elicit a response. Lastly, these data also only reflect a single site in a single year, and it is possible that year-to-year variation or differences in site could lead to different results. Nevertheless, the evidence provided here supports the conclusion that THC accumulation is proportional to that of CBD and is not strongly affected by environmental stress.

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DATA AVAILABILITY STATEMENT

Data will be made available upon request to the corresponding author.

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