FLORAL HEMP RESPONSES TO NITROGEN FERTILIZATION IN THE HIGH DESERT

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ABSTRACT

The performance of floral hemp under N fertilization is influenced by environmental conditions, management, and cultivar selection. Greenhouse and field studies evaluated the effect of different levels of supplemental N fertilization on hemp cultivars (Berry Blossom, Red Bordeaux, and Tahoe Cinco) in Northern Nevada. Nitrogen increased plant height, canopy cover, stem diameter, and shoot biomass, but other physiological parameters were dependent on the cultivar. We evaluated the use of a SPAD meter for ease of determining leaf N deficiency, and correlations with leaf chlorophyll content showed that the SPAD meter was a reliable tool in two of the three cultivars but not in Tahoe Cinco. Nitrogen treatment increased overall CBD yield, which was driven by increases in inflorescence biomass. Our studies suggest that hemp may have a positive response to soil N management, but adjustments based on genotype by environment interaction should be aimed at maximizing cannabinoid yield either by increasing biomass and/or CBD concentrations.

INTRODUCTION

Cannabis sativa L. cultivation rapidly increased in the US after federal and state regulations considered hemp (with a tetrahydrocannabinol or THC concentration <0.3%) an agricultural commodity. Although hemp has multiple uses, the three most promising from a market perspective are fiber, oilseed, and pharmaceuticals (Cherney and Small 2016). Different cultivars are selected based on the target use (i.e., cultivars for CBD production differed from fiber cultivars). For fiber hemp, the stem and fiber quality of the cultivars is emphasized for their use in the fabric industry, building materials, and automotive purposes, and research on fiber hemp in Nevada is being conducted as well (e.g., Solomon et al., 2022). CBD production has generated much interest from very diverse stakeholders. Cannabinoids such as CBD are becoming popular for relieving pain, and are potentially useful for epilepsy, schizophrenia, and Alzheimer's (Laverty et al. 2019). For ranchers and farmers, hemp can become an alternative crop to help them increase the profitability of their operations; yet, as a non-insured crop, producers are at higher economic risk without clear management strategies.

Scarce information exists on hemp production guidelines, and before 2015, agronomic research on industrial hemp in the United States was lacking (Williams and Mundell 2018). This information gap can lead to the adoption of inefficient production methods, which could result in environmental impacts from increased erosion to non-point source pollution, affecting crop profitability in the long term. Recommendations on nutrient management for hemp are broad, scarce, and sometimes extrapolated from other growing regions or other cannabis production systems (e.g., Backer et al. 2019; Bernstein et al. 2019); recommendations are between 50 and 240 lb of N per acre (Papastylianou et al., 2018; Williams and Mundell 2018). Studies have reported environment-by-cultivar interactions, and differences in N requirements depending on the purpose of hemp production (Wylie et al., 2020; Aubin et al., 2016; Struik et al., 2000).

Our main research objective was to evaluate the responses of floral hemp cultivars to nitrogen fertilization in the field. We consider that increasing understanding of agronomic

practices is pivotal to moving forward the hemp industry in regions with a challenging climate and environmental conditions (e.g., soils and short growing season) such as the Great Basin.

MATERIALS AND METHODS

Two separate studies on floral-hemp cultivar responses to N fertilization were conducted at the Valley Road Agricultural Experiment Station, University of Nevada, Reno. The greenhouse study included two cultivars Berry Blossom and Red Bordeaux, and the field study included those two cultivars and Tahoe Cinco. All cultivars were obtained from Plant Fuel Genetics, LLC. Seedling production was similar for both studies. Seeds were started in a greenhouse in seeding trays with a 3:2 ratio of potting soil (Miracle Gro Potting Mix, OH, USA) and medium, fine-grain sand (Quickrete, GA, USA). Two weeks after planting, seedlings were fertilized twice weekly with 0.28 ounces of 12-4-8 fertilizer per gallon of water (Miracle-Gro, OH, USA) until transplanted.

Greenhouse study: Seedlings were transplanted into three-gallon pots filled with the same 3:2 ratio of potting soil and sand (see above). After plant establishment (5 days), plants received, three times a week, two N treatments through watering: low N (42 ppm; N-) and high N (182 ppm; N+). The two N treatments consisted of modified Hoagland solutions with all other macro and micronutrients kept at the concentration of the full-strength Hoagland solution. At 38 days after transplanting (DAT), half of the plants in each N treatment were switched to the other N treatment to understand plant responses to changes in N availability. A total of nine to 12 plants were in each treatment group (N+, N+ to N-, N-, and N- to N+; 43 plants in the trials). Evaluations on canopy cover, biomass, and SPAD readings were conducted (see below) until the termination of the experiment at 15 weeks from the start of treatments.

Field study: Three-week-old seedlings were transplanted in the field in a randomized complete block design with four blocks total. Each block consisted of an N+ and a control (i.e., no additional N) treatment. Six drip lines (planting rows) conformed a block divided into two strips (main plots). Planting density was four feet between rows and three feet between plants (12 ft^2 per plant). Each plot had seven plants per row (21 plants per plot). Measurements were only conducted on the middle row or drip line within an N treatment, and the outer rows were considered buffers. The two most representative plants in the middle row were marked and used for all measurements (see below). Three rounds of 30 lb $acre^{-1}$ N were applied through fertigation (29, 46, and 59 DAT, totaling 90 lb $acre^{-1}$ N), using a urea solution injected with a fertigation pump (DEMA, MO, USA). Soil samples prior to the experiment indicated that organic matter was 2.89%, and nitrate, phosphorus, and potassium were 32 ppm, 10 ppm, and 156 ppm, respectively.

Morphological measurements

Plant height was measured from soil level to the apex of the main shoot. Stem diameter was measured 2.5 cm above soil level. Soil canopy cover images were taken every two weeks with an Agricultural Digital Camera, and pictures were pre-processed in PixelWrench2 (TETRACAM Inc., CA, USA). Images were then imported into R for analysis of canopy cover (R Core Team, 2019) as described in (Bristow et al., 2021).

SPAD measurements

SPAD measurements were conducted with the handheld portable fluorimeter MultispeQ (PhotosynQ Inc., MI, USA). The most recently mature leaf, third to fifth leaf down from the top of the canopy, was marked for measurements. The leaf was changed as needed between weeks of measurements as new leaves matured. Measurements were taken three times a week between 12:00 PM and 2:00 PM. Two measurements were recorded per leaf on the center leaflet avoiding the midrib.

Flowering time and inflorescence sampling

A plant was considered as flowering if clusters of pistils were present at the shoot apical meristem and axillary meristems (Spitzer-Rimon et al., 2019). The date of flowering was recorded and weekly monitoring continued until all plants reached the flowering stage. Inflorescence samples were taken at 90% of pistil dieback. The term dieback refers to the percentage of pistils that had turned from milky-white to orangey-brown, were dry and hardened. The 90% inflorescence samples were harvested on 111 DAT for Tahoe Cinco and 113 DAT for Berry Blossom and Red Bordeaux. Inflorescence samples were taken from two marked plants in the center of the plot and composited into one sample. Inflorescence samples were stored at -80 °C until processed.

Cannabinoid analysis

From each inflorescence sample, a ~5 g of fresh material was cut into smaller pieces and 1 g of it was placed into a glass scintillation vial with 30 mL of 100% ethanol (Koptec, PA, USA). The sample was homogenized with a handheld homogenizer (Pro Scientific, CT, USA) for 90 seconds. The solution was allowed to settle for 24 hours and 20 µL of supernatant was filtered through a 0.45 µm and 25 mm Nylon filter (Thermo Fisher Scientific, MA, USA) into a 2 mL amber glass vial (Thermo Scientific, TN, USA) with 80 µL of 100% ethanol for a 1:3 dilution. Glass vials were kept below 40ºC to avoid decarboxylation before HPLC analysis. Analytical cannabinoid standards were obtained from Restek (2018 Restek Corporation, PA, USA). External standards were prepared and HPLC methodology was followed as described in (Farnisa, 2022). The absorbance of cannabinoids was measured at $\lambda = 238$ nm for CBDA and THCA and 220 nm for CBD and THC with a bandwidth of 4 nm. Cannabinoid concentrations were calculated as total CBD = CBD + (CBDA x 0.877) and total THC = Δ^9-THC + (THCA x 0.877). CBD and THC concentrations were usually too low to routinely measure with a negligible concentration and peak area of approximately 0.05% or less.

Plant biomass

Plants were cut at 2.5 cm above soil level. Inflorescence clusters were harvested from the plants and fresh weight was recorded. Leaves and stems were combined and fresh weight was recorded. A subsample of the fresh inflorescence and shoot harvest were taken and dried in an oven at 60 °C and dry weights were recorded after 48 hours. Percent of subsample dry weights were used to calculate total dry biomass weights.

Statistical analysis

All statistical analysis was performed in R 4.0.2 software (R Core Team, 2019). The effect of ‘treatment’, ‘cultivar’, and their interaction (fixed effects) on the response variables was investigated using linear mixed-effect models (lme4 package). Random effects were chosen based on the Akaike information criteria (AIC) (ANOVA function, base R) and may have

included ‘block’ or ‘plot’ with ‘DAT’ nested for response variables with repeated measures. Data were transformed as needed, and model assumptions were checked and met (performance package). Root mean square error was used to determine the best-fit model (lmerTools package). For analyzing effects on time to flowering, a generalized linear mixed effects model (lme4 package) on binary data with ‘DAT’ as a repeated measure was used. The alpha for all models was 0.05. Post-hoc multiple-comparisons were conducted by the unrestricted least significant difference (LSD) test with the multcomp function (emmeans package).

RESULTS AND DISCUSSION

Nitrogen fertilization was found to be important for the vegetative development of floral hemp, especially early in the growing season (Figure 1A). Shoot biomass increased and earlier flowering was observed in plants that received more N (Figure 1B and 2; respectively). Under field conditions, the existing level of N in the soil could determine decisions on N requirements, timing, and fertilization strategies for hemp (Finnan and Burke, 2013). For instance, soils with high inorganic N and organic matter can show a lower response to N fertilization (Struik et al. 2000). Hemp trials at the University of Nevada, Reno, have shown little response to N additions above 100 lb acre^{-1} (Barrios-Masias and Solomon, *unpublished data*). Under greenhouse conditions, hemp showed to be responsive to additional N even after 38 DAT (Figure 1A; N- to N+ treatment), suggesting that a delay in N fertilization may not be detrimental to final biomass production, and could benefit nitrogen use efficiency.

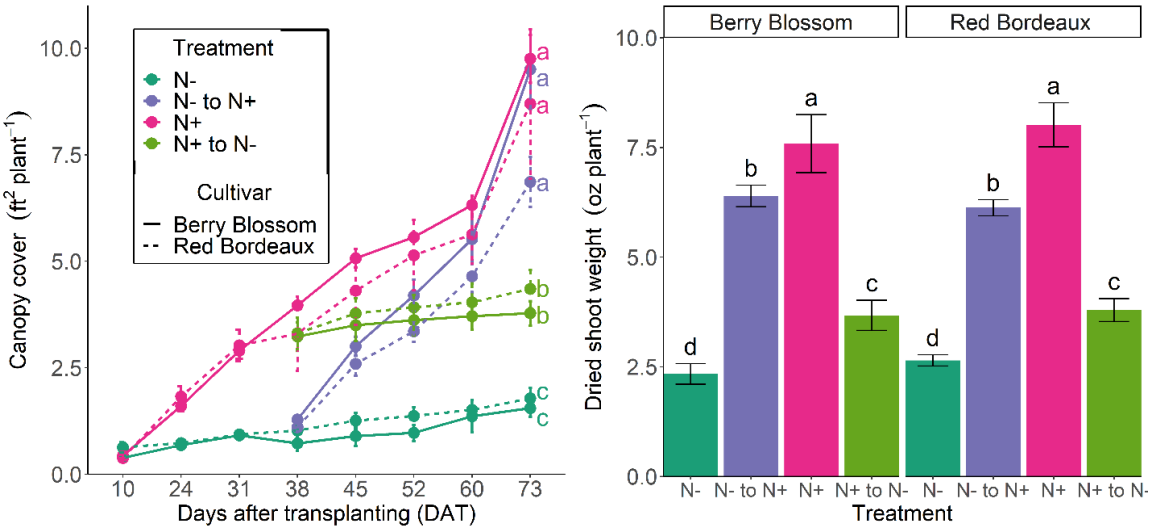

Figure 1. Greenhouse study showing aboveground biomass measured by soil canopy cover (A) and dried shoot weight (B) of hemp varieties grown with high or low N fertilization. At 38 DAT, half of the plants in each treatment were switched to the other N treatment to understand the effects of reduced or increased availability of N after plant establishment. Plants were harvested at 73 DAT before full flowering.

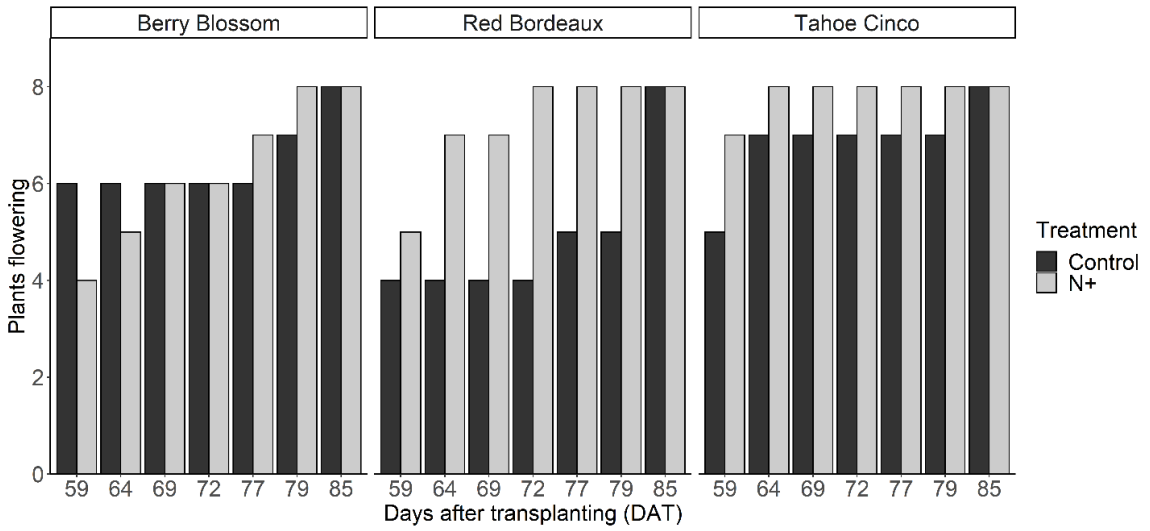

Figure 2. Field study showing the flowering time as days after transplanting (DAT) of three hemp cultivars under two N treatments.

Soil Plant Analysis Development (SPAD) measurements determine ‘greenness’ in a leaf and can be used as a proxy to indicate N deficiencies (Tang et al., 2017; Anderson et al., 2021). The greenhouse and field trials showed differences in SPAD readings between N treatments, except Tahoe Cinco, which showed no difference in SPAD (Figures 3A and 3B). Yet, differences in N concentration as a result of N treatment were observed in field hemp for all cultivars including Tahoe Cinco (Figure 3C). Overall, SPAD values, leaf chlorophyll, and leaf N concentration increased with higher soil-N availability, but cultivars responded differently, suggesting that the use of instruments based on ‘greenness’ should be calibrated to particular production conditions. For instance, the maximum SPAD readings of high N treatments in the greenhouse were lower than in the field, which could be a result of ~20% lower light intensity (i.e., photosynthetic active radiation) in the greenhouse than in the field. Our field SPAD measurements under control conditions were similar to Anderson et al. (2021) who suggested that SPAD readings below 44 indicated N deficiency. Thus, the use of a SPAD meter could be an easy tool to monitor nitrogen content in plants and help growers decide on their nutrient management plans if cultivar and environment are taken into account.

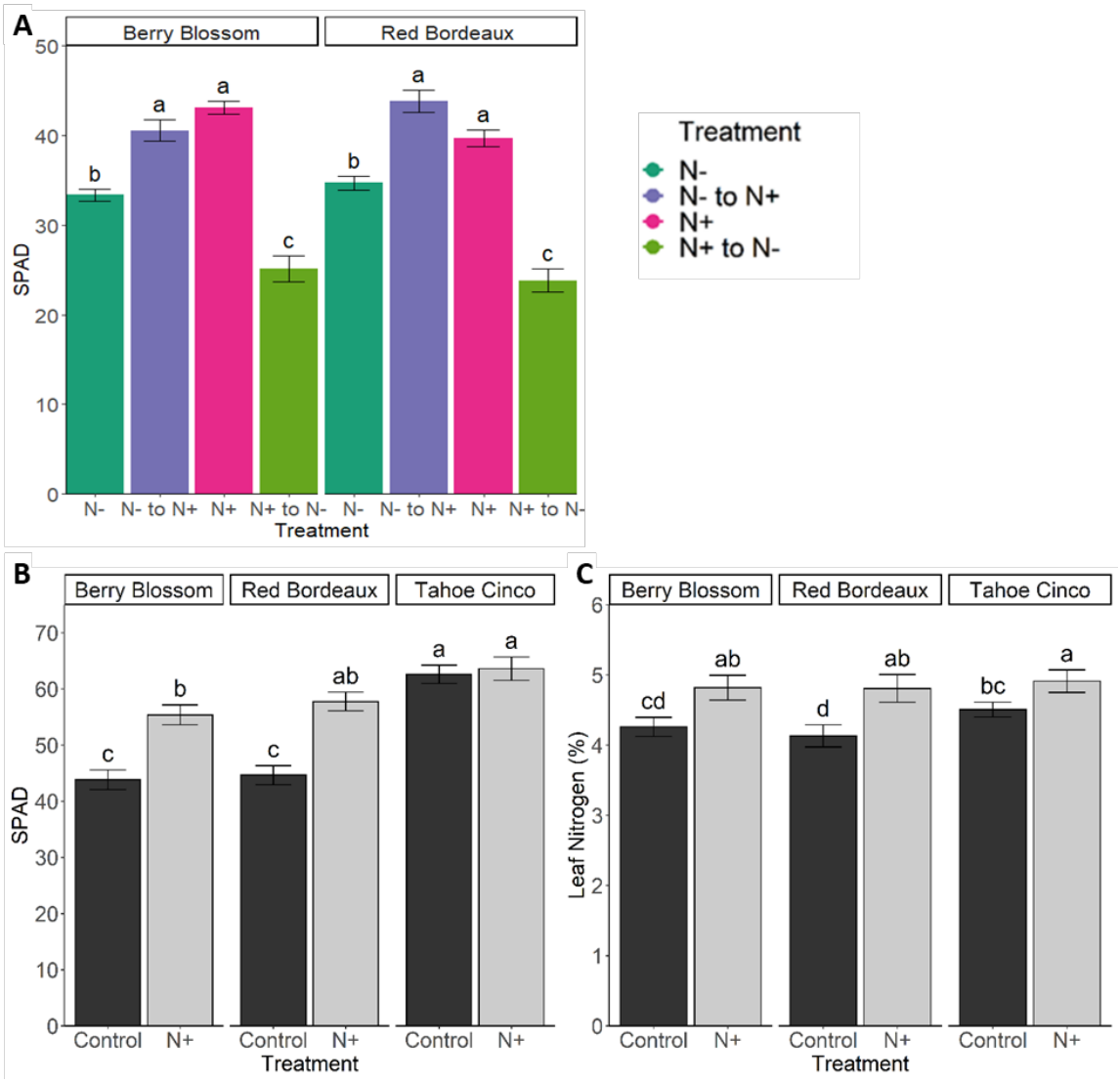

Figure 3. SPAD measurements in the greenhouse trial (A) and in the field (B), and leaf nitrogen content in the field (C) of hemp cultivars under low (N- or control) and high N (N+) availability. Measurements were taken during the peak of vegetative growth and initiation of flowering. Refer to Materials and Methods for specifics on greenhouse and field trial N treatments.

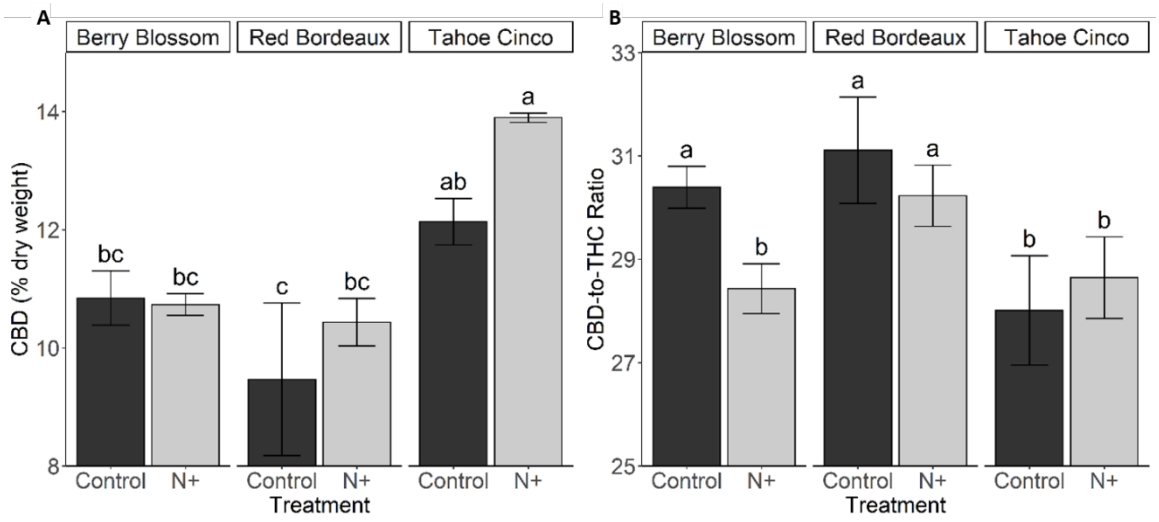

Figure 4. Field study showing CBD concentrations (A) and CBD-to-THC ratio (B) of three hemp cultivars under two N treatments. Measurements were taken when flowers showed a 90% pistil-die back.

CBD concentrations were found to be more dependent on cultivar than N fertilization (Figure 4). Yet, other studies have shown mixed effects of N on cannabinoid concentrations. Increases in N fertilization decreased CBD and THC concentrations by at least 10%, while overall higher cannabinoid concentrations have been reported in treatments receiving less N (Anderson et al., 2021; Saloner & Bernstein, 2021). On the contrary, Kakabouki et al. (2021) reported that CBD concentrations increased with N fertilization. In our study, the cultivar by N treatment interaction affected overall CBD yield as it was driven by increases in inflorescence biomass (data not shown), and suggests that cultivar is an important consideration to increase overall CBD yield per unit area while maintaining THC below 0.3%. Other studies have also shown that increasing N fertilization results in higher biomass and increases in CBD yield per plant (Atoloye et al., 2022; Caplan et al., 2017). Nonetheless, further understanding of how nitrogen affects cannabinoid accumulation patterns and inflorescence biomass can help growers select cultivars and plan nutrient management based on their particular environmental conditions.

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