Plant growth comparison of *Cannabis sativa* when grown in isolated containers versus communal bed systems.

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Introduction

The decision that all farms are faced with in choosing which containers (if any) to use for their crop(s) has both biological and financial implications. Not surprisingly, these implications are not mutually exclusive, as the type of containers used (e.g., hydroponics, potted soil, raised beds, ground-planted, etc.) inherently will express differences in ecosystem biodiversity. Furthermore, this decision as to which type of growing method used (e.g., isolated containers vs. communal beds) will inevitably impact how the crop plant perceives its neighboring community (Silvertown & Gordon, 1989; Karban, 2008; Novoplansky, 2009). The signals that plants pick up from neighboring (if any) plant species can be transferred via a range of stimuli, such as mechanical (i.e., touch) (Braam, 2005), or biochemical, such as organic compounds produced from the roots of surrounding plants (Biedrzycki et al., 2010; Chen et al., 2012).

The interpretation of these signals can have direct influence on important growth characteristics of the plant, which in turn can alter important chemical pathways via nutrient uptake capabilities, such as root growth behavior (Fang et al., 2013). Thus, the exposure to the ecosystems that are expressed by these varying grow methods can have dramatic impacts on the biology of the plant, thus influencing crop health and harvest outputs. As a result, deciding on how specific crops respond to these different container variants and associated ecosystems is an ongoing and heavily debated topic throughout horticultural societies, including the rapidly expanding cannabis cultivation industry.

Previous studies conducted on a range of crops have returned complex and often conflicting results, indicating that plants may express certain morphological traits and chemical compound compositions that are varied if grown in one type of container or growing methodology, while other traits of that same plant may be hindered (i.e., trait trade-offs). For example, tubers (*Solanum tuberosum* L.) grown in either beds, pots, or hydroponically, express a wide range of morphological responses (Correa et al., 2008). Briefly, the authors found that while the number of tubers per plant grown in hydroponics were 147% higher than the bed and pots systems, the actual tuber fresh weight itself was 51% larger when grown in beds than in pots or hydroponic systems. Similarly, as has been documented by numerous studies (see Ryser & Lambers, 1995; Garnier et al., 2001; Shipley & Almeida-Corzes, 2003), significant differences are observed in trait values for species as growing conditions change. As expected, most traits (e.g., canopy height, canopy width, shoot biomass) measured on both fertilized and unfertilized pot grown plants were significantly different than traits of the same species grown in natural grassland communities (Mokany & Ash, 2008). Thus, while these studies illustrate important insights into the relationships between plants and the growing methodology employed, it is clear that they do not apply universally across a wide range of plant taxa.

The aim of this study was to examine the growth of *Cannabis sativa* (hereafter: *C. sativa*) when grown in isolation versus that of *C. sativa* when grown in homogenous beds among conspecifics. We hypothesized that *C. sativa* would yield an increase in aboveground biomass as a result of being planted in a bed along with conspecifics of similar phenotypes (i.e., strains, varietals, cultivars) when compared to *C. sativa* planted in the identical soil but left isolated in pots.

Methods

Plant Growth

A total of 114 *Cannabis sativa* plants spanning three phenotypes were grown indoors under controlled conditions at Gold Leaf Gardens in Lacey, WA. All plants were transferred into their beds after vegetating for 10 days in #1 containers under 1000-W Double-Ended HPS light fixtures. Throughout this vegetative stage, all plants were fed and watered using Gold Leaf Garden's proprietary input recipes. Plants in both treatments were equally watered ad libitum by

gardeners (n=4) with no less than 4 years of cultivating *C. sativa* using these methods. After their initial vegetative stage, plants were then transferred into homogenous living-soil beds (n=6 plants per bed) where they were then allowed to continue to vegetate for an additional 10 days prior to inducing them to flower by adjusting the photoperiod of the flower room. Plants were then allowed to flower for an additional 60 days.

Experimental design

Three phenotypes (i.e., strains) were randomly selected for this experiment (Grateful Breath, Peppermint Cookies, and Koloa Sunrise). Aside from the photoperiod, all environmental parameters were held constant throughout the final 10 days of vegetative growth, and for the first 7 weeks of flowering. All clones were transferred into the flower room at the same time, and were randomly selected to be placed in either the homogenous beds, or solitary pots. The dimensions of the beds used measured 3 ft. wide by 3.75 ft. long, by 0.75 ft. tall, resulting in each bed filled with roughly 8.44 $ft.^3$ of living soil. The same soil was used to fill 12-gal. fabric containers that were used in the isolated replicates. To control for the amount of soil each plant had access to, each plant in the container had 1.55 $ft.^3$ of soil, while each plant in the communal bed had approximately 1.41 $ft.^3$ of soil per plant. Furthermore, to ensure that lighting was held constant across both treatments, identical phenotypes of *C. sativa* were planted next to one another (Fig. 1).

At the end of their flowering cycle (63 days), all plants were cut down at the base of their stalk (i.e., at the intersection of the stalk and the soil). Trellises were then inverted, containing the six plants within the gardening mesh. The trellis legs were removed, and the entire structure was then weighed using a digital Pesola scale (Fig. 2). Repeated measurements of the empty trellis with gardening mesh resulted in a tare weight of 3 lbs. Therefore, 3 lbs. were subtracted from all trellises and the remainder was recorded. This value was therefore the summation of the aboveground biomass (AGB) of six plant batches. Dry weights of the harvested plants were not analyzed, as these data could not be collected because of the workflow design of the processing facility.

Statistical analyses

All statistics were carried out in R 3.4.1 (R Core Team 2017). Differences in the three *C. sativa* phenotypes were tested using an ANOVA followed by a Tukey's honest significant test (HSD) to examine pairwise differences across strains. As statistical differences were detected within different strains (see Results), all phenotype data could not be combined relative to treatment (i.e., data for all beds could not be tested against the data for all pots). All data passed Levene's test for equal variance prior to the ANOVA analyses.

Fig. 1: Cultivation of *C. sativa* utilizing homogenous beds and individual pots. White PVC trellises woven with gardening mesh are used to aid in plant growth and support. Both the bed and pot treatments contained six plants per trellis.

Fig. 2: Weighing the aboveground biomass (fresh wet weights) of *C. sativa*. All trellises weighed contained six plants, regardless if grown in homogenous beds or solitarily in cloth pots.

Results

On average, groups of six plants grown in homogenous beds (n=12) contained ~13% more aboveground biomass (AGB) (mean: 4.01 lbs. per trellis) at the time of harvest (T_H) than those grown solitarily in cloth pots (n = 8; mean: 3.48 lbs. per trellis). ANOVAs and post-hoc Tukey's HSD tests revealed that strains grew significantly more than others even within treatments, and therefore a treatment-wide analysis comparing all plants grown in beds could not be compared to all plants grown in pots. For example, within the bed treatment, Grateful Breath grew significantly larger than both Koloa Sunrise and Peppermint Cookies (46% & 45% respectively; Tukey's HSD, $P<0.0001$, $F_{57.17}$; d.f. = 9). This same pattern was also observed within the pots treatment (Tukey's HSD, $P<0.001$, $F_{57.93}$; d.f. = 5).

When strain type was considered, significant differences were detected in the Grateful Breath strain, but not Peppermint Cookies, or Koloa Sunrise strains. However, while not significant (P<0.05), the maximum AGB for all three strains were grown in the bed treatment. Furthermore, the minimum AGB values for the bed treatment were slightly less (Grateful Breath: -0.01 lbs.), or better (Peppermint Cookies: +0.07 lbs.; Koloa Sunrise: +0.09 lbs.) than the maximum values for the pot grown groups (Fig. 3).

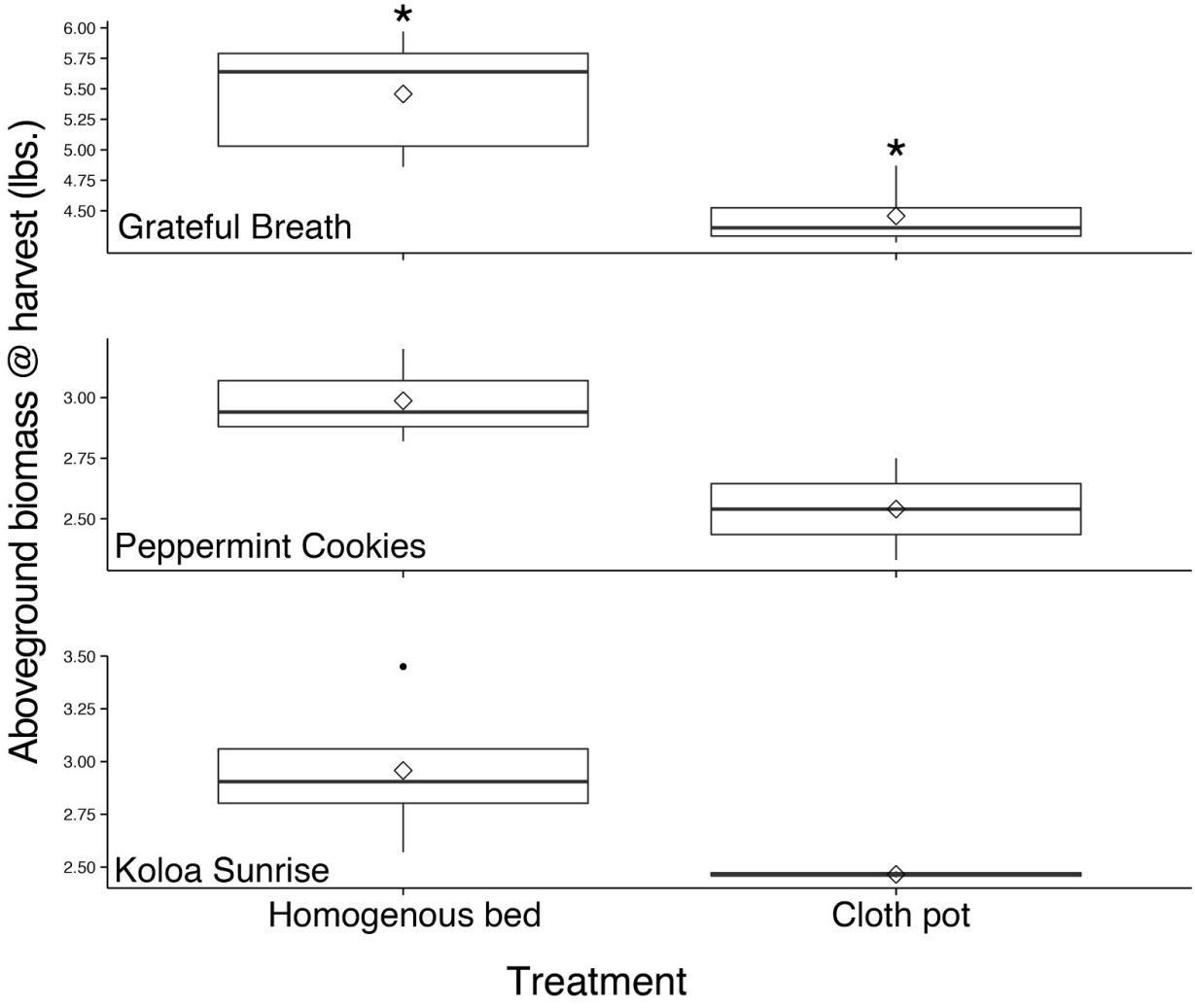

Fig. 3: Boxplot of three strains grown in groups of six plants across two different container types. Open black diamonds represent group means. Asterisks in the Grateful Breath strain indicate that this particular phenotype grew significantly more in the homogenous beds than in the cloth pots. Black dot in Koloa Sunrise indicates a statistical outlier. Note changes in Y-axis scaling across the three strains.

Discussion

The present study provides novel insights into the responses of *C. sativa* when grown on a commercial scale across two different commonly employed container types. As expected, the results indicate that all *C. sativa* varietals grown collectively in homogenous beds yield more AGB than when grown in isolation within cloth pots. While significance among the three phenotypes was only detected within Grateful Breath, a strong pattern was still observed in that the groups of plants grown collectively consistently outperformed those grown in isolation. Intriguingly, significance was detected in our largest of the three samples (n = 5 beds, n = 4 cloth pot groups), suggesting that an increase in sampling effort may lead to more significant results.

It has been well established that plants can not only detect the presence/absence of a nearby plant community, but also decipher the identity of such plants (Elhakeen et al., 2018). The identity of the surrounding plants has experimentally elicited fascinating plant behavioral responses, ranging from negative interactions such as allelopathy, to positive interactions, such as the release of volatile compounds as a warning to other plants (Bais et al., 2006; Dicke & Baldwin, 2010). Furthermore, there is mounting evidence that plants may also undertake kin selection where certain traits that are expressed only in the presence of strangers may indicate competition or selfishness, while traits expressed in the presence of kin may indicate cooperation or altruism (Murphy & Dudley, 2009; File et al., 2012; Dudley, et al., 2013). Thus, having similar phenotypes of *C. sativa* grown within close proximity of one another may provide a growing environment that may not only act to alleviate plant stresses, but also create an alert network for the invasion of pathogens, in turn boosting overall crop resiliences.

Another mechanism that may be being utilized by the six *C. sativa* plants grown collectively is that of the mycorrhizal networks (MNs) present within the soil. These MNs are composed of continuous fungal mycelia and can act to physically link two or more plants of the same, or even different species (Gorzelak et al., 2015). The tampering with, or interruption of these complex MNs has been found to alter behavior of plants. Moreover, how receptive a plant is to linking

into the MN also depends upon the identity of the plant neighbor (Gorzelak et al., 2015). Thus, an ecosystem that contains a community of the same *C. sativa* species (i.e., living-soil beds) would likely have access to more micronutrients via the pathways developed by the larger, and more complex MNs, whereas the plants grown individually in pots would likely have diminished access to such pathways. Furthermore, it is important to note that while the authors argue that any of the aforementioned biological mechanisms may be at play in helping explain the results gathered within the present study, we did not explicitly test them. Thus, much more research is needed to pinpoint the pathway(s) that are hypothesized to be more readily available to *C. sativa* to use when grown among kin within homogenous beds.

Additional trade-offs that lie outside the realm of soil ecology are also important for farms to consider when deciding between growing primarily in beds vs. pot systems. The utilization of communal bed systems do pose unique challenges that can be mitigated by growing in more isolated units such as cloth pots. For example, communal beds are typically less mobile, and since the soil is commonly static in the room and re-used each cycle, the risk for breeding populations of pests that utilize soil as part of their life cycle increases (e.g., fungus gnats, root aphids). Conversely, communal beds do offer additional benefits such as savings related to material costs and labor (see Hussey & Palmer).

Historical prohibition and its impacts on the research has led to a number of large gaps in our knowledge of cannabis cultivation. While limited in scope, the findings of the present paper do suggest that additional research into the following topics would advance our limited understanding of cultivating cannabis, particularly at an organic and commercially viable scale:

1. Introducing biological (e.g., mycorrhizal) inputs across isolated plants vs. communal plant environments and measuring plant responses.
2. Exploration into what container sizes are large enough to support multiple plants.
3. What container shapes would be most beneficial for C. sativa.
4. Replication of our experiment using other forms of media and nutrient applications.
5. Polycultures in communal beds vs monocultures.

While our goal with this experiment was to determine the optimal planting methodology for this commercial facility, we hypothesize that these results could be replicated in other controlled agriculture environments and hope this work would inspire further research by others on this subject.

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