**“Monitoring of trichome morphology and THC content of 3 *Cannabis sativa* cultivars throughout flowering and post-harvest periods”**

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A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science. Thesis process supervised by Dr. Anja Geitmann.

**Abstract**

The legalisation of *Cannabis sativa* L. has turned the illicit drug into the new cash crop in Canada. Crop yield and inflorescence cannabinoids content are the key aspects for producers to maximize their profit. The harvest timing impacts these characteristics significantly. Determining harvest date has colloquially been done based on the quantity of milky-white trichome bulbs, but in fact many other aspects of the cannabis production are considered. To analyze how cannabis producers decide the date of harvest, three metabolically distinct cultivars of cannabis were imaged over time for trichome bulb colour changes and tested for corresponding THC content. Cannabinoid variation between locations on a singular plant was also tested to better understand differences in metabolic content. Through the collection of images during the flowering period, the morphological structures that produce the so-desired secondary metabolites like THC, CBD, and terpenes are investigated. Cannabis trichomes were further imaged through scanning electron microscopy (SEM), and three methods of material preparation were compared for their ability to preserve delicate structures such as trichomes. The results of the imaging observations and THC testing provide a better understanding of how those managing cannabis production decide the harvest date of the crop and the impacts of harvesting on the trichome morphology.

La légalisation du *Cannabis sativa* L. a fait de cette drogue illicite une nouvelle culture commerciale au Canada. Les producteurs souhaitent maximiser leurs profits et donc maximiser la teneur des fleurs en cannabinoïdes. La date de récolte de la culture de cannabis est un aspect essentiel du système de production qui peut avoir un impact significatif sur la teneur du produit en cannabinoïdes. La détermination de la date de récolte a été précédemment effectuée par le ratio entre les bulbes de trichomes ambrés foncés et les bulbes de trichomes transparents. Cependant, de nombreux autres aspects de la production de cannabis sont pris en compte. Pour analyser comment un producteur de cannabis décide la date de récolte, trois cultivars différents de cannabis ont été imagés pour détecter les changements de couleur des bulbes de trichomes et testés pour la

teneur correspondante en cannabinoïdes. La variation des cannabinoïdes au sein d'une même plante a également été testée pour mieux comprendre les différences entre les différents niveaux de la plante (Bas-milieu-haut). Grâce à la collecte d'images pendant la période de floraison, les structures morphologiques qui produisent les métabolites secondaires tant recherchés comme le THC, le CBD et les terpènes sont étudiées. Les trichomes de cannabis ont ensuite été imagés par Microscopie Électronique à Balayage (MEB), et trois méthodes de préparation du matériel ont été comparées pour leur impact sur des structures délicates de la plante tels que des trichomes. Les résultats de la microscopie et des analyses des cannabinoïdes permettent de mieux comprendre les impacts de la date de récolte sur la production et la morphologie des trichomes.

## Acknowledgements

The research in this thesis is supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Mitacs Accelerate Program, and Cannara Biotech Inc. The project is a product of the Quality Assurance and Quality Control of Cannabis program (QAQCC) at McGill University.

I would like to express my gratitude towards Dr. Anja Geitmann for her supervision of this project. Her mentoring was extremely helpful for guidance and support throughout the process, and she has helped to mold the direction of my career. The Geitmann lab provided evaluation whenever asked and could not have been a better support system. Many thanks to Dr. Youssef Chebli, who was also an essential aspect of the project, aiding with microscopy work and working with me to decide the next step. I want to thank Dr. Donald Smith, who was always available to talk and provided great insight on the project. A special thanks to Dr. Sarah MacPherson for her help with cannabis research, securing funding, and working hard to maintain the interesting, important, and much needed QAQCC program.

I would also like to thank the team at Cannara Biotech for being inviting and generous during the research process; My supervisor in the corporate context, Dr. Safa Labidi, Dr. Isaam Ben Moussa, Cédric Guillaumain, Emy Poirer, Thomas Bossard, Jérôme Iler, and lastly, my close friend during the research, Geneviève Lanouette MSc. Thank you all for your help and advice.

## Contribution of Authors

Sarah M. E. Seay, MSc Candidate, completed the literature review, developed the project hypotheses and objectives, collected and interpreted the data, and is the primary author of the thesis. Dr. Anja Geitmann aided in the development of the project and provided feedback and edits throughout the writing process. Dr. Safa Labidi and Dr. Isaam Ben Moussa provided feedback about the thesis from the perspective of Cannara Biotech.

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## Introduction

### The Cannabis Plant

The dried cannabis flower, nug, bud, tree, etc. that is marketed looks substantially different to the cannabis inflorescence during the plant's flowering cycle. However, the cannabis plant and inflorescence are structured similarly to wildflowers and ornamentals (Spitzer-Rimon et al., 2019). The simplest structural explanation of the cannabis inflorescence is a "highly branched, compound raceme" with the induction of new flowering prior to "terminal inflorescence differentiation" (Spitzer-Rimon et al., 2019). A raceme inflorescence pattern is defined by budding on stalks along an elongated axis towards the apex, like the lily of the valley, *Convallaria majalis* (Spitzer-Rimon et al., 2019). With the plant growing upwards and outwards, the first growth originating from the main axis will be the largest, and inflorescence size decreases with increasing distance from the axis (Spitzer-Rimon et al., 2019). New inflorescence development begins before the previous inflorescence has finished development; the compounding structure of the flowers is due to the generation of new growths before the initial inflorescence finishes development (Spitzer-Rimon et al., 2019). Each branched stalk has a main foliage leaf and two calyces at the generation point; either an inflorescence or a stalk will develop from this point (Spitzer-Rimon et al., 2019). Bracts are vegetative, leaf-like structures with photosynthetic, protective, and metabolic potential (Tanney et al., 2021). Cannabis calyces make up the mass of the inflorescence as the protective tissue below inflorescence petals (Tanney et al., 2021). Glandular trichomes are found on the adaxial side of the bract in non-veinous regions or on the calyx of the inflorescence (Adebooye et al., 2012; Turner et al., 1977). When a plant is harvested, the foliage leaves, stems, and unnecessary mass are removed, resulting in a product that is made of calyces and bracts covered by a high number of trichomes.

Glandular trichomes are not unique to cannabis, but cannabis trichomes are unique. Understanding the morphology along with the chemical actions of the cannabis trichome is foundational for cannabis research. However, current research has yet to correctly define most terms. Due to the long and complicated social, political, and legal history of cannabis, the application of the scientific lens is minimal, which has caused fundamental discrepancies in aspects like terminology and source material. Often

language surrounding the cannabis plant can include medical or slang influence, but the agricultural perspective is often simpler to understand. The government of Canada defines cannabis and cannabis-related terms on the website. For cannabis legalisation and continued support for the scientific research, demystifying cannabis and cannabis terminology is essential. Once foundational vocabulary is understood, discussions about cannabis, and cannabis trichomes, are more accessible.

**Trichome Overview**

Trichomes are specialized unicellular or multicellular growths that originate from plant epidermal tissues, projecting outward from various plant organs (Dayanandan & Kaufman, 1976). Trichomes can be classified as “glandular” and “non-glandular” (Werker, 2000). Non-glandular trichomes may differ in length, size, shape, be branched or unbranched, and lack the ability to secrete secondary metabolites. The bristle-like hairs vary in length and circumference based on species. Glandular trichomes, which differ in both secretory metabolite composition and morphology, influenced by the plant’s genetics. Both glandular and non-glandular trichomes can form on all parts of a plant, including vegetative and reproductive organs (Werker, 2000). The overall distribution of trichomes varies based on species and can be different across tissue types with regards to their density or metabolic profiles. Often glandular trichomes are concentrated on intervein areas, while non-glandular trichomes are typically associated with veins and leaf margins (Kim and Mahlberg, 1991). The rate of trichome density development is dependent on genetic and environmental factors; research has investigated temperature, water stress, etc. effects on trichome production (Lyu et al., 2022; Caplan et al., 2019; Gonzáles et al., 2008; Gianfagna et al., 1992).

Classification based on secretory substances is complicated, as possible secretions include polysaccharides, sugars, salts, lipids, essential oils, resins, proteins, terpenes, et cetera (Werker, 2000). Secretions are divided into two groups: unmodified or synthesized by constituent cells of the secretory tissue. Significant difficulty arises in substance-based classification due to the ability of some plant species to secrete a combination of substances from the same gland (Werker, 2000). Morphology-based

trichome nomenclature is common due to the species-specific trichome shape. Luckwill (1943) initially developed a trichome identification system based on a species with significant trichome density, *Solanum lycopersicum* or the cultivated tomato. Attempts to completely classify trichomes are currently difficult with the discovery of new trichome structures that do not fit within the specified categories (Glas et al., 2012).

Modern hypotheses suggest that trichomes are beneficial to plants by acting as pest deterrence, dissemination of odours, or solar heat reduction (Duke, 1994). Trichomes have been found to trap insects or secrete a resinous substance to coat the plant surface, supporting the insecticide theory, but this only occurs in highly specialized scenarios (Dell & McComb, 1978). Specific secretions of glandular trichomes have chemical variations based on common species-pest interactions with insect repellant, behaviour-modifying, and feeding deterrent compounds (Duke, 1994). Glandular trichomes develop rapidly on immature trichome dense structures, further suggesting that trichomes often aid vulnerable tissues in defense against insects and pathogens (Duke, 1994). As these bioactive secondary metabolites require significant energy and resources for production, their benefits to the plant must be strong enough to justify the cost of development.

**Cannabis Trichome Complex**

The glandular trichome bulbs contain combinations of terpenes and cannabinoids that vary among cannabis cultivars, giving the specific cultivars their cannabinoid profiles and scent (Tanney et al., 2021; Dayanandan & Kaufman, 1976). Multicellular stalks protrude from the epidermis and their arrangement is without obvious patterning (see Figure 1) (Dayanandan & Kaufman, 1976). Trichome stalks thicken and lengthen with maturity (Tanney et al., 2021; Dayanandan & Kaufman, 1976). Each bulb is comprised of the secretory discs along the base of the trichome head, which secrete the secondary metabolites into a space beneath the cuticle (see Figure 1) (Tanney et al., 2021; Dayanandan & Kaufman, 1976). Mahlberg and Kim (2004) conducted much of the available research into the chemical composition of the cuticle and stalk, demonstrating a common walled structure of fibrous cellulose, hemicelluloses, pectins, and

glycoproteins with sub-cuticle cavities in the bulb. This research has further suggested that not only is there a cuticle layer made of cutin over the secretory discs, but a secondary subcuticular cellulosic wall. Following disc cell development, the disc cell walls thicken into a cuticle until an eventual fusion when they meet the subcuticular wall (Kim and Mahlberg, 1991). As the cuticle thickens with maturity, this suggests the bulb's cuticle may become stronger (Kim and Mahlberg, 1991).

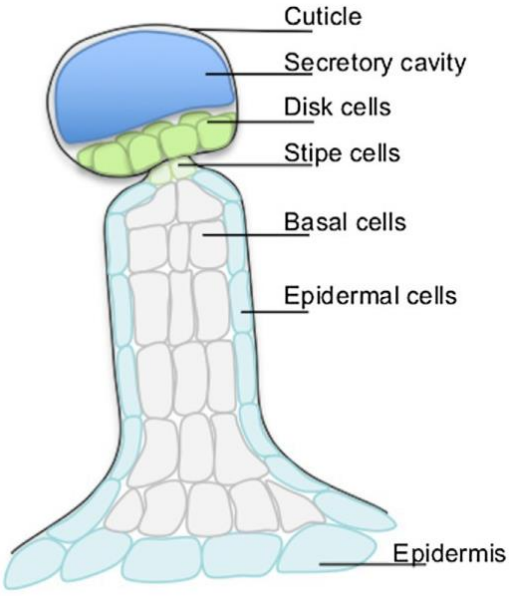


**Figure 1.** Illustration of *Cannabis sativa* stalked glandular trichome structure from Cailun Tanney et al. (2021).

Livingston et al. (2021) provide an updated understanding of trichome formation on cannabis. Stalk morphology agrees with Mahlberg and Kim's (1997) findings, however, additional insight was provided on the bulb's cuticle; the primary cell wall and cuticle are not separated by a clear boundary (Livingston et al. 2021). Bulbs are formed by the splitting of a specialized layer of the cell wall into two portions at the meeting of the secretory cells, but the composition of this specialized wall and the mechanisms that create the trichome bulb remain unknown (Livingston et al., 2021). As the trichomes develop, the cuticle thickens as Mahlberg and Kim (2004) previously determined, but Livingston et al. (2020) adds that the substantial expansion of the cavity also includes the

deposition of cuticular lipids. Further, there is evidence that droplets of lipidic cannabinoids and terpene metabolites within the cavity are encapsulated by glycoproteins and starches (Livingston et al., 2021). These droplets form the entirety of the bulb with emulsifying agents (Livingston et al., 2021). The final product is a delicate cuticle around an oil substance.

The morphological classification of cannabis trichomes currently consists of three distinctly different structures: bulbous, sessile, and stalked (Werker, 2000). Bulbous refers to epidermis level protrusions forming a blister-appearing growth, sessile trichomes appear very similar with an extremely short stalk and bulb, while stalked trichomes are notably longer than sessile (Hammond and Mahlberg, 1977; Livingston et al. 2020). Defining the three cannabis trichomes within Luckwill's (1943) trichome classifications is difficult due to the unique structures. The stalked and sessile glandular trichomes of cannabis resemble 'Trichome VI', but the tomato trichomes form the bulb with each secretory cell filling a singular cuticle to form multiple compartments (Bergau et al., 2015). *Nicotiana tabacum*, tobacco, is another cash crop that employs trichomes to develop flavor, smells, and volatile compounds. The tobacco trichome morphology is significantly thinner stalked, multicellular, and tipped with a petite bulb (Uzelac et al., 2021). Interestingly, two wild plant species have developed similar type 'VI' trichomes through convergent evolution. *Mimulus lewesii*, monkeyflower, is a perennial wildflower native to the mountain ranges of western North America with strong trichome stalks in a structure reminiscent of cannabis trichomes, however the bulbs are cleft similarly to tomatoes (Eguiguren et al., 2020). A specific variety of wild tomatoes *Solanum habrochaites*, aptly named hairy tomatoes, has almost identical 'IV' trichomes in similar high densities (Glas et al., 2012). In cannabis a continuous cuticle covers multiple secretory cells, creating a singular large bulb. While the terms 'bulbous', 'sessile', and 'stalked' are generally used as descriptors of distinct trichome types, there is discussion on whether sessile trichomes are simply young stages of stalked trichome (Brand & Tissier, 2022; Livingston et al., 2022). Rather than an individual category, 'sessile' trichomes may be immature, presecretory stalked trichomes (Livingston et al., 2022). To avoid overinterpretation, the trichomes observed within this project will simply be referred to as glandular.

**Chemical Deposition in Trichome Bulbs**

Δ9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) are the main two cannabinoids recognized by the human endocannabinoid system, causing the psychological and physical effects upon cannabis consumption. THC & CBD bind to cannabinoid receptors present in the adult central nervous system particularly, but also other organs including the liver and skin. Δ9-Tetrahydrocannabinolic acid (THCA) and Cannabidiolic Acid (CBDA) are the acidic precursors to THC & CBD found in the bulb of glandular trichomes (Happyana et al., 2013). The compounds must be decarboxylated through heat for consumption to induce the cannabinoid receptor signaling. THCA & CBDA are stored within the trichome bulbs, along with terpenes and phenolic compounds (Booth et al., 2017; Livingston et al., 2022). Cannabigerolic Acid (CBGA) is the sole precursor to THCA & CBDA found in the plastid surface (Livingston et al, 2022). CBGA is generated by olivetolic acid from the cytosol and geranyl pyrophosphate from the plastid but remains within the membrane bilayer due to its inherent lipophilicity (Livingston et al., 2022). Transfer proteins export CBGA to the disk cell surface wall, where THCA & CBDA synthase form the lipophilic metabolite droplets that enter the storage cavity (Livingston et al., 2022).

Changes in metabolic content are thought to be associated with the colour changes of trichome bulbs. Plant senescence is hormonally indicated by an increase in ethylene and abscisic acid, and species-specific morphological traits can develop with the hormonal changes (Turner et al., 1977). Tree leaves turn hues of reds, oranges, and yellows, but cannabis glandular trichomes turn from transparent to a semi-opaque white followed by a light and then dark, amber shade (Turner et al., 1977). Trials initially indicated a negative correlation between trichome darkening and THC content (Turner et al., 1977). The milky-white discolouration induced by a change in metabolite content throughout the flowering stage is indicative of cannabinoid deposition (Sutton et al., 2022). The transition of metabolites within the bulb from translucent to opaque may signal an important point within the flowering period, whether it be peak cannabinoid content or informative of a harvest in the near future. However, little scientific evidence exists on the

relationship between trichome hue and THC content (Sutton et al., 2022). As trichome maturity is inherently linked to THC content, fully understanding the relationship between the two features is an essential step in cannabis research and production.

Various means of detecting trichome maturity have been suggested, but similarly lack significant published research support. Sutton et al. (2022) provide evidence for distinguishing trichomes based on fluorescence and diameter and suggest that bulb hue changes are the most likely an indication of senescence. Fluorescence microscopy allows metabolic analysis within live samples by exciting the fluorescent particles at different wavelengths that indicate the samples components (Livingston et al., 2020). When excited by a two-photon microscope at 720 nm, cannabinoids fluoresce blue at 420-460 nm (Livingston et al., 2020), while green indicates flavonoids and red indicates undetermined compounds, possibly terpenes. Thus, younger trichomes that appear milky are expected to have the highest cannabinoid content with the high fluorescence and a blue shift. Fluorescence of both milky and brown glandular trichomes demonstrated a red shift at 490-530 nm for more mature trichomes, while green fluorescence increased linearly with maturity (Livingston et al., 2019). Therefore, evidence exists proving the shifting chemical contents of trichome bulbs during the maturation process.

The most popular method to identify the changing metabolites in cannabis material is high-performance liquid chromatography (HPLC), as major cannabinoids can be separated and identified without deterioration (Aizpurua-Olaizola et al., 2014; Moosmann et al., 2012; Salomone et al., 2012; Hazekamp et al., 2005). HPLC is a common methodology for compound separation in samples less than 5 mL; the machinery pushes air through a uniform column, dividing the mixture into individual solutions based on density (Aizpurua-Olaizola et al., 2014; Moosmann et al., 2012; Hazekamp et al., 2005). Material is analyzed for THC, delta-9-THC, THCA, cannabinol (CBN), cannabigerol (CBG), CBD, cannabigerolic acid (CBGA) and cannabidiolic acid (CBDA) (Patel et al., 2017). CBGA is the precursor to THCA, CBDA, and CBG; THCA degrades into cannabinol or delta-9-THC, while the other acids reduce to their specific decarboxylated forms (Addo et al., 2021). HPLC testing uses the THCA and THC values to determine a samples THC

content. Standardization regarding HPLC testing, and cannabis cannabinoid content is ongoing and a focus of cannabis market development (Patel et al., 2017).

**Harvesting and Drying Methods for Commercial Cannabis Production**

Post-harvest drying is essential for the preservation of cannabinoids in trichomes by controlling temperature and humidity. The cannabis inflorescence contains 70 to 80% moisture at harvest, classifying cannabis inflorescence as a perishable good (Das et al., 2022). Implementing a drying method is necessary for consistent product humidity and to breakdown excessive sugars, increasing the ratio of cannabinoids and reducing odours and burning sensations when inhaling (Das et al., 2022). Many experiments have evaluated cannabinoid quantities in dried cannabis over various exposure times and temperatures (Taschwer & Schmid, 2015; Trofin et al., 2011), but most companies 'slow-dry' at 18-21°C and 50-55% humidity for 10-14 days (Das et al., 2022). Plants are hung with the apical inflorescence pointing downwards to increase the accumulation of metabolites in the flowers. Methods of drying the material for testing can have a significant impact on cannabinoid and terpene profiles (Addo et al., 2021). Hot-air drying has been shown to cause the loss of secondary metabolites through evaporation in hemp, while freeze drying and microwave-assisted drying reduces quality deterioration (Addo et al., 2021). Cannara Biotech uses drying rooms and microwave-assisted drying for the preparation of material for in house cannabinoid testing; this method is easy to replicate, low cost, and generates more consistent product humidity than only implementing hang-drying methods. Excitation of the remaining water molecules creates a temperature gradient within the material, removing bound water through the outer layers of the cannabis (Addo et al., 2021). The hang-dried and microwaving processes maintains colour and nutrients when compared to non-microwave-assisted drying of other plants (Nghia et al., 2018)

Trimming can occur at various points in the drying process; 'dry-trimming', as opposed to 'wet-trimming' before drying, is more common as the fresh plant material is more difficult to remove due to the sticky excretes (Das et al., 2022). Whichever process a producer chooses to employ, the final product is the whole cannabis inflorescence with

little leaf material (Al Ubeed et al, 2022). The inflorescence is then manipulated and sold in many forms, including dried flower, but also extractions, pre-rolled marijuana cigarettes, or edibles (Al Ubeed et al, 2022). The dried inflorescence product exiting the drying process is cleaned of leaves and non-trichome dense material to have a product ‘ready-to-smoke’ (Al Ubeed et al, 2022). Due to the high labour demand of processing many pounds of infloresence a day, the processes of cleaning and packaging are left to machines and ‘bud-trimmers’ using the silicone brushes at a rapid pace. The quickest way to remove excess material is by machine tumbler, which rotates a cylinder with circular holes to detach leaves, stems, etc. while preserving the cannabis inflorescence (Das et al., 2022). Hand-trimming is the more time-consuming method; individuals will use silicone brushes to gently brush the surface of the flowers (Das et al., 2022). Due to the known delicacy of cannabis trichome stalks after the drying process, the aggressive method of inflorescence preparation seems contrary to product metabolic content and appearance.

Consumer opinion is considered in the decision making with regards to processing and final product. Markets vary on the demand for certain THC values based on geographic location (Donnan et al., 2022), but metabolic content is a singular aspect of the product. Cannabis quality has yet to be qualified in literature, and opinions on what constitutes a quality cannabis product vary significantly. Perceived quality of the inflorescence involves the THC content, aroma, visual appeal, and moisture content (Donnan et al., 2022). Metabolic content is dependent on inflorescence cultivar and moisture content is dependent on drying methodology, but the aroma and visual appeal is subjective to the individual. Aromas are a result of the natural scent of cannabis and the cultivar’s combination of terpenes, as well as many monoterpenoid compounds (Oswald et al., 2023); as scent interpretation by the individual is not accurate, quantification of aromas can only occur analyzing the chemotype of the cannabis. Visual appeal is also very difficult to quantify due to the general public's lack of cannabis morphological understanding. Throughout the history of illicit cannabis, low consideration was given to the appearance of the dried inflorescence and protection of the trichomes; packaging was focused on reducing odour and maximizing the amount of product in a contained space. Packaging is now intended for educating a consumer on the product,

warning about the effects of cannabis, and potentially drawing a customer in with interesting colours and brands. The transition occurred without a mass education about the appearance of high-quality cannabis, and for the casual consumer of cannabis, the transition into machine packaging represents a significant increase in quality. Thus, most consumers depend on price and cannabinoid content as the only understood aspects of the cannabis flower, and the Canadian market has reacted with a similar focus (Donnan et al., 2022). The lack of understanding about cannabis trichomes and chemical changes within the bulbs can cause false beliefs among consumers, with some considering the darkened bulbs to indicate over maturity. Informing consumers about trichome development and metabolite changes is the first step to address the lack of knowledge about visual cannabis product quality.

**Scanning Electron Microscopy for Cannabis Trichomes**

Electron-based imaging of cannabis material allows for imaging of trichome dense material for visualization and analysis at an ultrastructural level. However, preparation of biological materials for electron microscopy requires significant preparation. The steps involve: fixation, ethanol rinsing, critical point drying (CPD), sputter coating, and SEM. Chemical fixation maintains the structure of biological material by stably bonding proteins for viewing through electron microscopy or preserving the sample immediately after sourcing (Yuan et al., 2023; Eltoum et al., 2001). Halting the sample degradation and preserving the structures prevents the subsequent dehydration steps or the microscope's vacuum settings from collapsing cells and tissues. For preparation of samples for electron microscopy, the primary fixatives are formaldehyde, glutaraldehyde, and osmium tetroxide (Yuan et al., 2023; Eltoum et al., 2001). Formaldehyde forms cross-links in and between proteins and preserves the peptides of the cellular proteins to stabilize structures (Eltoum et al., 2001). Glutaraldehyde cross-links proteins as well, but the additional aldehyde groups, more cross-links are formed than when formaldehyde is employed (Eltoum et al., 2001). Both aldehydes react with other macromolecules like amino acids, nucleic acids, and nuclear proteins, resulting in the possible formation of undesired reactive groups (Eltoum et al., 2001). Rinsing tissues with a solvent after removes

reactive groups formed by formaldehyde and any remaining water in a sample is replaced with ethanol in preparation for CPD (Eltoum et al., 2001). Osmium tetroxide reacts with protein side chains, causing linking like aldehydes, but primarily works to polymerize unsaturated lipids by the cross-linking of ethylenic bonds (Eltoum et al., 2001). Primary fixation with another chemical fixative and the subsequent application of osmium tetroxide as a secondary fixative is a common method to ensure all compounds within the cell are fixed (Yuan et al., 2023; Eltoum et al., 2001). Osmium tetroxide as a primary fixative is uncommon due to the slow rate of reaction and low penetration abilities. Methanol has been investigated as another chemical for fixation purposes. Samples immersed in methanol are rapidly dehydrated and cellular proteins precipitate to preserve the cellular architecture (Talbot & White, 2013; Kim, K.W., 2020). The rapid fixation of the material may be beneficial for timing purposes, but folding and shrinking of the cells can occur and lipids and carbohydrates can be extracted during the process (Talbot & White, 2013; Kim, 2020).

Duration of the fixation period is an important factor in choosing the most suitable fixative for a sample. The rate of fixative diffusion within a tissue is dependent on the size of the sample and a fixative's coefficient of diffusion (Eltoum et al., 2001). Osmium tetroxide has a notably higher coefficient of diffusion than formaldehyde, and thus osmium tetroxide is better applied to thin materials that can be penetrated entirely (Eltoum et al., 2001). Often it is used as a secondary fixative. Formaldehyde requires less time to penetrate the tissue, but the rate of fixation for the two fixatives is similar; formaldehyde requires 1 hour per 1 mm of material thickness, and osmium tetroxide fixes within 60 to 90 minutes within a stabilized tissue (Eltoum et al., 2001). Without prior fixation, osmium tetroxide fails to penetrate samples thicker than 1 mm well and can cause loss of large portions of proteins and carbohydrates (Eltoum et al., 2001). In contrast, methanol replaces water within a sample rapidly, reaching the entirety of the sample in minutes (Kim, 2020). However, the rapid nature of the fixation is a source of potential damage to the cells that undergo rapid protein denaturation and precipitation (Talbot & White, 2013; Kim, 2020). Temperature and pH can both further impact the effectiveness of any chemical fixation process. At higher temperatures, the rate of fixation penetration within tissues is increased, and the rate of fixation for formaldehyde specifically occurs more

rapidly (Eltoum et al., 2001). Fixation with aldehydes may occur up to 45°C before denaturation occurs, but room temperature is effective (Eltoum et al., 2001). An acidic pH can cause targeted groups like amines and hydroxyl alcohols to become unreactive to the hydrated formaldehyde formed by the aldhyde linkages (Eltoum et al., 2001). Ensuring a neutral pH reduces the occurrence of fixation failure or discolouration (Bone et al., 1973). Additives of a vehicle buffer can reduce the chance of pH caused errors and maintain osmolarity of the cells. Fixative solutions with a difference in osmolarity to the samples can cause distortion of the cells; one benefit of osmium tetroxide is the higher osmolarity rate (Bone et al., 1973). When fixing with formaldehyde, the fixative vehicle is essential to ensure full penetration as formaldehyde exerts a much higher osmotic pressure than osmium tetroxide (Bone et al., 1973). Decreasing the osmotic pressure and ensuring a slightly acidic or neutral pH is essential when using aldehydes, while osmium tetroxide naturally provides better conditions for fixation (Bone et al., 1973). Methanol fixation requires no pH or temperature adjustments to ensure fixation (Kim, K.W., 2020). Deciding which chemical fixative to employ is dependent on cell type and the methodology required for operating the CPD and SEM machinery. Undesired results such as cell shrinkage or folding is most commonly from human error during sample preparation or the lack of chemical fixative permeation. Determining a proper fixation of the sample based on previous research is essential for success in SEM preparation.

Following the fixation step, the protocol involves replacing water with ethanol performing repeated rinses with increasing concentrations of the solvent. The initial rinsing after a chemical fixation by formaldehyde is with the vehicle buffer solution before the introduction of ethanol, beginning with 30% and gradually increasing to 100% rinses (Tsotsas & Mujumdar, 2011). The rinsing ascension ensures water within the sample is replaced with ethanol; ethanol works as an intermediate fluid with a closer critical point to $CO_2$ and is miscible with $CO_2$ unlike water (Tsotsas & Mujumdar, 2011). The liquid ethanol in the sample is exchanged for liquid $CO_2$, and the $CO_2$ gas is evaporated at a temperature and pressure past the critical point, removing the $CO_2$ when there is no distinction between gas and liquid (Tsotsas & Mujumdar, 2011). Removing the $CO_2$ in this manner avoids damage by surface tension and facilitates retaining delicate structures (Tsotsas & Mujumdar, 2011). Before imaging, a thin coating of metal, traditionally gold or

platinum, is applied with a Sputter Coater to provide reflection points for the electrons (Echlin et al., 2013). Samples must be electrically conductive at the surface to reflect an electron beam emitted from a tungsten filament cathode (Echlin et al., 2013). Excited ions of the metal are directed at the sample under vacuum and high voltage, depositing the metal onto the sample to ensure the sample can be viewed (Echlin

et al, 2013). Within the SEM machine, the electron beam is focused by one or two condenser lenses and is directed through scanning coils or deflector plates, depending on the microscope model (Echlin et al., 2013; Mohammed & Abdullah, 2018). Electrons are directed at the sample within the chamber, and specialized detectors determine the emission of the scattering primary and secondary electrons, the emission of electromagnetic radiation, and the proportion of the beam absorbed by the specimen to generate the image (Echlin et al., 2013; Mohammed & Abdullah, 2018). Computer imaging is synchronized with the position of the electron beam, aided with electronic amplifiers (Echlin et al., 2013; Mohammed & Abdullah, 2018). The resulting image is a distribution of the intensity of the electron signals being emitted from and absorbed by the sample (Echlin et al., 2013; Mohammed & Abdullah, 2018).

Chemical fixation methodology for preparation of a biological sample for SEM is time-consuming, requiring liquid changes often, and one cannot discern if a sample has been prepared correctly until the process is completed and the sample imaged. However, chemical fixation is easily replicated and can be done in a lab space with less equipment prior to the CPD stage; this method is the most accessible way to prepare biological samples. High-pressure freezing is the method of material fixation for electron microscopy that immediately flash freezes the samples using liquid nitrogen, high pressures, and/or cryogenic temperatures (Issman & Talmon, 2012; Kim & Mahlberg, 1997). The benefit of this method is rapid, non-selective, and the processes are performed in semi-automated equipment, limiting human error in sample preparation. Further, there is current discussion as to whether chemical fixation results in accurate portrayal of the cannabis cells structures. Livingston et al. (2023) compared the effects of chemical fixation with glutaraldehyde and osmium tetroxide to high pressure frozen cannabis material. Glutaraldehyde was used as a primary fixative for cellular immobilization, and osmium

tetroxide as a secondary fixative to preserve the cytoplasm and maintain cell permeability. The chemical fixation process is slower than high pressure freezing, which occurs in 20 milliseconds, at an extremely low temperature, and at 2100 bar with liquid nitrogen. The conditions transform the water within the sample from a liquid to an amorphous stage to avoid the development of ice crystals and allow the removal of the water with air pumps. When compared to the rapidly fixed frozen samples, chemically fixed glandular trichomes had “intracellular inclusions found on the surface of plastids” (Livingston et al., 2023). The high pressure freezing of samples result in a lack of inclusions without erroneous spaces in the cytoplasm (Livingston et al., 2023). Samples prepared under the high-pressure freezing fixation conditions have homogenous cytoplasmic contents with a lack of lipidic inclusions seen in the chemically fixed material, suggesting a better fixation of the cells (Livingston et al., 2023). Further, the frozen samples had no obvious porosity within the cell wall structures, while chemically fixed cell walls had separated (Livingston et al., 2023). Damage occurs during the lengthy chemical fixation process, and Livingston et al. suggests there is excessive mobility of internal compounds, and the drying process causes cells to separate from the pressure of CPD. Analysis of the cytoplasmic and cell wall structure of trichomes on chemically fixed samples may therefore be more prone to artifacts than high pressure frozen fixed samples. However, chemical fixation may be satisfactory for the morphological analysis of the trichome stalk and outer structures. As materials and methods for chemical preparation are less expensive and require little sophisticated equipment, developing a reliable fixation method that is more accessible and replicable for simple morphological investigations is a worthwhile effort. As cannabis research develops, the development of chemical fixation methodology is important for labs without the access to high pressure freezing fixation machinery.

## Hypotheses and Objectives

Correlation between Trichome Transformation and Cannabinoid Content

One important point in cannabis cultivation is the harvest date, as metabolite content and trichome appearance are dependent on the length of flowering time. The harvest date was previously determined by the discolouration of the bulbs, but with HPLC technology, some producers prefer to test inflorescence cannabinoid content repetitively

during flowering to evaluate their trend and determine the harvest date. There is time and expenses associated with consistent HPLC testing of flowers, and there may be a possibility of determining the harvest date at a flower's maturity through the trichome bulb colour changes. This thesis hypothesizes that the harvest date of the cannabis plant for maximizing THC potency occurs prior to trichome senescence, as indicated by the metabolite darkening, but after the onset of discoloration to milky-white. The first objective (Objective 1.1) is to determine whether the maturation of the harvested organs is associated with a change in trichome density. To understand if there is a correlation between the colour shifts and THC %, the THC content of three cultivars was tested with HPLC every 3 to 4 days beginning at flowering day 40 and ending at the cultivar's harvest date as set by the company. An HPLC test was performed on material that has undergone the hang drying process to compare to the fresh material. The second objective (Objective 1.2) is to visualize the changes in trichome morphology and density at each sampling date, digital photography was employed to image the material prior to cannabinoid analysis. 5 images of each cultivar at each sampling date were chosen for trichome density calculation from regions of interest sized 1 mm x 1 mm, and ImageJ was used for image analysis and processing. As a complementary approach the morphology of trichomes was analyzed using SEM.

Impact of Post-Harvest Trimming Methods on Trichomes and Cannabinoid Content

Another critical step in the cannabis production system is the manipulation of the dried inflorescence after harvest and drying. Companies often employ large machine tumblers and/or 'bud-trimmers' for manual trimming. At Cannara Biotech, premium grade flowers are hand trimmed by bud-trimmers using silicone brushes and medium grade buds are machine trimmed. Trichomes are not durable structures and become brittle during the drying process, and thus the intense and rapid removal of the excess leaf material may have a negative impact on the appearance and metabolite content of the dried flowers. The consequences of each cannabis trimming method on THC content and trichome density of the final product have yet to be investigated. This thesis hypothesizes that there is a reduction in THC content due to the loss in trichomes from the manipulation

of the dry flowers. Again, HPLC testing (Objective 2.1) and macro-photography (Objective 2.2) were employed to compare the physical state of cannabis organs prior to and after the manipulation of material that has been trimmed using either machines or manual silicone brushes.

## Optimization of Chemical Fixation Procedures for Scanning Electron Microscopy of Cannabis Trichomes

Preparing biological samples for SEM is elaborate and requires meticulous attention to ensure a successful image. Chemical fixation is the primary step of petrifying cellular structures and preventing that any material degrades over time, preserving the sample rapidly after collection. SEM and other electron microscopy techniques have been applied to cannabis material recently (Livingston et al., 2022, 2023), but often employ high pressure freezing for optimal results. High pressure freezing requires expensive equipment such as a Leica EM ICE high pressure freezer ($331,498 CAD), the Leica EM VCT500 cryo-transfer system ($123,920 CAD), and the Leica EM Vacuum Cryo-Manipulation system ($53,959); chemical fixation is an accessible, easily replicable method of fixing materials that can be completed in any conventional laboratory setting. However, as there is limited research on chemical fixation of cannabis tissue for SEM, there has yet to be a defined method for obtaining the best images. Due to the numerous steps in sample preparation, there are various points in the methodology that could result in poor sample preservation; choosing the proper fixative, the method of rinsing, and the critical point drying machinery can each have profound impacts on the quality of the sample. This thesis hypothesizes that the chemical preparation method for cannabis tissue is applicable for high-quality SEM imaging. To do so, the experiment consists of three different fixative mixtures applied and multiple setting variations for the critical point drier to determine an improved methodology for the processing of chemically fixed cannabis material for SEM (Objective 3.0). Samples were analyzed for quality of fixation, focusing on cannabis epidermal layer for inclusions, distortions, etc. and trichome structures were assayed for their structural integrity. One specific goal of the optimization of the fixation methodology was to maintain the integrity of trichome bulbs located at the ends of the glandular trichome stalks. Current research fails to investigate the chemical

fixation process with cannabis tissue thoroughly, and this thesis aims to provide a starting point for further investigation.

## Material and Methods

Plant Material - Cultivation and Sampling

Some material for objective 3 was sourced from female *Cannabis sativa* L. plants of the cultivar “CBD Kush” at Macdonald Campus, Sainte-Anne-de-Bellevue, Quebec, in a Canada Revenue Agency and Health Canada approved research laboratory (license no. LIC-5AZZW7S4GM(2019)). Cultivation practices were performed as per Tanney et al., 2023. Cuttings were grown in 3 cm pre-soaked rockwool cubes and under vegetative conditions for four weeks: 18-hour photoperiod at 150 µmol $m^{-2}$ $s^{-1}$, 20-22 °C, and 65% relative humidity. Plants were in flowering conditions for eight weeks: 12-hour photoperiod at 150 µmol $m^{-2}$ $s^{-1}$, 20-22°C, and 65% relative humidity. Stim Root No. 2 (Master Plant-Prod Inc., Brampton, ON, Canada) was applied to cuttings and the producer recommended values of VeloKelp nutrient solution (pH 5.6, Remo Nutrients; Remo Brands Inc., Maple Ridge, BC, Canada) were used in the vegetative period. The nutrient regimen throughout the flowering period included MagNifiCal, Micro, VeloKelp, Grow at pH 6.3, and Remo Nutrients as per manufacturer guidelines and Lyu et al. (2022). The same amount of nutrients were used for the first 7 weeks, with only water given in the final week of development. Plants were spaced 1.5 feet apart to minimize competition. Methods for fixation and imaging are identical to methodology applied to Cannara Biotech sourced material.

The cultivars grown at Cannara Biotech, Terple (TER), Early Lemon Berry (ELB), and Triple Burger (TBU), were used for all objectives of the thesis. Female *Cannabis sativa* L. plants were grown from cuttings sourced from mother plants at the Farnham facility of the company. The facility employs fertigation for daily watering, but nutrient regime and scheduling are proprietary information. Cuttings develop roots for 14 to 21 days in rockwool cubes with HPS lighting and high humidity before being transplanted in a larger rockwool cube for vegetative growth. Vegetative growth occurs in a separate room for 2 to 3 weeks, and then the plants are moved to flowering rooms. Each flowering room has 1000 individual plants on 5 rolling tables with each plant having at least 6 inches spacing between individuals. Netting is placed around and through the tables to avoid entire crop or individual branches falling due to inflorescence overweight. Inducing flowering requires the change of photoperiods, but the exact temperature, humidity, and

lighting of each designated room is proprietary information. Once the batch of plants in a flowering room has reached an adequate number of days, entire rooms are harvested at one time. The netting is delicately removed, plants are cut at the base of the stem to remove the rockwool blocks, and the remaining material is hung to be dried. Cannara uses rolling drying racks that can hold up to 50 plants at one time, and plants hang vertically with cut stem upwards and flowers pointing downwards. After a preliminary removal of the main stalk, individual plant branches are classified according to the grade of their inflorescence. Branches holding premium grade buds are hand trimmed and branches with medium grade flowers are processed through large cannabis trimming tumblers to remove excess leaves. Determination of quality considers aesthetic aspects of cannabis including the size of the inflorescence, density of calyces, and appearance of trichomes.

Sampling occurred from day 40 of flowering to the harvest date to ensure adequate inflorescence and trichome development in all growing conditions. The same room of a single cultivar was sampled on six to nine evenly spaced occurrences based on the flowering schedule. With sterile clothing, plant trimming scissors, and labeled bags, one inflorescence was taken from the top, middle, and bottom portions of three randomly selected plants within one room. The top inflorescence sampled was always the apical inflorescence of the plant, the bottom buds were sourced from the lowest 2 branches, and the middle buds were sampled from in between the apical and bottom buds. The Triple Burger cultivar initially began cultivation at the Farnham facility, but after Cannara Biotech acquired a new facility in Salaberry-de-Valleyfield, QC, Triple Burger production was moved. The two facilities differ in size, plants per room, lighting and air movement enough to be considered different growing conditions for experimentation purposes. Therefore, only two groups of TBU could be investigated for this project. Samples were weighed for recording before being separated for analysis, and individual buds were divided for HPLC analysis or imaging. The samples waste was destroyed as per Health Canada regulations.

Determination of Trichome Density

Organ pieces 1 mm to 5 mm in size were delicately removed from inflorescences. Individual calyces have the most trichome development when compared to bracts (Tanney et al., 2021) and can be removed without disrupting the structures. Surface areas measuring 1 $mm^2$ of the calyces were captured using Aven Cyclops 2.0 Digital Microscope at 35x lens magnification and corresponding software. ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/, 1997-2008) was used to count trichome density for fresh cannabis samples. For each date of sampling, at least 10 images were taken of each sampling location, and for each set of images, 5 high quality images were randomly chosen (*n=5).* Imaging was repeated with 3 independent groups of each cultivar over the 8-month period.

Analysis of Post-Harvest Methodology

For analysis of post-harvest methodology, the final Terple growing room was imaged after drying but prior to treatment, after being hand trimmed, and after being machine trimmed. Samples labeled 'without manipulation' or 'control' used very delicate and slow removal of inflorescence portions, broken down into small pieces, and prepared carefully for cannabinoid analysis. Hand trimmed plants were cut with hand trimmers rapidly and brushed with the silicone brush to mimic the rapid processing of material by a hand trimmer. Machine trimmers were loaded with a few randomly chosen plants, turned at the recommended speed and pressure for obtaining a clean product. Photography focused on trichome structure and density before and after the common product trimming methods. Samples will be analysed with HPLC potency testing after imaging to determine if there is a significant impact on metabolic content after manipulation.

Quantification of Cannabis THC Content

THC content refers to cannabinoid concentration (% dry weight) of the sample. To obtain the values and prepare the samples, the freshly sampled inflorescence is hand-trimmed further to remove as much trichome-free material as possible. Maintaining the

division of samples based on the location on the plant (top, middle, bottom), the inflorescence is cut into smaller pieces with a handheld cutter. The ground material is dried by microwave for 10 minutes at 70% strength, and then again in a convection oven for 10 minutes to ensure removal of water. At least 0.800 g of tissue is loaded into a Cole-Parmer HG-400 MiniG Bead-beater Homogenizer and ground into a powder; 0.200 g of powder is mixed with 20mL of an 80:20 methanol:dichloromethane solution. The mixture is centrifuged and diluted 100x with 100% methanol before analysis. Three technical replications of each test were performed, and the corresponding THC values collected are an average of the three values. Cannara Biotech employs a validated method for the quantification of 11 cannabinoids in fresh cannabis samples with an Agilent 1290 Infinity Ultra High-Performance Liquid Chromatography (UHPLC). Though CBD is analyzed, the focus of the metabolic analysis is THC, and thus CBD values will not be considered.

Sample Preparation for Scanning Electron Microscopy

Individual calyces and bracts were sampled from each cultivar, noting the time and location of each sample point. Sampling and chemical fixation procedures occurred at room temperature. Three fixation methods were tested. In Method 1, pieces were submerged in two mL of 3.5% v/v formaldehyde in 0.025 M PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid)) buffer with a pH of 7.0. Samples were placed in a rotor for slow agitation for 30 minutes followed by three rinses with 0.025 M PIPES buffer. Samples underwent ethanol ascensions of 30, 50, 70, 80, 95, and 100% for 30 min each, with three additional 100% ethanol rinses. Method 2 employed 3 mL of formaldehyde to 27 mL of glacial acetic acid buffer, commonly known as the Formalin Acetic Acid fixative (FAA) with a buffered pH of 7.0. These samples were rinsed with ethanol after an initial 30-minute rotation, and due to the presence of ethanol within the formalin glacial acetic acid (FAA) buffer, the ascension was as follows: 60, 70, 80, 95, and 3x 100% ethanol rinses. For Method 3, samples were fixed with 100% methanol. Samples rotated for 5 to 10 minutes, and the 30-minute ethanol ascensions included 30%, 50%, 70%, 80%, 95% and 3x 100% ethanol rinses. All chemically fixed cannabis samples were critical-point dried with solvent-substituted $CO_2$ (Leica EM CPD300, Leica Microsystems, Concord, ON, Canada)

after ethanol rinses. Samples were mounted on aluminum stubs with carbon mounts and rotary coated with 4 nm gold layer (Leica EM ACE200, Leica Microsystems, Concord, Canada).

Imaging

Fixed and coated samples were imaged under vacuum with a Hitachi TM-1000 scanning electron microscope operated at 15 kV (Hitachi Ltd., Chiyoda City, Japan) and a backscattered electron detector. Stereomicroscopy imaging was used between fixation steps to inspect the physical status of the samples. The material was imaged fresh, after the first fixative rotation, and after the ethanol rinses on a clear petri dish lid under darkfield conditions (0.63x, 2.5 optivar; Zeiss SteREO Discovery V8, Carl Zeiss Canada Ltd, Toronto, ON, Canada). All microscopy work occurred at Macdonald Campus' Multi-Scale Imaging Facility. Images taken of trichome covered material are manipulated within ImageJ to primarily count individual trichomes for colour changes or density or to analyse the structural state of trichomes.

Statistical Analysis

All data sets were analyzed using the statistical software GraphPad Prism 7. Trichome density statistical analysis was done by two separate Kruskal Wallis (repeated) one-way ANOVA tests: one concerning on the independent variables cultivar and one concerning the location. A linear regression was applied to analyze the trend in trichome density over the change of time (Table 1). A linear regression was applied to analyze the trend in the change in percentage of milky-white trichomes per the total trichome density over the flowering period (Table 2). For THC content analysis, a repeated one-way ANOVA test compared the means between location of samples on the plan. A linear regression was applied to analyze the trend in THC content % per dry weight over the change of time (Table 3). Triple Burger 2505 data were not included in the calculations due to the change in conditions.

## Results

Trichome Bulb Density & Colour Change

In order to monitor the development of trichomes on cannabis inflorescences over time, bract surfaces were imaged starting at 40 days, at which trichome development is known to be well underway and ended at the decided harvest date. Of the three cultivars imaged, Terple (TER), Triple Burger (TBU), & Early Lemon Berry (ELB), trichome density was consistent in range and random distribution (Figure 2). Trichome density varied between 17 per $mm^2$ and 55 per $mm^2$ during the flowering periods (Figure 2). To analyze the non-normally distributed data, a Kruskal-Wallis H test was applied for determining if cultivar or location of the sample impacts trichome density. The means between cultivars have a significant difference ($p < 0.05$) when comparing ELB or TBU to TER. The density values at each time frame were similar for ELB and TBU, while TER demonstrates consistently lower trichome density values. The apical and middle region inflorescence did not have a significant difference in trichome density, but the lower region inflorescence had a significantly lower average values, suggesting that trichome development is lower in this area. Once divided into blocks of cultivar and location (Figure 2), a slightly increasing trend over time can be seen in the top and middle regions, while the lower region appeared to remain constant over time. When a linear regression was applied, the correlation values were insignificant for each group ($R^2 < 0.5$)(Table 1). Each regression had a positive rate of increase but very low values ranging between 0.589 % THC (ELB Top) and 0.002 % THC (TER Bottom) (Table 1).

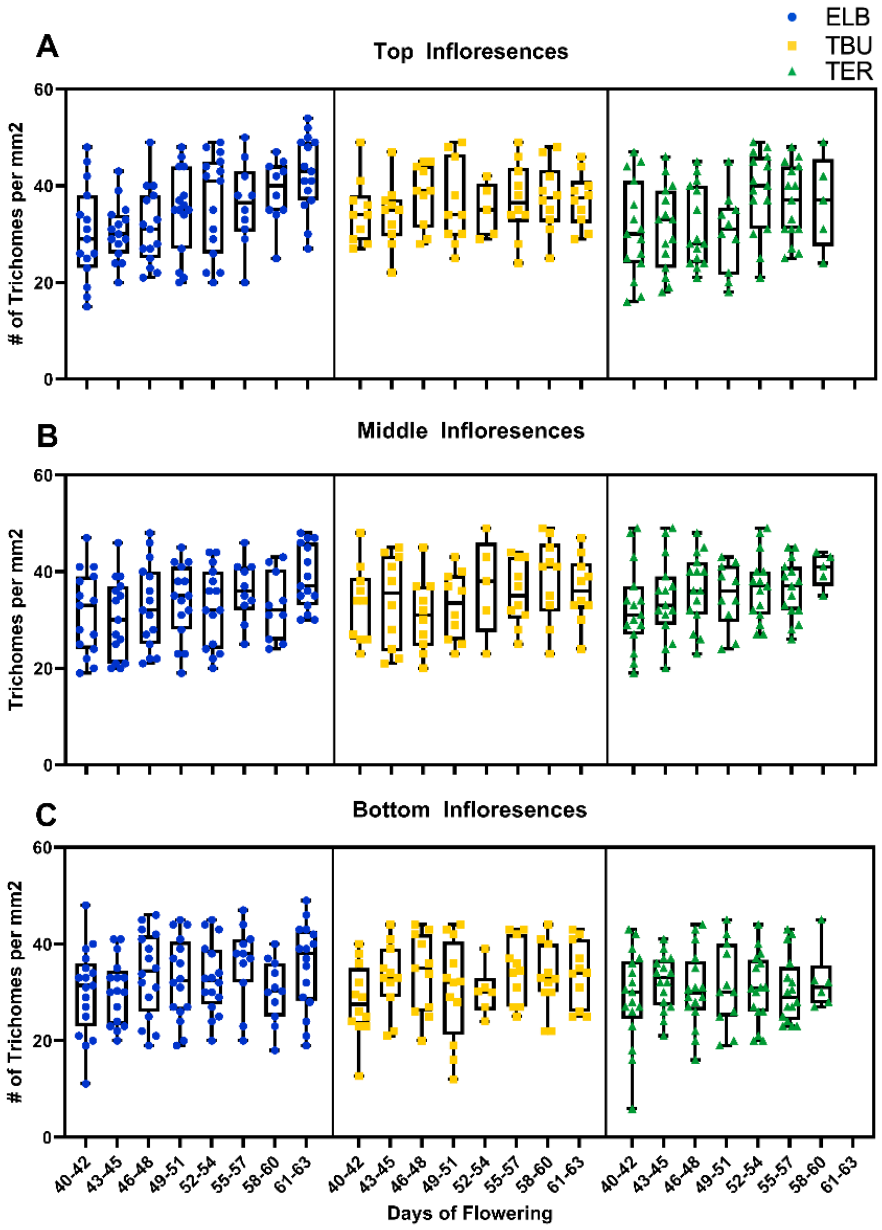


**Figure 2**. Trichome density on cannabis calyx. Each data set is divided by cultivar, and each data point represents the number of trichomes in a 1 $mm^2$ region of interest. Box plots indicate median values with 25% quartile ranges. Inflorescences were taken from the apical (A), middle (B) or lower (C) part of the plant.

**Table 1. Average glandular trichome count per 1mm$^2$ area of three cannabis cultivars**

| Cultivar | Location | Linear Regression | $R^2$ |
|---|---|---|---|
| ELB | Top | Y = 0.5887*X + 4.297 | 0.1957 |
| | Middle | Y = 0.2987*X + 18.02 | 0.0670 |
| | Bottom | Y = 0.1521*X + 24.92 | 0.0165 |
| TBU | Top | Y = 0.1240*X + 29.80 | 0.0170 |
| | Middle | Y = 0.2406*X + 22.05 | 0.0472 |
| | Bottom | Y = 0.1211*X + 26.31 | 0.0136 |
| TER | Top | Y = 0.4444*X + 11.30 | 0.0787 |
| | Middle | Y = 0.2823*X + 21.35 | 0.0473 |
| | Bottom | Y = 0.0017*X + 30.13 | 1.783e-006 |

**Table 1.** Average glandular trichome count per 1mm$^2$ area with standard deviation of three cannabis cultivars: Early Lemon Berry, Triple Burger, and Terple. A linear regression was applied to analyse the trend in data, and the $R^2$ values are compared to a significance level of 0.5.

Capturing the shift in metabolite content in the trichome bulb, as indicated by the opaque white deposition, involved considerable scrutiny. The trichome structure is devoid of colour until the metabolites accumulate within the bulb. When imaged, a collection of trichomes may overlap, causing transparent trichomes to appear opaque or to have a discolouration while immature. To focus on the white colour shift, Early Lemon Berry trichomes were analyzed beginning at the 40-day period with an understanding of possible inaccuracies due to the limitations of the imaging approach on the densely populated bract surfaces. The location of the sample had a significant impact on the rate of metabolic deposition (Table 2). At each location on the plant, the percentage of milky-white trichomes increased over time with similar y-intercept values (Figure 3). However, trichomes on the middle inflorescence demonstrated the milky-white discolouration shift at an increased rate compared to the other two locations but also showed a higher variability. For the apical and middle area inflorescences, the ratio milky-white:transparent trichomes increases as the number of milky-white trichomes increases, but the number of transparent trichomes is consistent. The bottom inflorescence, delayed in development, increases in transparent trichomes at a rate higher than the milky-white trichomes. Over half of the trichomes at harvest remain transparent at the harvest date for the bottom inflorescence, while the rest of the plant will have over 65% of the trichomes turned milky-white. Further, the apical inflorescence demonstrates the beginning of senescence through a slight increase in amber trichomes.

**Table 2. Average percentage of milky-white glandular trichomes from the total trichome count**

| Location | Linear Regression | $R^2$ | p-value |
|---|---|---|---|
| **Top** | Y = 0.0775*X + 3.99 | 0.7633 | <0.0001 |
| **Middle** | Y = 0.0904*X + 4.656 | 0.6565 | <0.0001 |
| **Bottom** | Y = 0.0610*X + 3.142 | 0.7558 | <0.0001 |

**Table 2.** Average percentage of milky-white glandular trichomes from the total trichomes per 1mm$^2$ area of cultivar Early Lemon Berry. A linear regression was applied to analyse the trend in data, and the $R^2$ values are compared to a significance level of 0.5. False Discovery Rate controlled by Prism software.

The cultivar Terple is harvested at 59 days of flowering, and after this time, few trichomes bulb had initiated senescence. The one group of Terple analysed at the 63-day point had an increased rate of 12.4% of the trichomes darkened to amber. The cultivar Triple Burger is harvested at 63 days of flowering but also displayed few bulbs, if any, that turned to amber by the harvest day. Early Lemon Berry trichome bulbs began turning amber in small numbers at 57 days, with more obvious colour changes between 61 and 63 days. However, the average number of bulbs that underwent senescence was 5.4 per $mm^2$, which is less than 15% of the average number of trichomes for ELB for the final flowering days (Figure 3). Prior to the senescence point of colour change, isolated trichomes may be discoloured, but otherwise bulbs remained transparent or milky-white. Isolated, darkened bulb color was found to be associated with physically damaged trichomes and thus did not reflect the overall maturity of the plant.

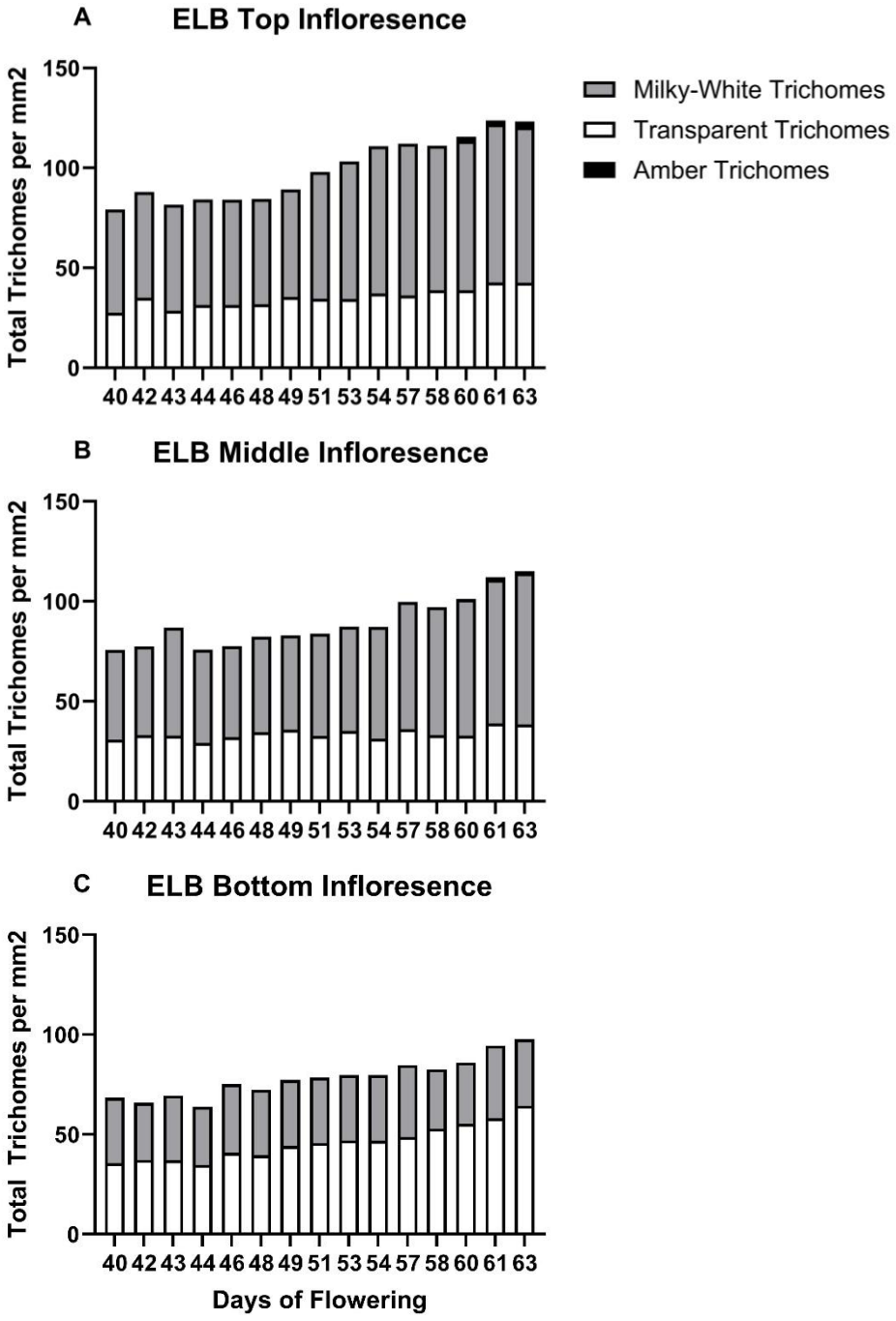


**Figure 3.** Ratio of transparent trichomes to milky white trichomes to amber trichomes out of the total trichome density on a calyx from the Early Lemon Berry cultivar. Inflorescences were taken from the apical (A), middle (B) or lower (C) part of the plant.

After Cannara Biotech's hang-drying process, trichome bulbs appeared clear with a concentration of yellow metabolites around the secretory cells (Figure 4). The slight yellow discolouration occurred in bulbs that had not yet begun senescence. No metabolite testing was done to correlate colour change during drying with the change in metabolite composition.

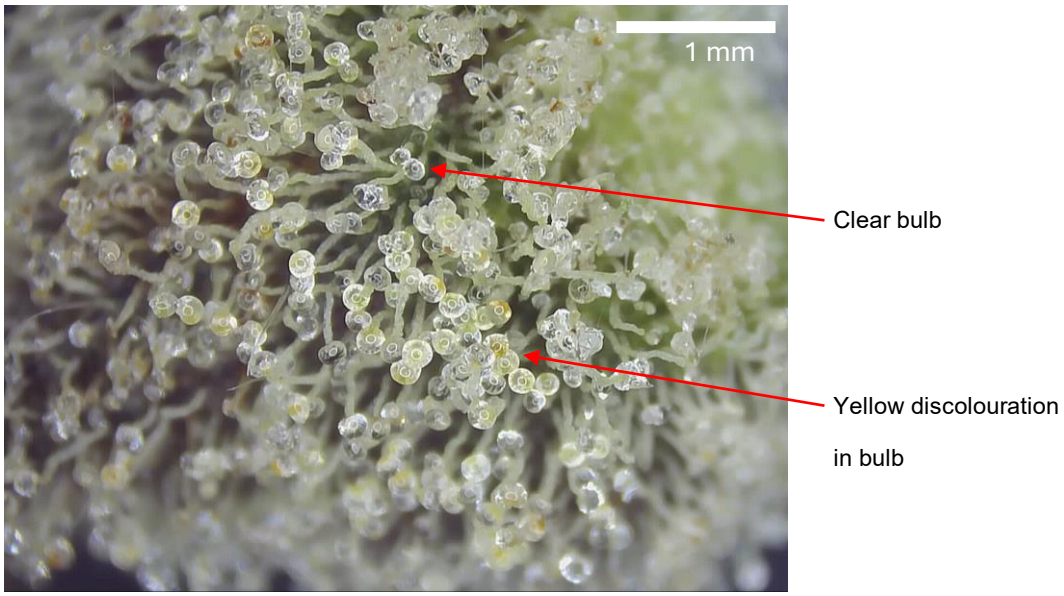


**Figure 4**. Stereomicrograph showing slight yellow discolouration of trichome bulbs after drying process concentrated around the secretory cells. Red arrows point to clear and discoloured bulbs.

Changes in Cannabis THC Content during Development

Previous research has determined cannabinoid content is dependent on cultivar type (Hazekamp & Fischedick, 2012; Hazekamp & Tejkalová, 2016). When analysing Cannara Biotech's cultivars, ELB and TBU cultivars produced overall higher THC content than TER, consistent with the information provided by the seed producers. On average, THC content of ELB and TBU was 10.3% and 10.8% higher than TER, respectively (Figure 5); these values compare the THC content of inflorescence located in every region of the plant, but it is well understood that there is variation among locations of inflorescence sampled within a given plant (Massuela et al., 2022). The authors note that for the cultivar examined, the inflorescences taken at the top of the plant had a CBD

concentration of 9.9% ± 0.5%, compared with 8.2% ± 0.5% for the middle and 7.4% ± 0.5% for the bottom inflorescences. As shown in Figure 5, ELB and TER followed the same trend with the THC concentration decreasing with lower position of the inflorescence on the plant. The middle-sourced inflorescence from the TBU cultivar averaged over 1% higher than the top bud, but this value had a higher standard deviation (0.97% higher). Overall, the standard deviations were extremely high, ranging from 0.30% (TBU Top; Figure 5A) to 3.92% (TER Top; Figure 5A) suggesting that there is high variability in cannabinoid content among plants within the same growing room. Linear regressions applied to the changing THC content over time demonstrates the high variability similarly. It is well understood that cannabinoid content increases over the flowering period until senescence, and Massuela et al. (2022) analyzed the cannabinoid content of inflorescence after four different harvest points: 5, 7, 9, and 11 weeks. The cannabinoid trend described by Massuela et al. (2022) indicated a relative increase of 1.06% between 5 to 7 weeks, an increase of 0.01% between 7 to 9 weeks, and a decrease of 0.89% between 9 to 11 weeks. However, the standard deviation for the cannabinoid values is between 0.37% to 0.77%, suggesting that the parabolic trend also has high variability among samples (Massuela et al., 2022). In Table 2, the $R^2$ values for the linear regression of the THC content over time for ELB and TER were all lower than 0.20, regardless of location. Figure 5 visually displays the high variability, while the low $R^2$ values statistically exhibits the inability to assume linearity of THC content over time. The $R^2$ values for TBU are higher: 0.72 (Top), 0.84 (Middle), and 0.47 (Bottom); a linear model could be applied to the top and middle inflorescence THC content. Due to the change in growing conditions, the third group of TBU could not be tested for metabolic content, and thus *n=2* or *1* for each flowering period.

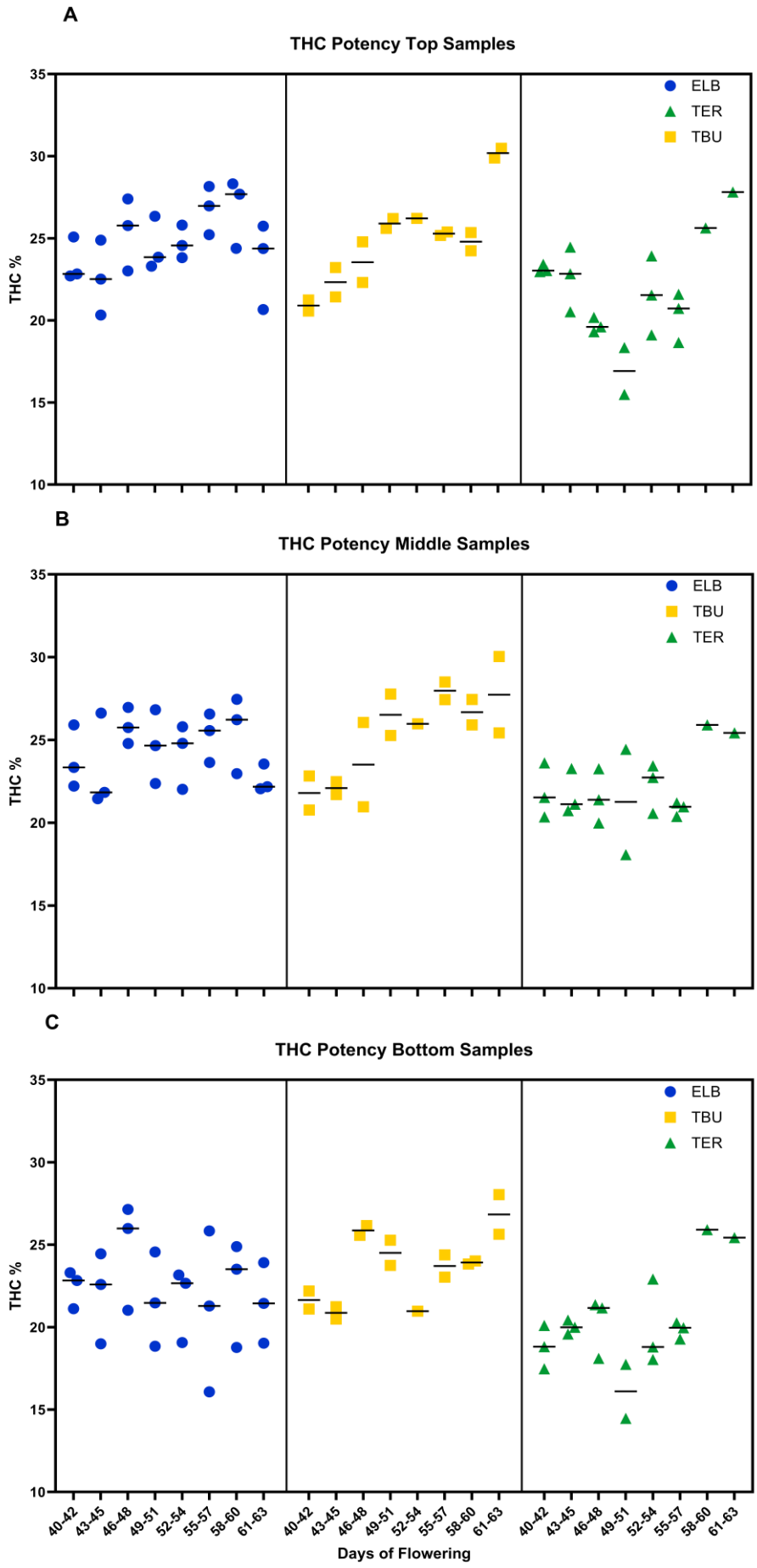


**Figure 5**. Change in %THC per dry weight over time determined by HPLC. Each data point represents the average of 3 replicates. Triple Burger has only 2 growing groups tested due to environmental condition changes. Buds harvested from the apex of the plant (A), the middle region (B) and the bottom region (C) One-way ANOVA, (p-value<0.05) was used to determine statistical differences.

**Table 3. Average THC % per dry weight of three cannabis cultivars**

| Cultivar | Location | Average and Std Dev at Harvest (THC %) | Linear Regression | $R^2$ |
|---|---|---|---|---|
| ELB | Top | 26.14 ± 1.63 | Y = 0.1049*X + 19.32 | 0.1265 |
| | Middle | 24.40 ± 2.24 | Y = 0.003623*X + 24.21 | 0.0002 |
| | Bottom | 23.41 ± 1.45 | Y = -0.04960*X + 24.72 | 0.0177 |
| TBU | Top | 30.19 ± 0.30 | Y = 0.3275*X + 8.035 | 0.7220 |
| | Middle | 31.27 ± 1.23 | Y = 0.4453*X + 3.188 | 0.8338 |
| | Bottom | 26.84 ± 1.21 | Y = 0.1960*X + 13.42 | 0.4673 |
| TER | Top | 22.40 ± 3.92 | Y = 0.06118*X + 18.52 | 0.0192 |
| | Middle | 22.33 ± 2.21 | Y = 0.09861*X + 17.16 | 0.1051 |
| | Bottom | 21.12 ± 1.43 | Y = 0.1339*X + 13.15 | 0.162 |

**Table 3.** Average THC % per dry weight with standard deviation of three cannabis cultivars: Early Lemon Berry, Triple Burger, and Terple. A linear regression was applied to analyse the trend in data, and the $R^2$ values are compared to a significance level of 0.5.

The metabolite profile also changes following harvest, during the drying process (Figure 6). Each batch's THC content changed inconsistently from the harvest to the 2 weeks of hang drying point, regardless of cultivar or location (Figure 6). The greatest loss was observed in inflorescences taken from the middle position of Terple plants. This

sample exhibited a decrease by 5.01% THC during the drying period (Figure 6). Variation exists among the metabolite values for each subset (cultivar and location), and thus it is possible for the cause in variation post-drying to be a result that is more influenced by these factors rather than the drying process. The existence of a metabolic change after a drying period is important to understand the full impact of the post-harvest methods.

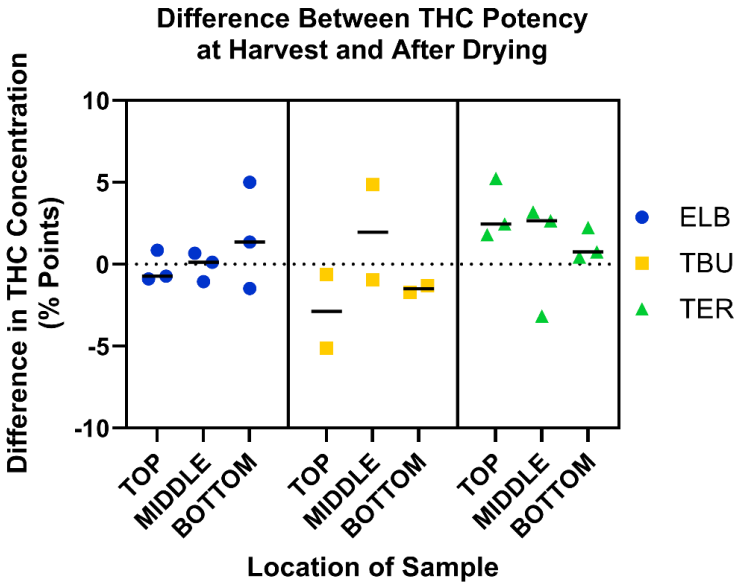


**Figure 6.** Difference between THC % at harvest and THC % after the drying period. Each data point represents the difference for a singular growing group. Positive values indicate an increase in THC after 2 weeks of hang drying, while negative values indicated a decrease in THC after 2 weeks of hang drying.

Effects of Post-Harvest Methods on the Structural Integrity of the Cannabis Bud

To compare the impact of two different, commonly employed, post-harvest methods, they were applied to TER material which was then analyzed for THC content and damage to the dried inflorescence and trichomes. After 7 days of drying, the average THC % with standard deviation was 20.23 ± 3.28% in material that was trimmed delicately with hand-trimmers and a close attention to avoid damaging trichomes. Material treated with only the tumbler method averaged 17.44 ± 1.34%, and material treated with only the silicone brush method averaged 15.88 ± 2.08%. Due to the high variance, the difference of means is not statistically significant, but there was a notable decrease in THC content

for both post-harvest methods compared to minimal hand trimming. 3 potency tests per trimming treatment were completed and further analysis is required to validate these preliminary results. Further, the samples were not divided based on location on the plant, and thus the values represent an average of the entire plant. The evaluation of THC values separated by an inflorescence's location on one plant suggests that the method of using a singular inflorescence to represent metabolic values of a plant or growing room is inaccurate. Imaging by the inspection scope demonstrated damage to the outermost and protruding structures on the outside of the dried bud. Entire calyx faces showed total trichome loss in areas that had come in contact with the machinery or silicone brushes (Figure 7). Machine trimming caused more tears to the material and removal of entire trichomes or bulbs when compared to the hand-trimming with brushing (Figure 7). Although the THC analysis showed a high variation among the location of samples, there is evident damage to trichome structures and the consequential removal of trichome bulbs. Trichome stalks become more delicate and brittle after the drying process, and there is a negative impact on the retention of the trichome structures by either post-harvest methodologies.

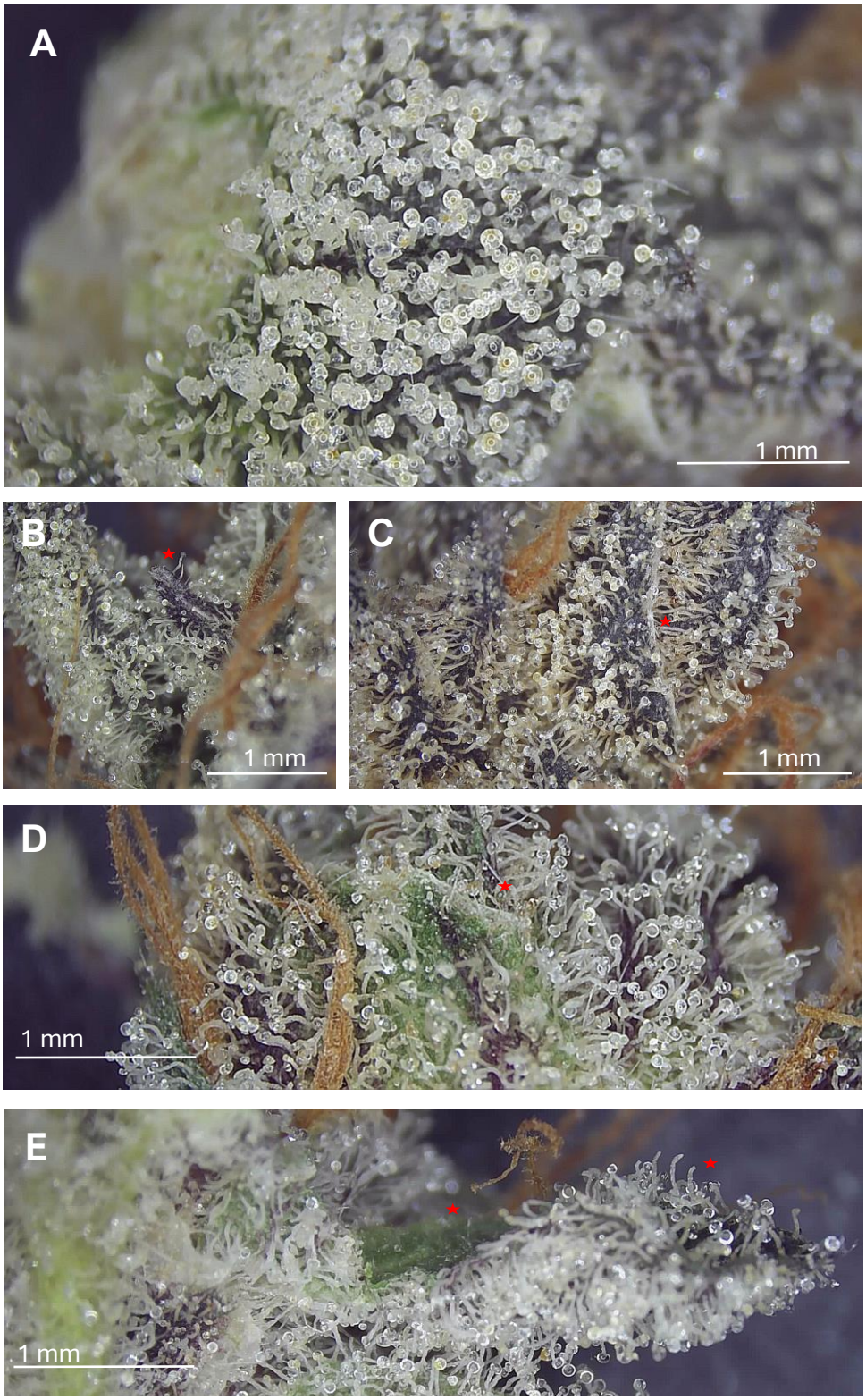


**Figure 7**. A comparison of images of Terple cannabis calyx material that has undergone different post-harvest treatments. Processing refers to the removal of excess plant material that is not trichome dense, like sugar leaves and stems. (A) Terple material that was processed extremely delicately with hand trimmers to avoid any trichome disturbance. (B & C) Terple material that was processed by Cannara workers with hand trimmers and silicone brushes. (D & E) Terple material that was processed by a machine tumbler and then by Cannara worker with hand trimmer and silicone brushes. Red stars indicate locations of destruction, focusing on loss of trichomes within an area, loss of trichome bulbs, and tearing of the calyx material.

Structural conservation of cannabis trichomes under different sample preparation methods for SEM

In order to optimize the preparation of cannabis material for SEM by chemical fixation, three variations of sample preparation methodology were tested for the degree of structural conservation (Figure 8). Qualification of adequate fixation looks for flaws in epidermal cells, such as loss of turgidity or wall crimping (Yuan et al., 2023). The formaldehyde and methanol-based methods produced a turgid and consistently shaped epidermal layer on the cannabis material (Figure 8). When prepared with the FAA solution, cells demonstrated some wrinkle-like deformation (Figure 8D). Further analysis of fixation of delicate tissues looks for open, turgid, stomatal structures, and each fixative produced strong, clear stomatal openings (Figure 8A & 8C).

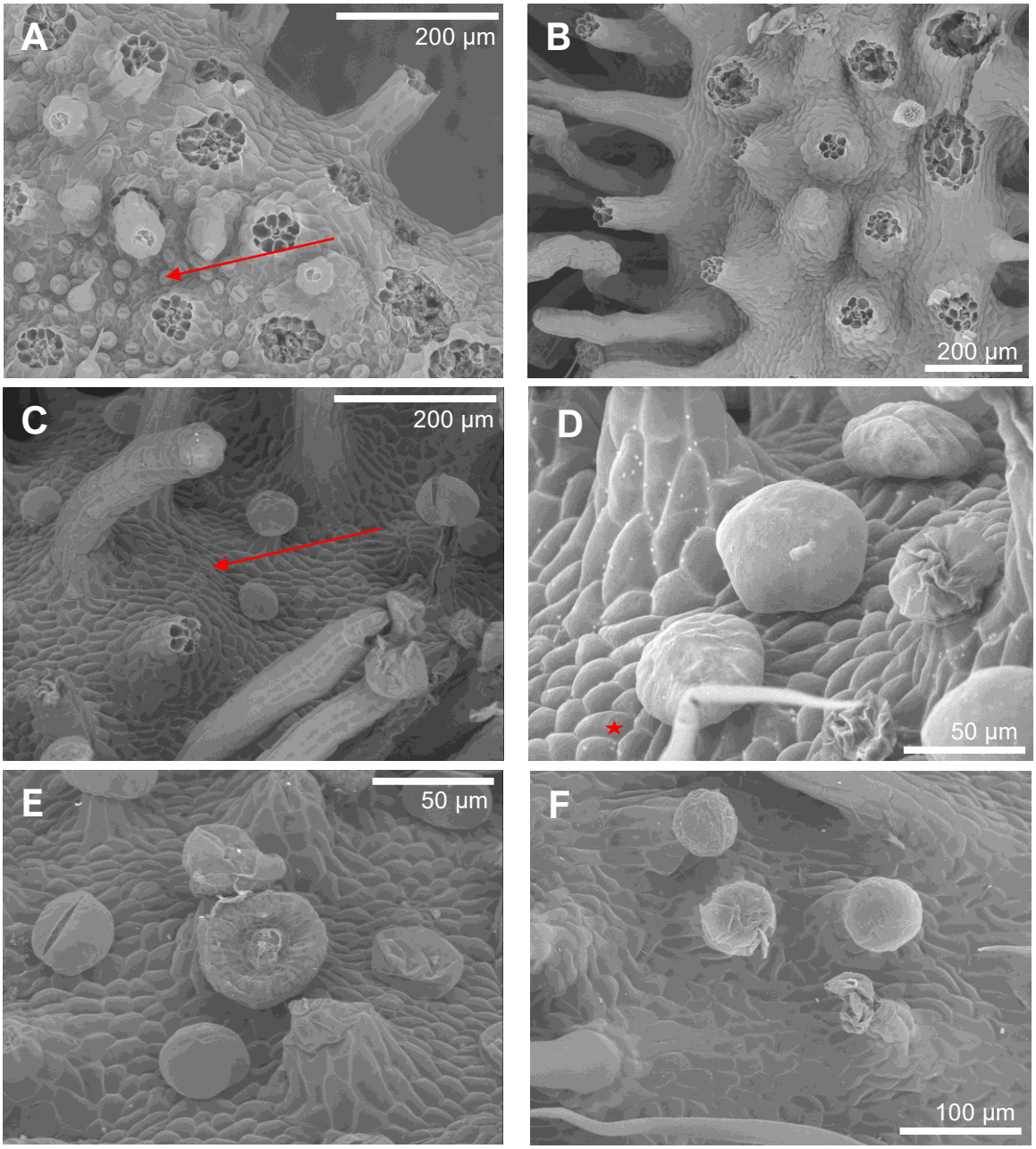


**Figure 8.** Scanning electron micrographs of surface of cannabis calyx material after preparation with 3 different chemical fixatives followed by critical point drying and sputter coating. (A & B) Methanol fixation (C & D) Formalin Acetic Acid fixation (E & F) Formaldehyde fixation. Damage to the epidermis is denoted by red stars. Stomatal structures are visible in images A & C, as indicated by a red arrow.

Regardless of fixative applied, there was a negative correlation between trichome stalk length and structural preservation of stalk structure (Figure 9A). There was significant wrinkling, desiccation, and loss of structural integrity to longer stalks. Stalks longer than 400 µm were almost always damaged with desiccation or deformity, regardless of fixative. However, the methanol-fixed material resulted in further damage to the trichome stalks with entire removal from the epidermis or truncated stalks with low cuticle retention. While the epidermis was adequately preserved, the damage to the trichome stalks was prominent with methanol fixation in comparison to the formaldehyde-fixed material. Most notably, after samples have undergone the entire preparation process, trichome bulb cuticles rarely remained intact. On most samples, less than 5 trichome bulbs remained intact on the entirety of the calyx, with many samples had only 1, 2 or 0 whole bulbs (Figure 9C & 9D). To understand which step in the fixation process the trichome bulb cuticle was torn, material was imaged in a stereo microscope prior to fixation, after fixation/before ethanol ascensions, after ethanol rinses, and after critical point drying and sputter coating (Figure 10). Stereo-imaging demonstrated the presence of intact cuticle at all sample preparation stages, as shown by the discoloured bulbs throughout (Figure 10A-C). Understanding that the damage apparently occurred within the critical point dryer or the sputter coater, samples were imaged without the thin metal coating. While the images had issues with contrast due to lack of conductivity, trichome bulb cuticles were abundant (Figure 10E & 10F). The younger or shorter trichome structures maintained better, similar to when sputter coating was used, but without coating, there were many intact cuticles (Figure 10E & 10F). Whole cuticles attached below the secretory cell structures without the tearing seen in other samples. The images of samples that had not undergone sputter coating failed to generate adequate contrast from the lack of conductivity. Without the thin metal layer, the sample will fail to reflect the electrons adequately, resulting in electron absorption by the material. A ‘charged’ sample with electron accumulation causes error in image capture due to an abundance of signals. For high resolution and high contrast imaging, sputter coating is essential.

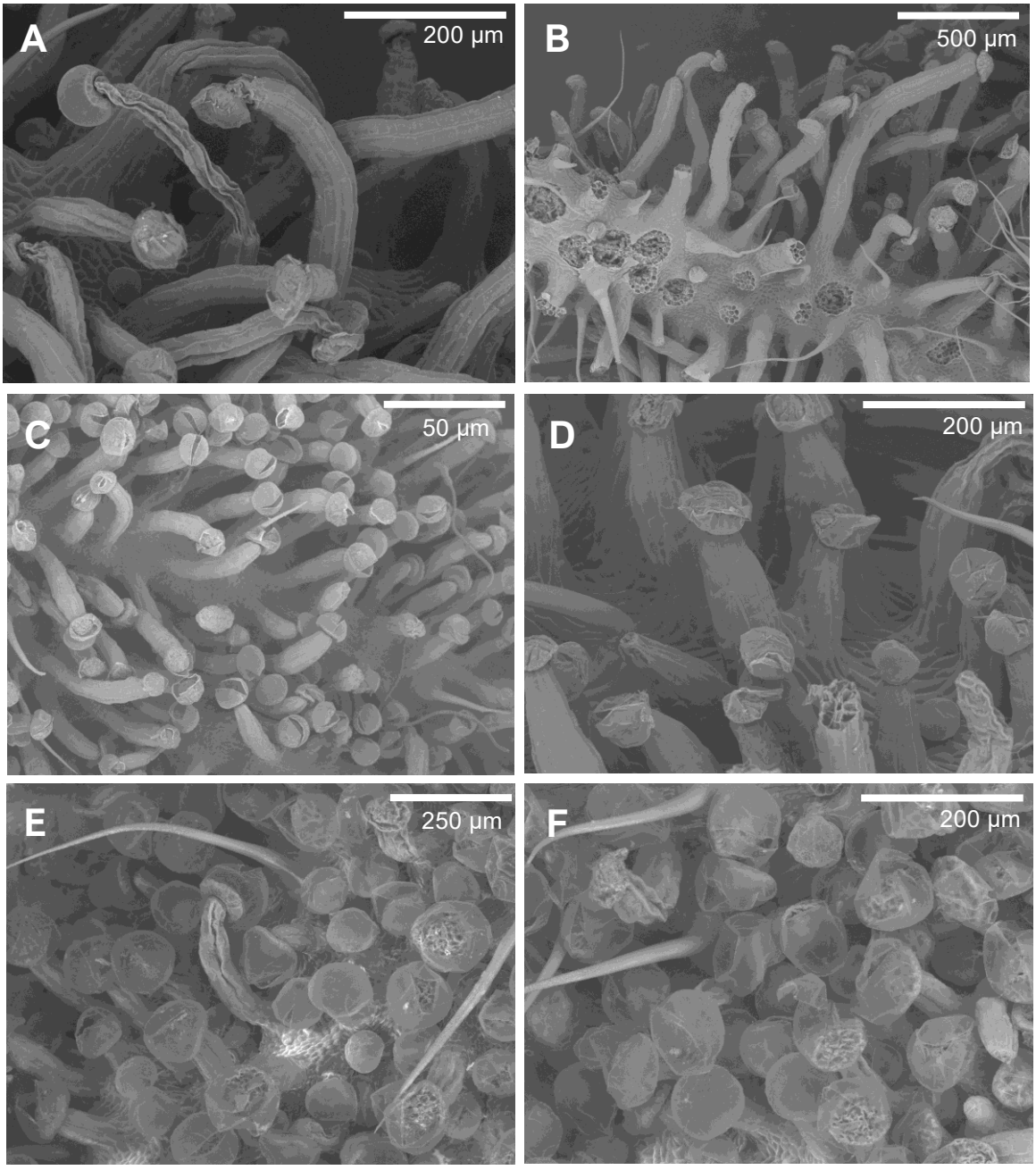


**Figure 9**. Scanning electron micrographs of surface of cannabis calyx material after preparation with 3 different chemical fixatives followed by critical point drying and sputter coating. (A) Formalin Acetic Acid fixation (B) Methanol fixation (C & D) Formaldehyde fixation. (E & F) Formaldehyde fixation with critical point drying and no sputter coating.

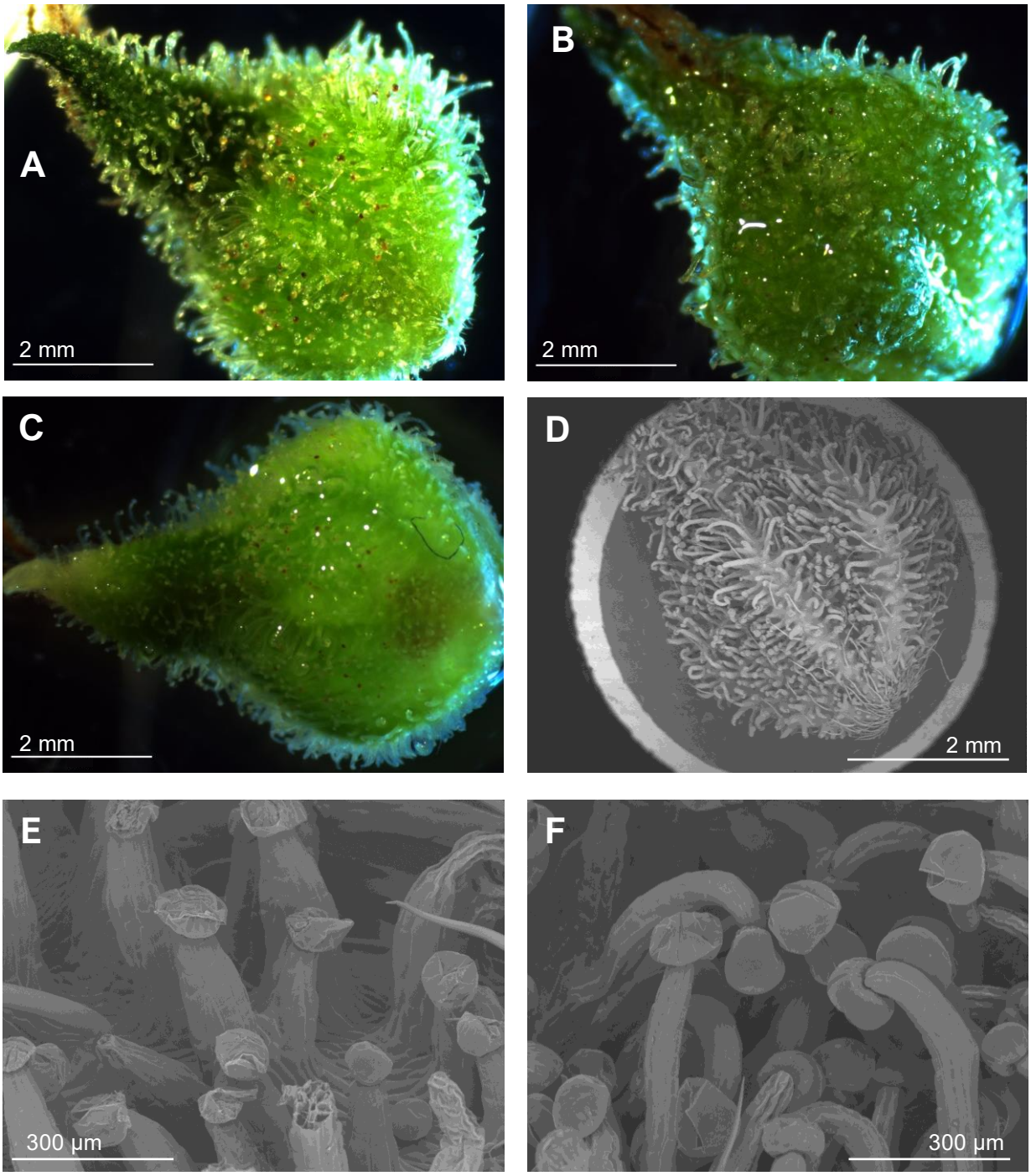


**Figure 10.** A comparison of images of cannabis calyx material that has undergone the stages of preparation for Scanning Electron Microscopy. (A) Material that has been imaged with Zeiss SteREO Discovery V8 stereomicroscope immediately after harvest with no manipulation. (B) Material that has been imaged with Zeiss stereomicroscope after formaldehyde and PIPES fixation. (C) Material that has been imaged with Zeiss stereomicroscope after treatment with formaldehyde and PIPES buffer, and the water in the sample has been replaced with ethanol through rinses. (D, E, & F) Scanning electron micrographs of material that has been treated with formaldehyde and PIPES buffer, dried with Critical Point Dryer, sputter coated, and imaged by Hitachi TM-100.

## Discussion

Considerations for Future Trichome Imaging

Cannabis trichomes can be seen with the naked eye as fluffy hairs on the surface of plant organs, but for their structural analysis, the minuscule cannabinoid producers need to be magnified at least 10x. The method of capturing images with a 2-megapixel sensor and LED illumination at 30 to 50x magnification was the simplest way to capture the entire calyx organs for image analysis at the Cannara Biotech facility. Imaging for trichome density requires a clear, distinguishable surface area to record data; obtaining an area 1 mm by 1 mm in focus requires stereomicroscopy or an imaging method with high resolution. However, the shape of the trichome-dense plant organ, the calyx, is not flat; it has folds and protrusions. With trichome stalks of different lengths, imaging a larger epidermal surface for the purpose of counting trichomes may require observation on more than a single focal plane. Further, the transparent nature of the trichome structure can create difficulty in the correct classification of metabolite content. Overlapping trichomes within the dense collections caused by the calyx shape may cause some trichome bulbs to appear opaque without any metabolite accumulation. Variation among trichome counts could therefore be attributed to the changing angles of imaging, a lack of consistency among samples, and the transparent nature of the trichome complex. For calyx analysis, a composite of multiple images at varying focal planes is optimal or the use of stereomicroscope, as seen in Tanney et al. (2023).

Recent work with Transmission Electron Microscopy and SEM (Livingston et al., 2022 & 2023) has demonstrated the value of electron microscopy in imaging the morphology of trichomes. Of the three methods selected for chemical fixation of cannabis material here, the use of formaldehyde fixative with the PIPES buffer proved best for conservation of structures. In comparison to methanol as a fixative, formaldehyde better maintains structural integrity and epidermal turgidity, likely due to a better compatibility of the solution with the cell turgor and improved fixation action. The number of cuticles that stayed attached was higher in formaldehyde treated material, but complete intact glandular bulbs were uncommon with all methodologies. Rather than this being due to the chemical fixation method, the damage to stalked bulbs seems to be introduced after the fixation and rinsing steps. Imaging using stereomicroscopy demonstrated that up until

critical point drying, there are intact trichome bulbs on most samples. If not damaged during chemical fixation or dehydration, damage to cuticles must occur during critical point drying or sputter coating. The critical point drying using $CO_2$ involves application of a pressure of 1072 p.s.i. and the cuticle may not be able to maintain under said pressure, causing it to burst. Alternatively, it may be the agitation that sometimes occurs during gas exchange that causes the cuticles to rupture by mechanical action. Ethanol and glutaraldehyde are known to remove lipids or weaken the integrity of thin plant tissue (Kiyoto et al., 2022), leaving the cuticle very susceptible to damage under the critical point drying or sputter coating conditions. However, other species' trichomes and delicate structures have been shown to be maintained throughout the drying process (Yuan et al., 2023; Iriti et al., 2006). Trichomes with intact cuticles observed in the cannabis material prepared here were often highly protected by the natural shape of the calyxes. Bulbous and short stalked trichomes were more likely to retain the cuticle and bulb structure than the extended trichomes. To determine the cause of the damage to the bulbs' cuticles, samples were imaged without sputter coating. The difference was drastic, with most trichome stalks maintaining the cuticle, either as an attached strip of material, a torn bulb, or as a full bulb. Yet, due to the lack of metal coating, the contrast in the SEM was poor. There was no impact to the stalk structures with the lack of metal coating; longer trichome stalks were still more likely to become twisted and distorted. The stalks thin towards the base of the bulb, reducing structural integrity with excess trichome length. The further a structure protruded from the epidermal surface, the more likely mechanical damage occurred. As trichome length is a question of leaf maturity, younger material would likely be more suitable for capturing more intact trichome structures. Avoiding the use of a sputter coater, but optimal imaging would require lowering the voltage, an option that was not available in the SEM used. Sputter coating is an essential aspect of the SEM sample processing for biological material, but as the cause of the damage to the trichome bulbs, it is imperative to find another method of generating charge within the sample without sputter coating. Damage to samples is not uncommon, with the possibility of tears in the cuticles by stray gas ions or metal particles hitting the delicate surface with force. Using SEM for trichome counting and classification of trichome types after chemical fixation is therefore not as suitable as stereomicroscope imaging of live samples (Livingston et al.,

2020), but it does provide the ability to investigate the cellular structures in more detail. High pressure drying is likely the best methods of cannabis sample preparation for the retention of trichome bulbs. Freeze-drying removes frozen water from a sample by sublimation, employing extremely low temperatures within a vacuum chamber to gently remove the water over a period of 24 to 48 hours (Das et al., 2022). While both freezing methodologies require expensive and not easily accessible machinery, maintaining the trichome bulbs with the chemical fixatives and critical point drying is unlikely due to the delicate cuticle bulb cover and the lipid metabolite content of the bulbs.

Determining Harvest Time & the Impact of THC Variations

In corporate cannabis cultivation, the harvest date is based on many factors including the information provided by the seed/genetics provider, intended purpose of the crop, and organization of the production plant. Many producers conduct in-house HPLC testing to ensure the cannabinoid content meets the expectations and projected harvest date provided by the seed vendors. My data suggest that the trichome colour shift from transparent to the opaque white is a consistent indication of an aging plant, while THC content is less predictable. All 3 cultivars demonstrated a lack of a statistically significant trend in THC content. The THC values, separated by location of inflorescence on the plant, increased slightly over the flowering period, without indicating a plateau or negative shift associated with over-development. The lack of reduction of THC content may suggest that peak cannabinoid content has yet to occur at the 63-day harvest point, but this hypothesis cannot be confirmed with the data collected. Metabolic content variation in cannabis plants is a novel trait being investigated genetically (Adamek et al., 2022), and thus a wide cannabinoid range is not uncommon among inflorescences at different locations on a given plant. Determining the average cannabinoid content of the harvest should be done by HPLC on pooled samples from various locations on the plant and various placements of the plant in the growing space to accurately gauge a batch. Visual analysis of trichome discolouration provides a proxy measure for the maturity of the crop but cannot confirm whether the desired cannabinoid content has been reached.

Trichome density analysis and THC content are both characteristics of cultivar type, as determined by the data and previous research (Hazekamp & Fischedick, 2012). For this project, only one cultivar was analyzed for the milky-white shift in bulb colour, but likely this aspect of the trichome development is applicable to all cultivars (Sutton et al., 2023). The change is determined by time of flowering rather than cultivar, but analysis of other cultivars would be able to confirm this hypothesis. Further, an investigation into the metabolic shift well past the point of harvest and even past total senescence would be beneficial to determine the impact of over-maturity on cannabinoid and terpene content. The amber darkening of trichome bulbs is more obvious and visible to the naked eye, thus providing a better visual indication of the changing metabolic content. However, the hypothesis claiming that peak cannabinoid content can be determined by analyzing the ratio of transparent:white:amber trichomes has yet to be proven.

### Trichome Development through the Production Process and the Impact of Post-Harvest Manipulation of Cannabis Flowers on THC Content

Throughout the flowering period, a trichome that has completed development of the bulb grows through stalk extension. The bulb maintains the spherical, globus shape throughout metabolite deposition, maintaining turgidity with adequate watering practices and absent physical damage. There is no way to determine the maturity of a cannabis plant using the shape of the trichome bulb as a reference. It may be possible to use the stalk length as a measurement for plant maturity, as the stalk continues extending throughout development. An investigation well past trichome senescence would determine if there is a finite height at which stalks stop elongating. Due to the organic nature of calyces, imaging would need to capture the trichome from the side rather than face on as this project has done. Similar to the white deposition within the bulbs, it is likely that the stalk length is an indicator of maturity rather than a characteristic of cultivar.

Regardless of the drying method chosen for reducing the moisture content of the cannabis flower, there is an impact on trichome morphology. Trichome stalks lose cell turgidity during drying, becoming contorted and thinner. SEM images and digital images demonstrate this change in structure (Figures 7 & 9), further confirmed by Livingston et al. (2022 & 2023) imaging. The bulb maintains a spherical structure, but the support to

the bulb by the stem lessens with water loss. The impact of drying on THC content is less obvious and few published articles exist describing the relationship (Das et al., 2022). Metabolic analysis of the cultivars determined the lack of consistency between inflorescence locations on the plant, and thus variations are expected. The HPLC testing post-drying and without manipulation revealed a change in THC values after harvest compared to prior to harvest, but the direction and magnitude of change is inconsistent. Previous research demonstrates the differences in cannabinoid content throughout flowering (Massuela et al., 2022) but fails to address a shift after harvest. Further research is required into the metabolic shifts once the cannabis plant has been dried.

The decision to use hand trimming or machine trimming is another choice by cannabis producers that involves many variables like timing, costs, and ease of production (Das et al., 2022). Trichome damage or removal should be of concern when considering trimming methods, and hand trimming with the silicone brush may generate similar levels of damage as those inflicted by machine trimming. The major indications of damage are areas without trichomes on calyx material or distinct tearing of outermost protrusions (Figure 7). Tumbling the delicate dried material within a moving grater removes entire areas of trichome development (Figure 7). Similar patches of destroyed trichomes were found on hand trimmed, brushed material. Between the friction of the silicone material and speed at which the brush is applied, it is likely that trichomes are removed in concentrated areas, yielding tissue that closely resembles that treated with the tumbler method. Hand trimming with extreme care for the preservation of trichomes is the only method tried that provided nearly undamaged cannabis flowers. Obtaining the trained labour force to trim the cannabis at a rapid pace without using aggressive methods of trimming is a big challenge for producers. Conflict arises when consumers look to trichomes as indication of quality for a cannabis product, and the widespread damage by both methods could negatively impact a producers' image (Donnan et al., 2022). The cannabis industry is largely influenced by consumer opinion, and thus the use of damaging post-harvest methods does not align with consumer desires. To avoid damaging the product and aiming to provide the highest quality product, Cannara Biotech delicately hand-trims inflorescence intended for sale as whole inflorescence. Informing consumers on what constitutes a good product and how trichomes appear throughout the

flowering process is an important aspect of cannabis education. The goal of cannabis education is to ensure consumers can make a knowledgeable decision that benefits everyone involved in the production system, and cannabis research about trichome development and testing is the first steps towards an informed public.

**Conclusion**

Understanding the process of cannabis maturation is an essential aspect of the cannabis production system but much foundational research remains to be done. It is well known that the darkening of the cannabis glandular trichome bulb indicates senescence, but it is unclear whether the milky-white discolourations are indicative of peak THC content. The data confirmed that trichome development is indicated by the deposition of metabolites, but the THC content was too variable to establish a correlation. Image analysis of trichome density demonstrated the impact of cultivar type and location of the inflorescence sampled, while bulb discolouration is a common trait among all cannabis cultivars. Capturing an entire calyx face with all trichomes in focus was not possible by inspection microscope, but scanning electron microscopy provided high resolution images of the material. The chemical fixation methodology for sample preparation has yet to be optimized, and thus the comparison of three chemical solutions is a step towards an improved fixation process that maintains the trichome bulbs.

Potency testing of the same cannabis material revealed that the location of the inflorescence on the plant also impacts THC % values. Not only does the cannabinoid content change during flowering, but the drying and post-harvest processes can change the inflorescence potency. The glandular trichome loses structural support with a desiccated stalk, and the use of a tumbler or silicone brushes during inflorescence processing damages the dried inflorescence tissue and removes entire trichomes. Quality in cannabis has different meanings between producers and consumers, with many consumers unsure of what an inflorescence at the correct maturity for peak cannabinoid content would look like. Education and research about cannabis trichomes throughout the flowering period and within industrial production helps to promote a better relationship between consumers and producers and allows consumers to make safer, educated decisions.

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