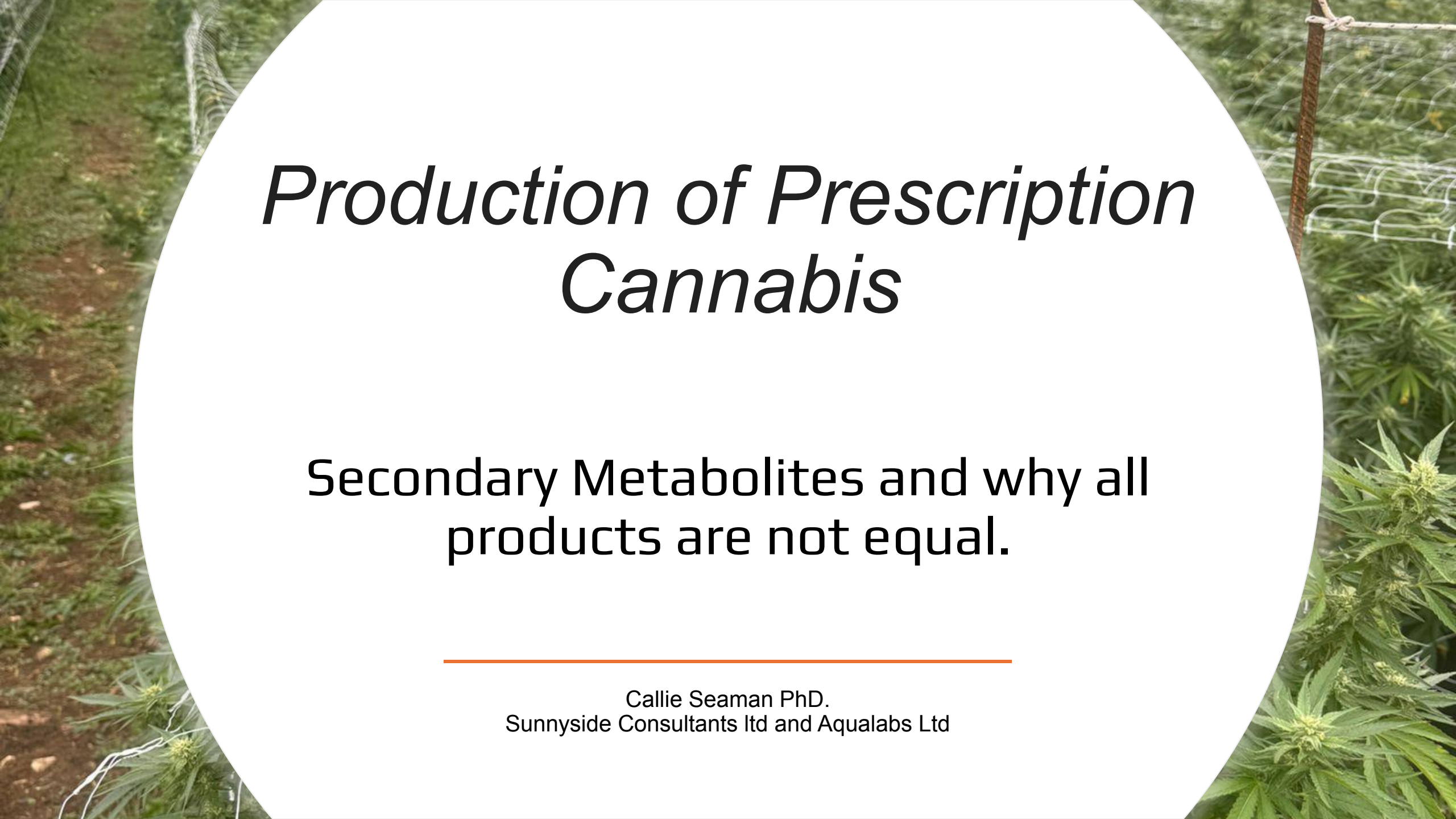


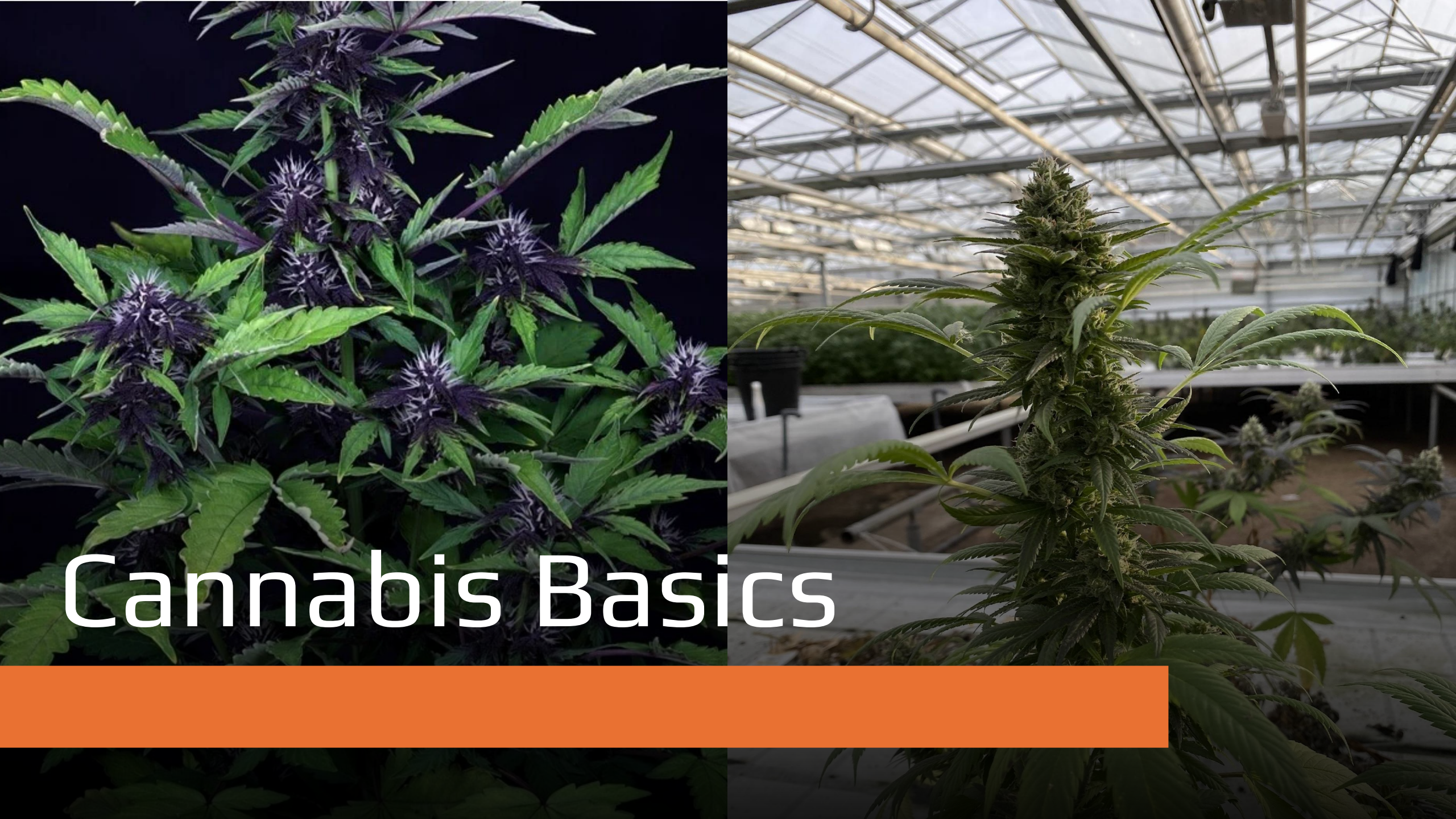
Production of Prescription Cannabis

Secondary Metabolites and why all products are not equal.

Callie Seaman PhD.
Sunnyside Consultants ltd and Aqualabs Ltd

Content

- Cannabis basics: Anatomy & Habitat
- Active Ingredients: Cannabinoids, Terpenes & metabolic pathways
- The Process of Producing a medicine
- Cultivation Methods & Extraction
- The Standards
- Patient expectation
- Conclusion & Q&A



Cannabis Basics

Hemp vs Cannabis

- **Hemp**
  - Industrial use to produce fibers, animal bedding, building materials, clothing and seeds.
  - Different licensing
  - Can Not extract from
- **Cannabis**
  - Produced for its flowers and oils that contain cannabinoids such as CBD and THC.
  - Controlled substance

Cannabis the Plant

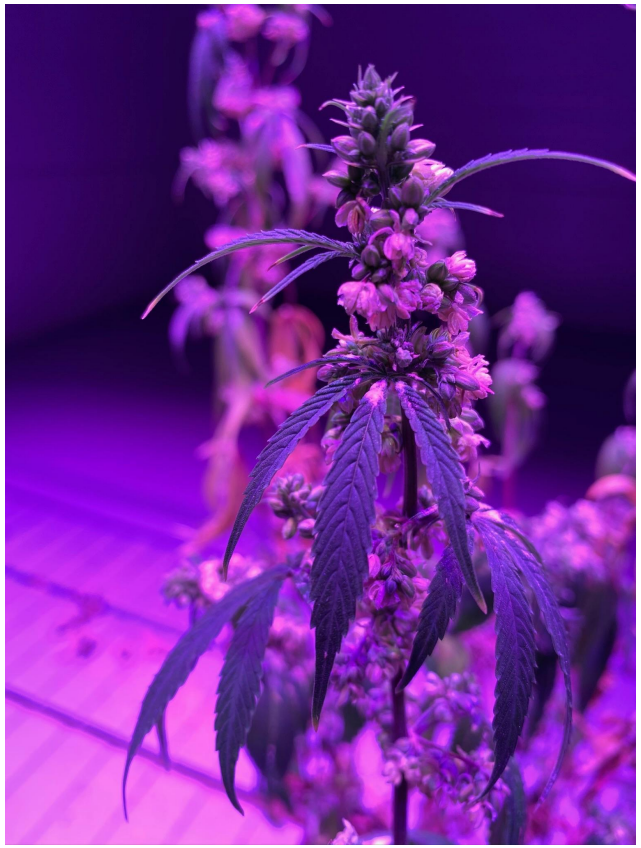
- Three Species
  - Sativa
  - Indica
  - Ruderalis - Auto-flowers
- Hybrids
- Photoperiod plants - Short day flowers
- Dioecious – Male and Female
- Phytoremediator

Native Habitat

- Has adapted to grow around the world.
- Sativa – subtropical
  - East Asia
  - Central America
- Indica – tropical climate
  - Afghanistan
  - Turkey
  - India
  - Pakistan

Chemovar's / Chemotypes

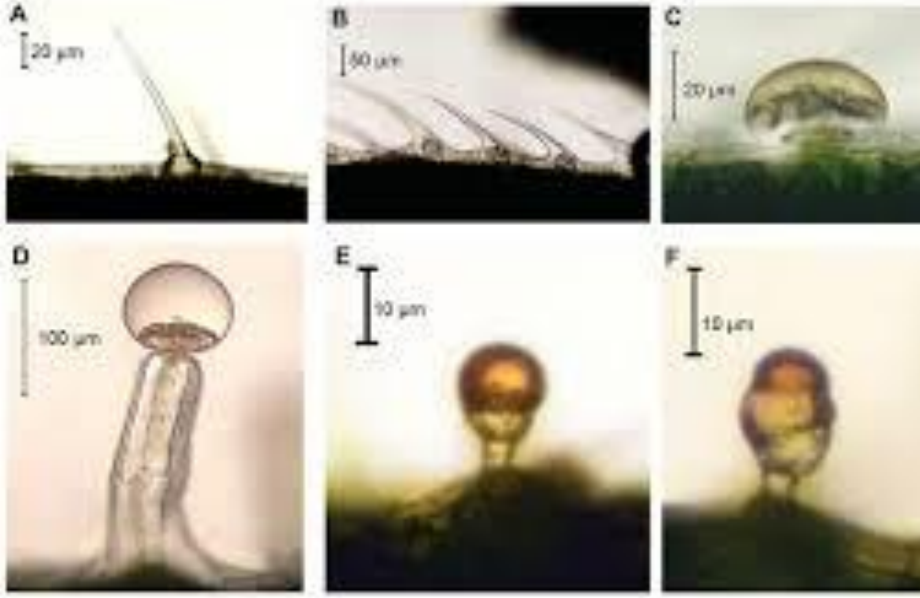
Type I	Type I –THC-dominant with a concentration of >0.3% and CBD content of <0.5%.
Type II	Type II – Equal THC:CBD - a mixed ratio profile with CBD and THC in varying moderate concentrations, almost always presenting as CBD-forward
Type III	Type III – High CBD, low THC content that provides little to no intoxication.



The Flower

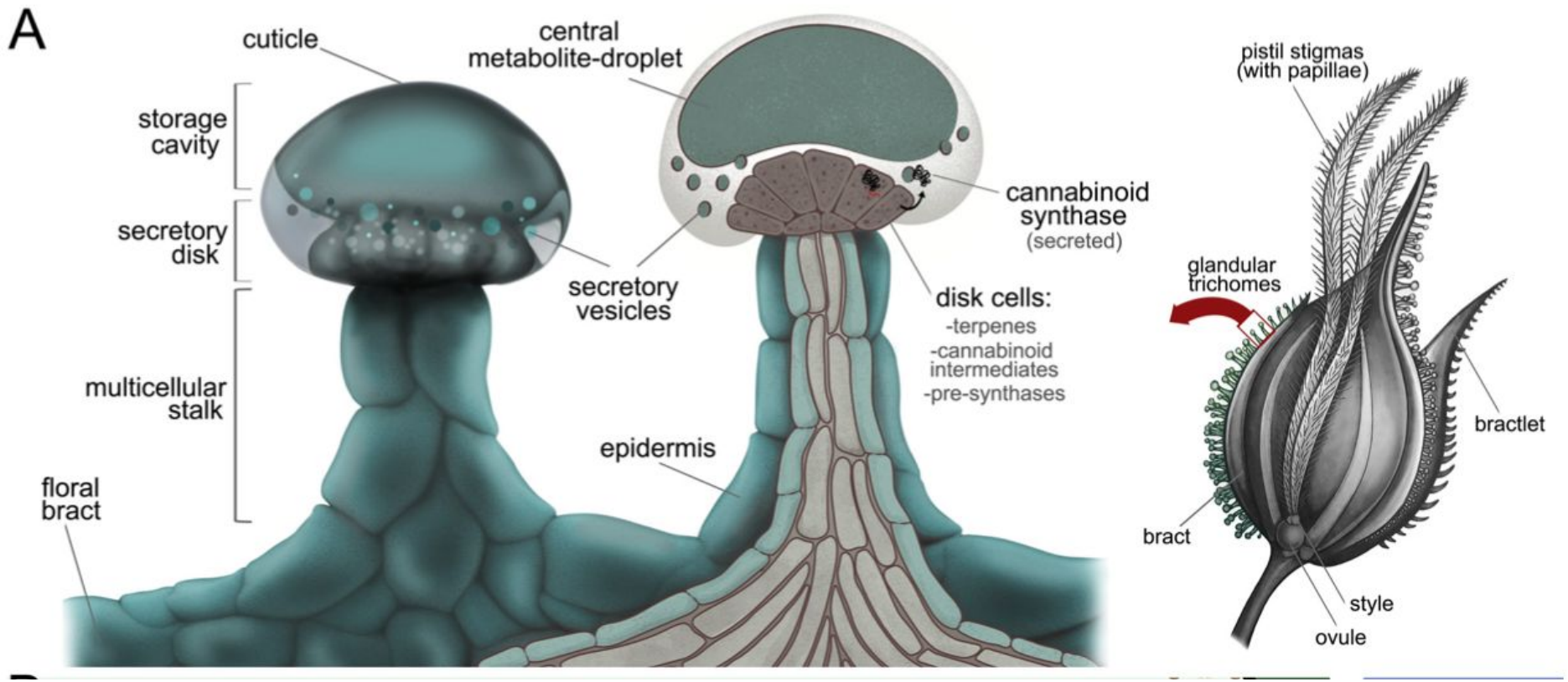
Trichomes

- Glands on the leaves
- Site of cannabinoid and terpene production.
- 6 types



Hemp trichome types. **(A)** Unicellular non-glandular trichome; **(B)** cystolythic trichomes; **(C)** capitate sessile trichome; **(D)** capitate-stalked trichome; **(E)** simple bulbous trichome; **(F)** complex bulbous trichome

The Antimony of Trichomes



Active ingredients

The Polypharmacology

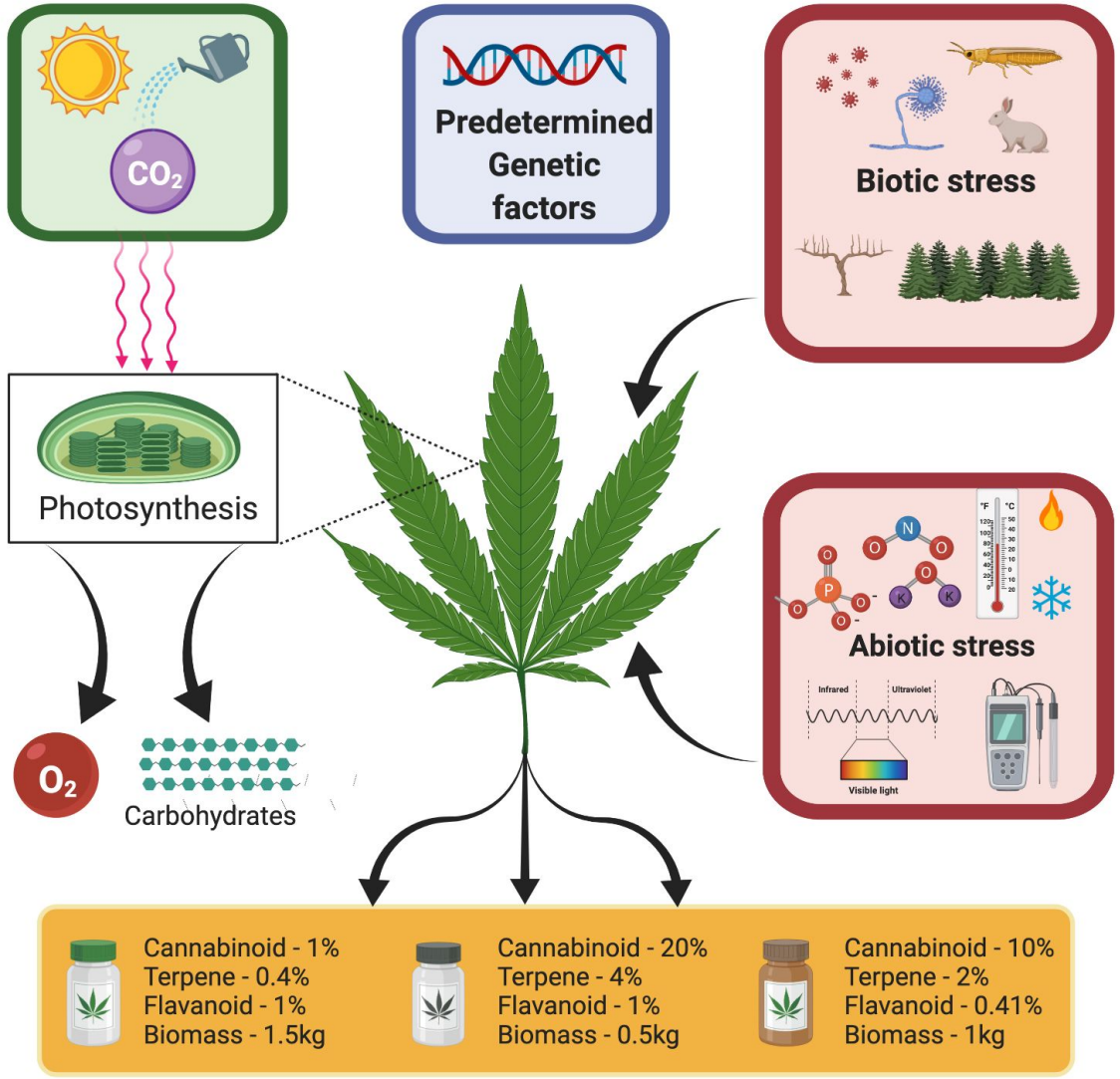
Phytochemicals

- These are secondary metabolites.
- Made up of 100s of medicinal compounds including:
  - Cannabinoids - 147
  - Terpenes - 200
  - Flavonoids - 20
  - Amino acids - 21
  - Fatty Acids
- Many different strains/cultivars
- Entourage effect
- One size does not fit all!

Why are Terpenoids produced?

- Stress
- Defense
- Repair
- Reproduction

•Ref: Seaman, C., 2021. Cultivation Stress Techniques and the Production of Secondary Metabolites in Cannabis sativa. In *Recent Advances in the Science of Cannabis* (pp. 1-30). CRC Press.



JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Evolution of the Cannabinoid and Terpene Content during the Growth of *Cannabis sativa* Plants from Different Chemotypes

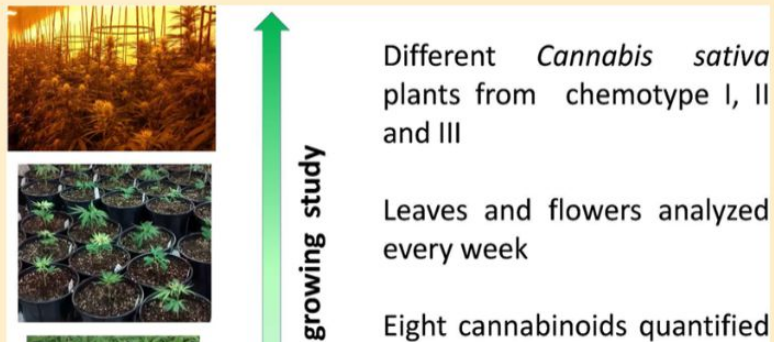
Oier Aizpurua-Olaizola,[†,‡] Umut Soydaner,[†] Ekin Öztürk,[†] Daniele Schibano,[†] Yilmaz Simsir,[†] Patricia Navarro,[‡] Nestor Etxebarria,[‡] and Aresatz Usobiaga*[,‡]

[†]Aifame GmbH, Tüfi 450, 9105 Wald-Schönengrund, Switzerland

[‡]Analytical Chemistry Department, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Spain

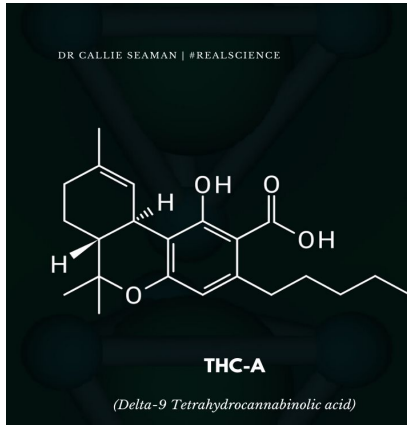
Supporting Information

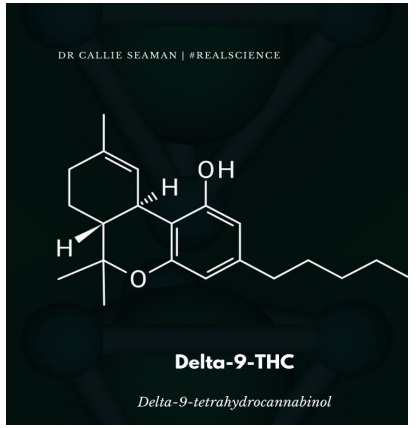
ABSTRACT: The evolution of major cannabinoids and terpenes during the growth of *Cannabis sativa* plants was studied. In this work, seven different plants were selected: three each from chemotypes I and III and one from chemotype II. Fifty clones of each mother plant were grown indoors under controlled conditions. Every week, three plants from each variety were cut and dried, and the leaves and flowers were analyzed separately. Eight major cannabinoids were analyzed via HPLC-DAD, and 28 terpenes were quantified using GC-FID and verified via GC-MS. The

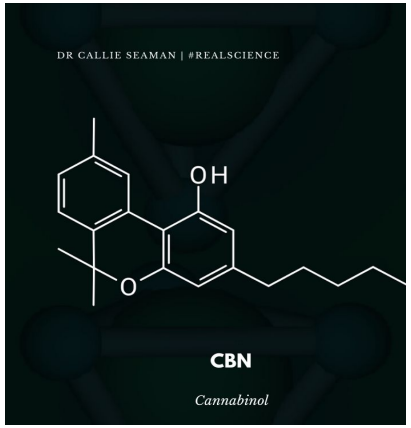


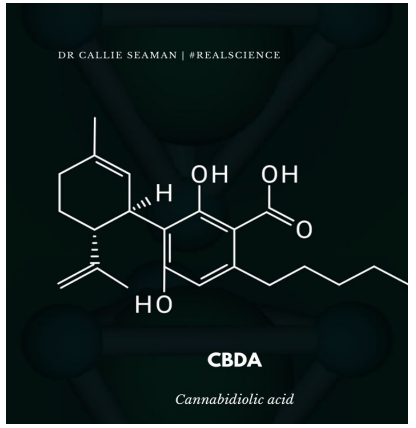
Cannabinoids

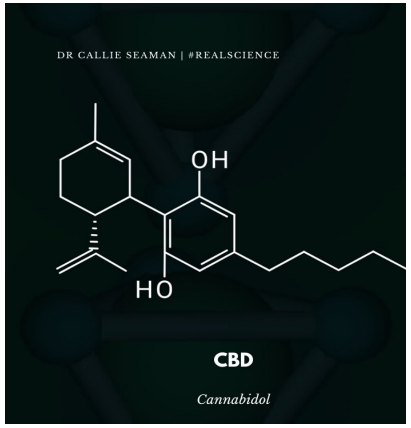
- 147 different ones
- Major – < 1%
  - THC-A
  - CBD-A
- Minor - > 1%
  - CBG
  - CBN
  - THCV
  - CBC
- Produced in Trichomes
- Found predominately in the acid form in the plant
- Effects
  - Analgesic
  - Anti-inflammatory
  - Anti-seizure
- Properties
  - Lipophilic



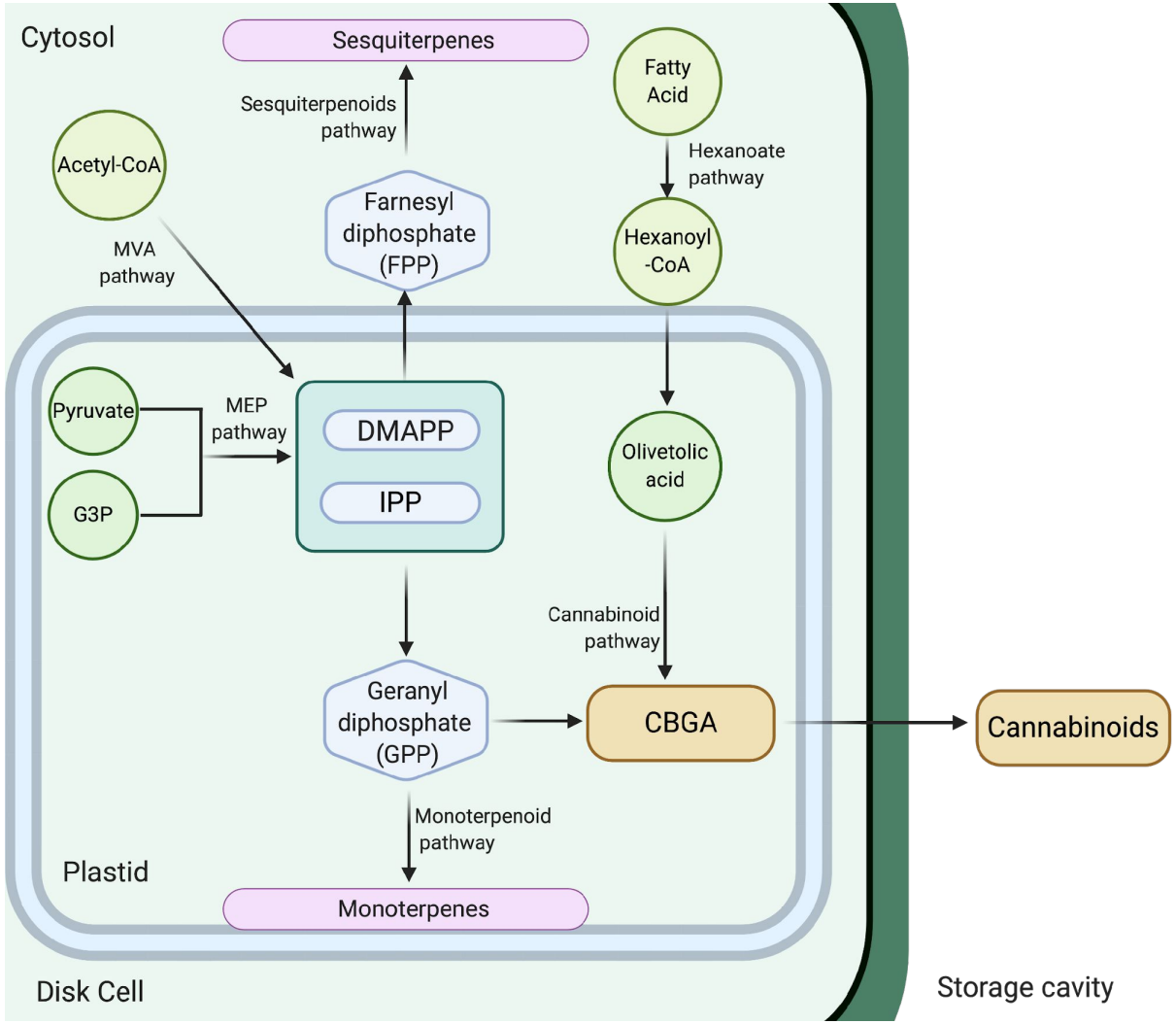








Biosynthetic pathway in the plant



Ref: Seaman, C., 2021. Cultivation Stress Techniques and the Production of Secondary Metabolites in Cannabis sativa. In *Recent Advances in the Science of Cannabis* (pp. 1-30). CRC Press.

Metabolism of cannabinoids

Gene Networks Underlying Cannabinoid and Terpenoid Accumulation in Cannabis[1][OPEN]

Jordan J. Zager,[a] Iris Lange,[a] Narayanan Srividya,[a] Anthony Smith,[b] and B. Markus Lange[a,2,3]

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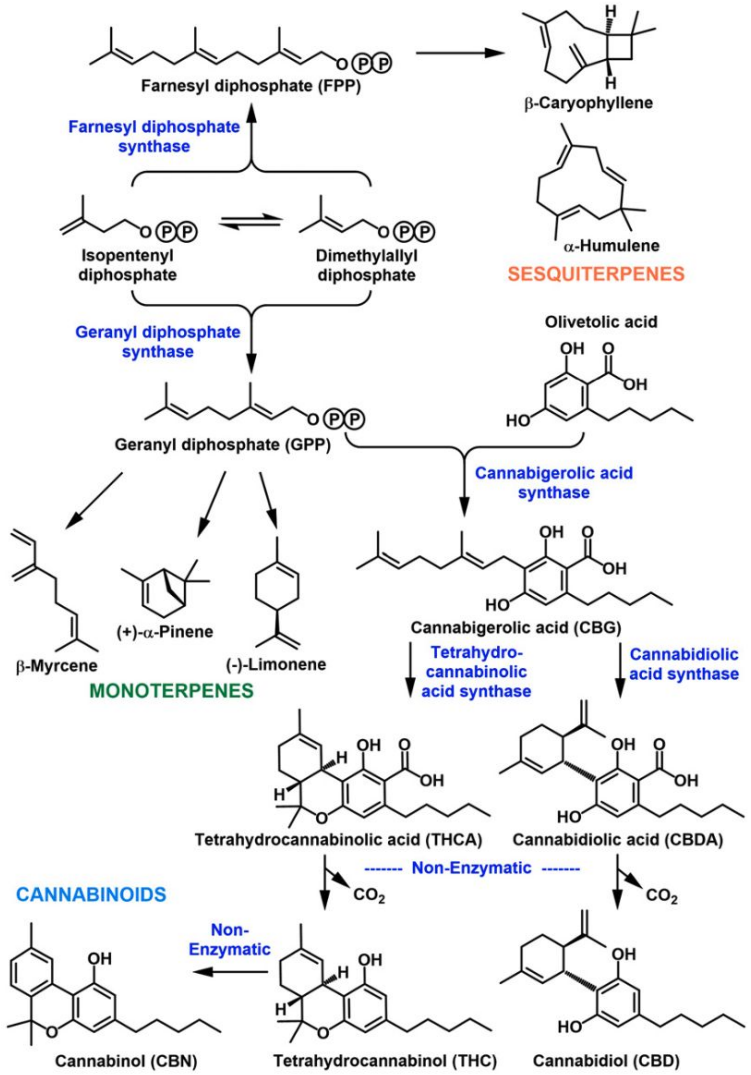
[b]Evio Labs, Central Point, Oregon 97502

ORCID IDs: 0000-0001-6970-5832 (J.J.Z.); 0000-0001-7934-7987 (N.S.); 0000-0001-6565-9584 (B.M.L.).

Glandular trichomes are specialized anatomical structures that accumulate secretions with important biological roles in plant-environment interactions. These secretions also have commercial uses in the flavor, fragrance, and pharmaceutical industries. The capitate-stalked glandular trichomes of *Cannabis sativa* (cannabis), situated on the surfaces of the bracts of the female flowers, are the primary site for the biosynthesis and storage of resins rich in cannabinoids and terpenoids. In this study, we profiled nine commercial cannabis strains with purportedly different attributes, such as taste, color, smell, and genetic origin. Glandular trichomes were isolated from each of these strains, and cell type-specific transcriptome data sets were acquired. Cannabinoids and terpenoids were quantified in flower buds. Statistical analyses indicated that these data sets enable the high-resolution differentiation of strains by providing complementary information. Integrative analyses revealed a coexpression network of genes involved in the biosynthesis of both cannabinoids and terpenoids from imported precursors. Terpene synthase genes involved in the biosynthesis of the major monoterpenes and sesquiterpenes routinely assayed by cannabis testing laboratories were identified and functionally evaluated. In addition to cloning variants of previously characterized genes, specifically *CsTPS14CT* [(−)-limonene synthase] and *CsTPS15CT* (*β*-myrcene synthase), we functionally evaluated genes that encode enzymes with activities not previously described in cannabis, namely CsTPS18VF and CsTPS19BL (nerolidol/linalool synthases), CsTPS16CC (germacrene B synthase), and CsTPS20CT (hedycaryol synthase). This study lays the groundwork for developing a better understanding of the complex chemistry and biochemistry underlying resin accumulation across commercial cannabis strains.

ure 1. Shared origin of the cannabinoid and penoid biosynthetic pathways. A circled P notes phosphate moieties.



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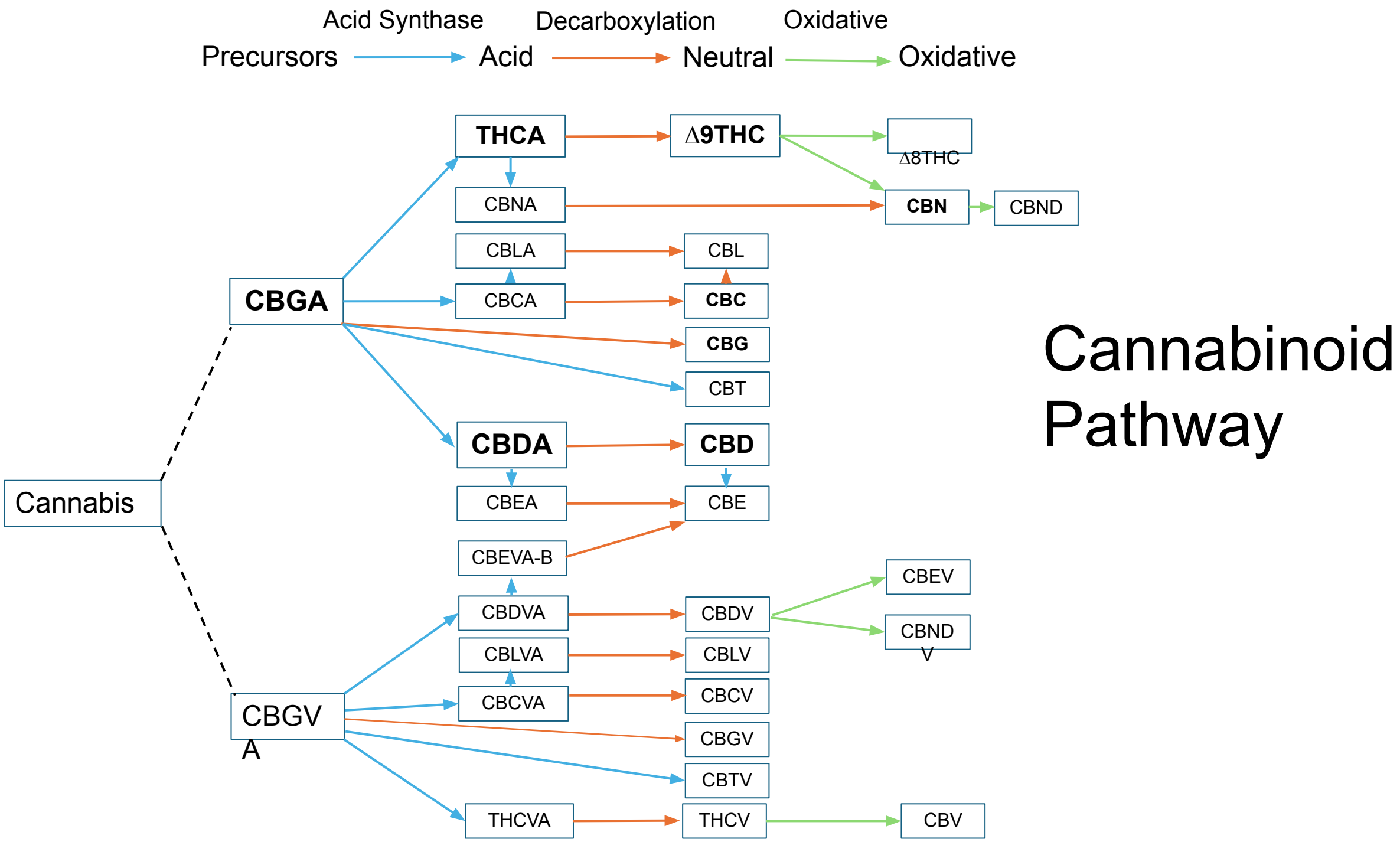

Acid Synthase
Decarboxylation
Oxidative
Precursors
Acid
Neutral
Oxidative
THCA
Δ9THC
Δ8THC
CBNA
CBN
CBND
CBLA
CBL
CBGA
CBCA
CBC
CBG
CBT
CBDA
CBD
Cannabis
CBEA
CBE
CBEVA-B
CBEV
CBDVA
CBDV
CBND V
CBLVA
CBLV
CBGV A
CBCVA
CBCV
CBGV
CBTV
THCVA
THCV
CBV
Cannabinoid Pathway

Terpenes

Over 200 –

- *α-Pinene, Camphene, β-Myrcene, Sabinene, ß-Pinene, Linalool , Caryophyllene, Limonene*

Aromatic compounds

Volatile

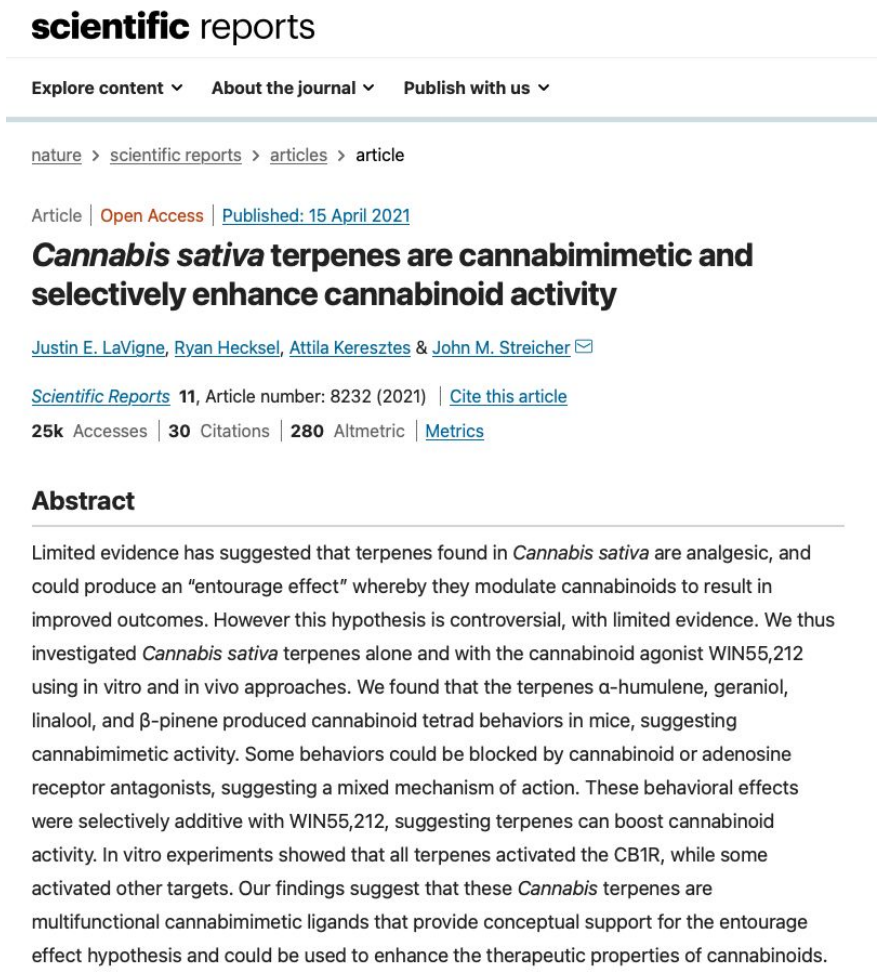
Found many plants

Anti-microbial properties

Often cause the allergies

Camphene binds to the CB1 receptor

Produced in the plant through the same metabolic pathway as cannabinoids.



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Article | Open Access | Published: 15 April 2021

***Cannabis sativa* terpenes are cannabimimetic and selectively enhance cannabinoid activity**

Justin E. LaVigne, Ryan Hecksel, Attila Keresztes & John M. Streicher

Scientific Reports **11**, Article number: 8232 (2021) | Cite this article

25k Accesses | **30** Citations | **280** Altmetric | Metrics

Abstract

Limited evidence has suggested that terpenes found in *Cannabis sativa* are analgesic, and could produce an "entourage effect" whereby they modulate cannabinoids to result in improved outcomes. However this hypothesis is controversial, with limited evidence. We thus investigated *Cannabis sativa* terpenes alone and with the cannabinoid agonist WIN55,212 using in vitro and in vivo approaches. We found that the terpenes α-humulene, geraniol, linalool, and β-pinene produced cannabinoid tetrad behaviors in mice, suggesting cannabimimetic activity. Some behaviors could be blocked by cannabinoid or adenosine receptor antagonists, suggesting a mixed mechanism of action. These behavioral effects were selectively additive with WIN55,212, suggesting terpenes can boost cannabinoid activity. In vitro experiments showed that all terpenes activated the CB1R, while some activated other targets. Our findings suggest that these *Cannabis* terpenes are multifunctional cannabimimetic ligands that provide conceptual support for the entourage effect hypothesis and could be used to enhance the therapeutic properties of cannabinoids.

Flavonoids

- Oxygen free radical scavengers
- Anti-oxidants
- Produce pigments
- Result of temperature stress
- Produced in the leaves and roots
- Cannabis specific include Cannflavin A and B
- Migrate into the Trichomes

Other compounds

- Flavonoids – the colours
- Lignans
- Chlorophyll
- Lipids
- Sterols
- Sulphuraphanes

frontiers in Plant Science

REVIEW
published: 04 February 2016
doi: 10.3389/fpls.2016.00019

Cannabis sativa: The Plant of the Thousand and One Molecules

Christelle M. Andre*, Jean-Francois Hausman and Gea Guerriero

Environmental Research and Innovation, Luxembourg Institute of Science and Technology, Esch-sur-Alzette, Luxembourg

Cannabis sativa L. is an important herbaceous species originating from Central Asia, which has been used in folk medicine and as a source of textile fiber since the dawn of times. This fast-growing plant has recently seen a resurgence of interest because of its multi-purpose applications: it is indeed a treasure trove of phytochemicals and a rich source of both cellulosic and woody fibers. Equally highly interested in this plant are the pharmaceutical and construction sectors, since its metabolites show potent bioactivities on human health and its outer and inner stem tissues can be used to make bioplastics and concrete-like material, respectively. In this review, the rich spectrum of hemp phytochemicals is discussed by putting a special emphasis on molecules of industrial interest, including cannabinoids, terpenes and phenolic compounds, and their biosynthetic routes. Cannabinoids represent the most studied group of compounds, mainly due to their wide range of pharmaceutical effects in humans, including psychotropic activities. The therapeutic and commercial interests of some terpenes and phenolic compounds, and in particular stilbenoids and lignans, are also highlighted in view of the most recent literature data. Biotechnological avenues to enhance the production and bioactivity of hemp secondary metabolites are proposed by discussing the power of plant genetic engineering and tissue culture. In particular two systems are reviewed, i.e., cell suspension and hairy root cultures. Additionally, an entire section is devoted to hemp trichomes, in the light of their importance as phytochemical factories. Ultimately, prospects on the benefits linked to the use of the *-omics* technologies, such as metabolomics and transcriptomics to speed up the identification and the large-scale production of lead agents from bioengineered *Cannabis* cell culture, are presented.

Keywords: fibers, hemp, *Cannabis*, cellulose, lignin, cannabinoids, terpenes, lignans

OPEN ACCESS

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Specialty section:
This article was submitted to Plant Biotechnology, a section of the journal

The Process of Producing a Medicine

Cultivation	Harvest	Curing	Packaging	Processing
Propagation Selection –(Pheno-hunting) Vegetation Flowering Breeding Stabilisation	Removing all leaves Separating the flowers Trimming the flowers Grading of the flower	Drying of the flower Hanging Freeze Drying Until water activity is is below 0.4 or 13% moisture content	Product sterilized Packaged into required units	Extraction API production

Cultivation Methods & Standards

Indoor vs Outdoors

Indoors	Outdoors
Closed system	Natural light
Artificial Lights	Grown in the ground
Expensive to run	Less overheads
Less risk of contamination	High risk of contamination
Hydroponic systems	Weather damage risk
Closely controlled environment	Seed production risk

Extraction – Making the medicine

- Types of products
  - Full plant extract
  - Isolate
  - Distillate
- Types of Extraction
  - CO_2
  - Butane
  - Rosin Press
  - Chromatography
  - Rotary evaporation Ethanol

The Standards

GACP – Good Agricultural and Collection Practice, applies to cultivation and harvest

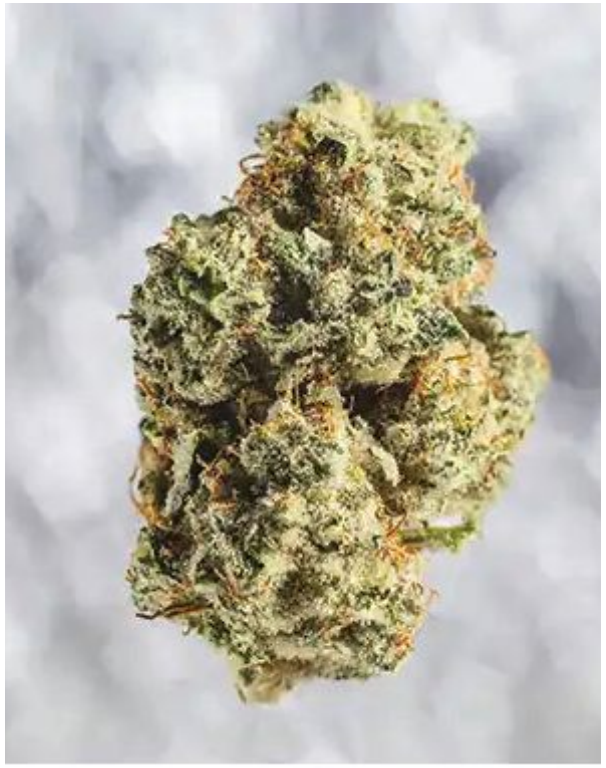
GMP – Good Manufacturing Practice applies to packaging and processing

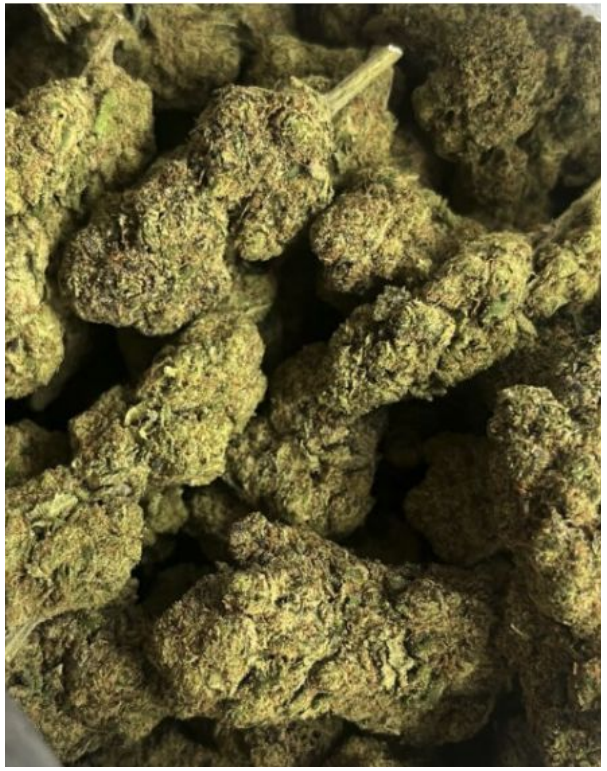
EU-GMP – Meets the European pharmacopeia requirements and can be prescribed in the the UK

What the Patient expects

Bag Appeal

- Neatly trimmed
- Sparkling/sugar frosted
- Large buds
- Aromatic
- Sticky





Types of Street cannabis

*Image adapted from Woah Storks visual guide to cannabis

Thank you for Listening

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