

## INTRODUCTION

A field of dreams is a fitting metaphor symbolizing the remarkably full and distinguished academic career of Professor Robert Sievers. He retired from CU on January 1, 2022 after ~ 45 years of service to the University. From analysis of moon rocks to development of novel means for vaccine delivery, his research has been most fruitful and one might even say, "supercritical."

It is my pleasure to have worked for Professor Sievers multiple times. I'm honored to be able to share a reflection of his career and to be able to present a summary of his most recent research in which I have been involved.

Some of Professor Sievers's impressive career achievements:

- He mentored 44 Ph.D. students, numerous Masters and undergraduate students, as well as post-docs and visiting professors
- Served as Director of CIRES, Interim Dean of the Graduate School and was a CU Regent for Colorado's 2nd Congressional District
- He helped establish Anschutz and Boulder South and East Campuses
- Won numerous academic awards including Colorado Governor's Award
- He directed numerous research programs including an over \$20 million grant from the Bill and Melinda Gates Foundation for execution of a Phase 1 clinical trial, demonstrating needle-free measles vaccine delivery
- He has coauthored over 220 papers, several book chapters, and edited a few books. His publications have been cited more than 5000 times
- Was awarded more than 30 U.S. and international patents
- He founded several CU spinoff companies, creating hundreds of jobs. One company, Sievers Instruments, was created by him, Misha Plam and Ric Hutte in 1984. Through acquisitions, first by Ionics, Inc., then by General Electric, it is now part of the French engineering firm, Suez, and has so far manufactured over 30,000 analyzers accounting for over \$2 billion of economic activity in Boulder and 2 analyzers delivered to and installed onboard the International Space Station
- Somehow, he found time to become an artist and he has graced CU campuses with several of his marble sculptures

Since hemp research became legal in Colorado in 2014, Professor Sievers turned some of his attention to the amazing plant, *Cannabis Sativa L.* I've helped him in this endeavor for the past couple of years and here describe some of this work.



Sara Spacek and Jan Hu  
Volunteer to Plant Seedlings

## OBJECTIVES AND METHODS

We researched various aspects of hemp cultivation and processing in the field, which included: varietal selection for maximum yield of cannabinoids; weed control *via* use of organic herbicides; production of high purity cannabinoid isolates, such as CBD and CBDA; and study of consumer products for purity/safety.

A focus has been on process improvement in order to supply other researchers who study physiological benefits of cannabinoids. A chief aim is to help those researchers find solutions to mental and physical ailments, from reduction of anxiety for aiding the treatment of PTSD, addiction and for pain management. We also work with ASTM to standardize test methods for quality management.

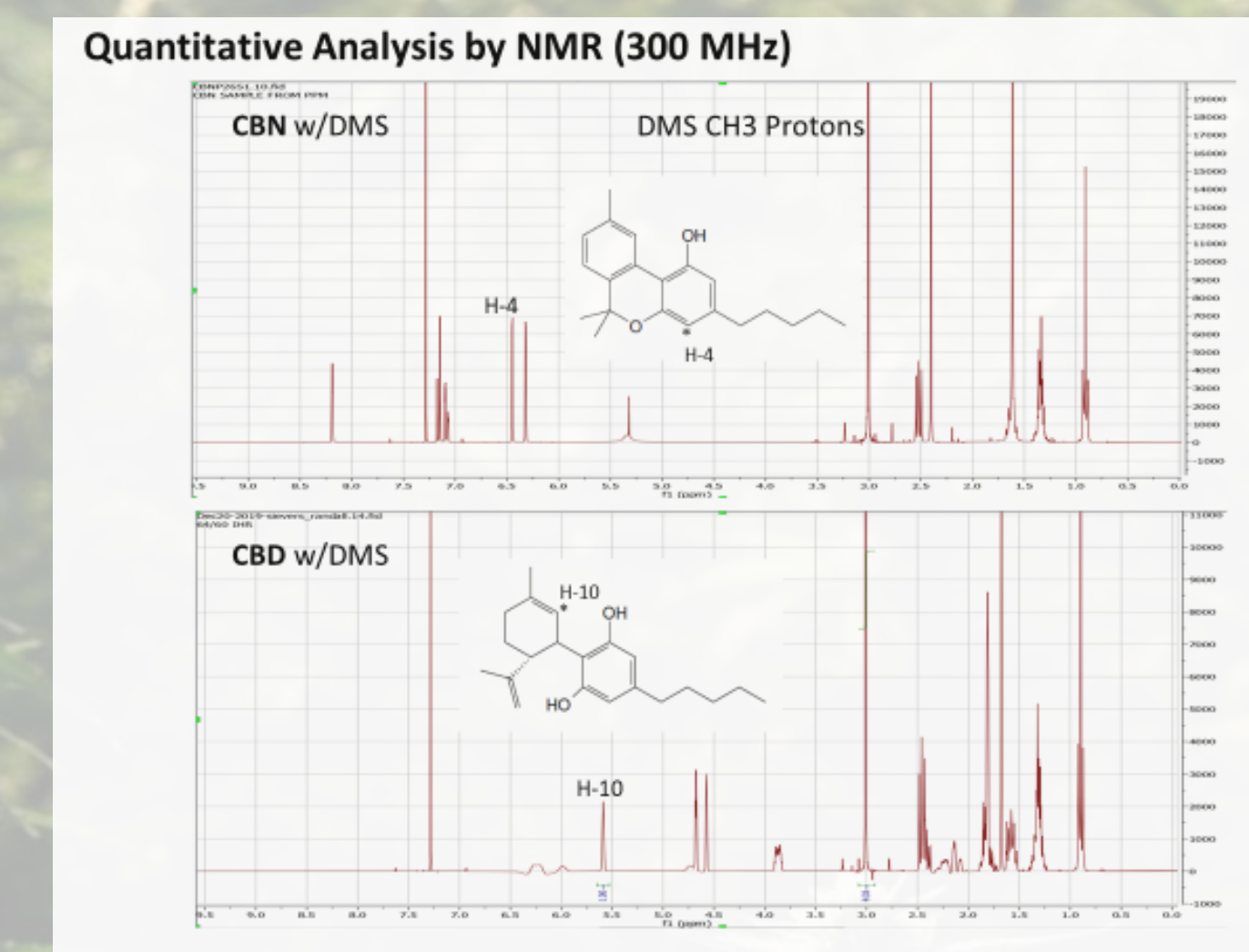
Our experimental plot is located near Lafayette. Undergraduate students assisted with several aspects of cultivation and analysis. GC, HPLC and other standard analyses were performed in Professor Sievers' Lab. HPLC-TOFMS analysis was performed at CU's Center for Environmental Mass Spectrometry and <sup>1</sup>H-NMR was performed on a Bruker Avance 300 MHz Spectrometer in the Chemistry Department's NMR Facility.

Major techniques used for purification include solvent extraction, distillation, and especially crystallization from n-pentane for isolation of CBD. CBDA was derived through a Kolbe-Schmitt type reaction, adding CO<sub>2</sub> to CBD.

## FINDINGS

The Colorado climate provides good growing conditions for hemp along the Front Range when weather produces adequate water for irrigation. The Cherry Wine hemp variety yields relatively high concentrations of CBD/CBDA in its biomass, ~ 10-12% by dry weight, and reasonably large plants, often > 10 kg at harvest, and importantly they contain < 0.3 mass % THC (tested by Colorado Department of Agriculture). Terpene oil derived from hemp itself is a somewhat effective herbicide, but it is not as potent as commercial limonene based formulations against common weeds found within Boulder County.

Analysis of commercial isolates by LC-TOFMS and qNMR agree quite well. They show that most are of high cannabinoid purity. Regulations also require toxic metals, pesticides and mold toxins not to exceed hazardous levels. We are able to produce CBD and CBDA of higher purity than that available commercially. In fact, when analyzed externally, our isolates assay at more than 100% because they are of higher purity than available analytical standards. We provided CBD isolate from our plot to several researchers, both internal and external to CU.



## Analysis and Results from Examination of Commercial CBD Isolates

Cannabinoids	Mass % by LC-MS Normalized to CBD					Purity by NMR %	Ext. Solvent
	THC	CBT	CBDV	CBK*	Sum of Impurities		
22-1	0.32	0.97	0.35	0.9	2.54	97.5	Ethanol
22-2			0.21		0.21	99.8	Ethanol**
22-3			0.32	0.73	1.05	99.0	Isopropanol
22-4			0.55	1.04	1.59	98.4	Isopropanol
22-5	0.25	0.17		1.18	1.6	98.4	Supercrit. CO <sub>2</sub>
22-6	0.26			0.38	3.44	96.3	Ethanol

\*Work to definitively identify CBK (exact mass 333.2424) is on-going, 1 literature candidate  
\*\*Proprietary purification step effectively removes THC and CBK

- LC-MS and NMR results are complimentary and independently equivalent, neither requiring authentic standards
- Commercial CBD isolates are pure but can be made purer
- Differences result from choice of extraction solvent, ethanol extraction as practiced tends to produce a less pure product



Preparing for Harvest – Early October 2019  
Imma Ferrer, Mike Thurman, Ku Thomas,  
Bob Sievers and Randy Shearer



Professor Sievers Admiring a Big  
Mama Plant Hiding a Smart Car

## ACKNOWLEDGEMENTS

We acknowledge support from Randy Greaves and from the Danny Alberts Foundation for funding this work. We thank CIRES, the Chemistry Department and CU in general for the opportunities making this research possible. A special thanks goes to Garrett Hause of Ball Acres for his stewardship of the land and to Imma Ferrer and Mike Thurman for our productive collaborations. Most of all, I am grateful to Professor Sievers for his mentorship and continued support. Science would not have been the same without him.

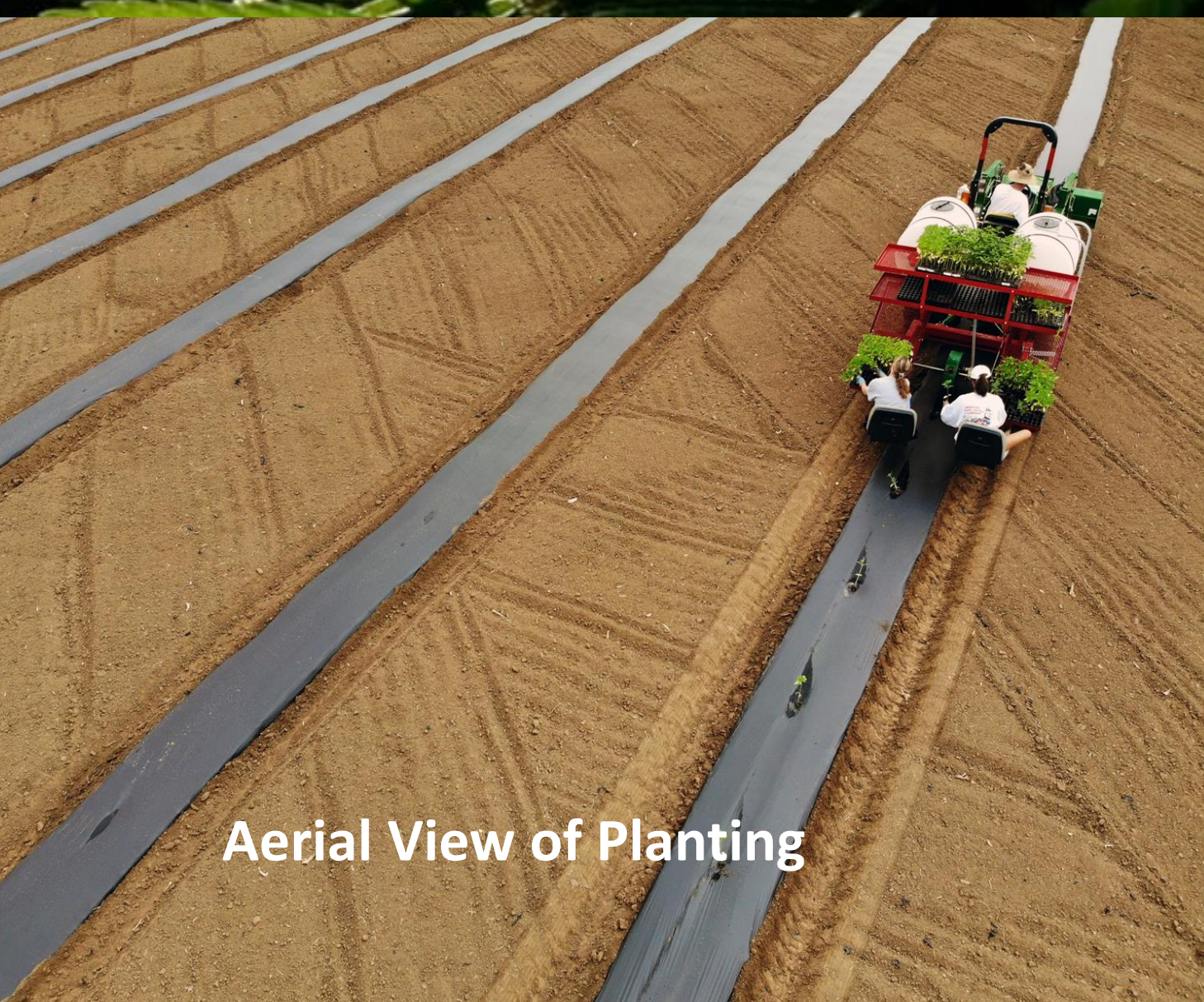
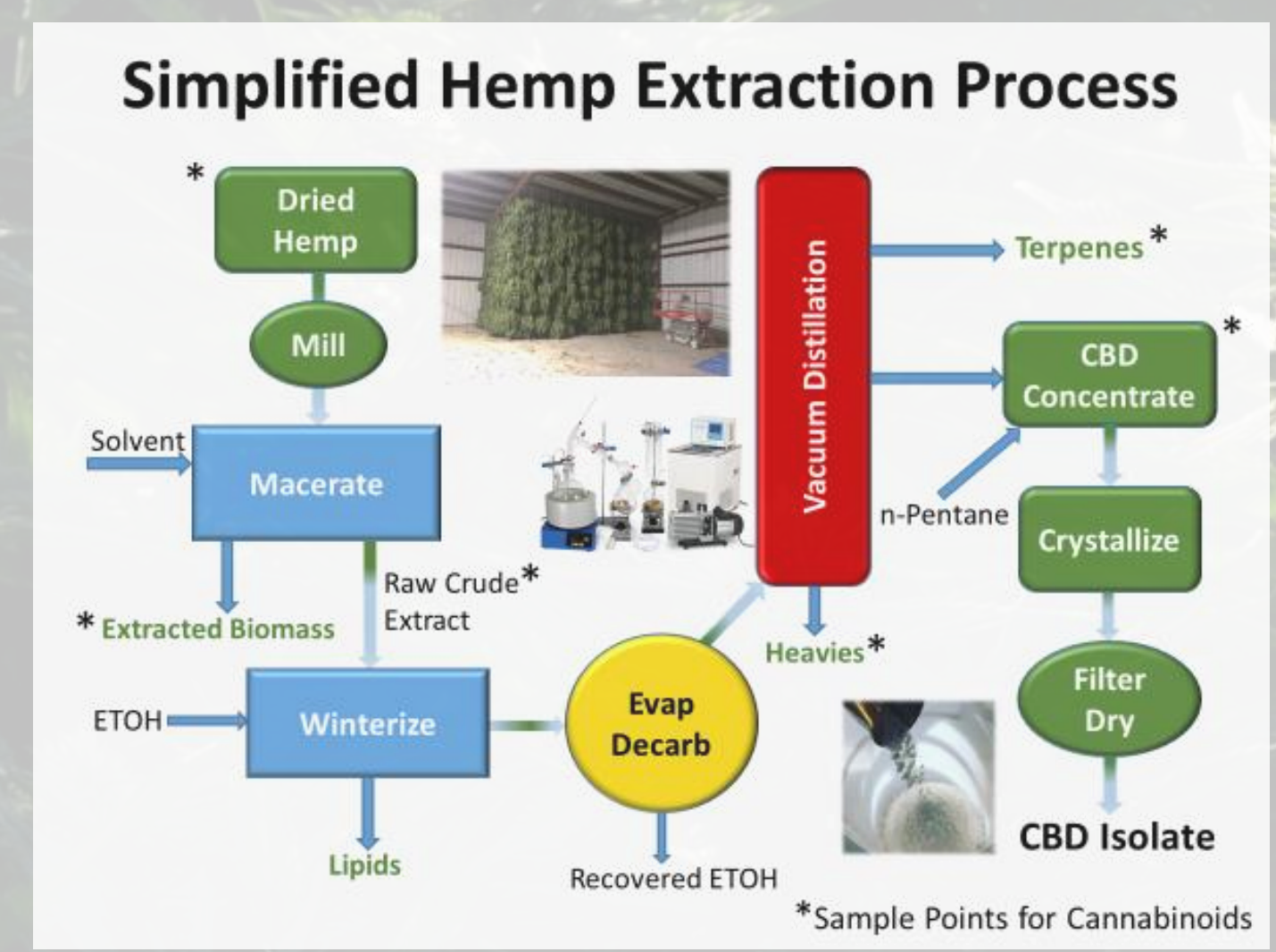
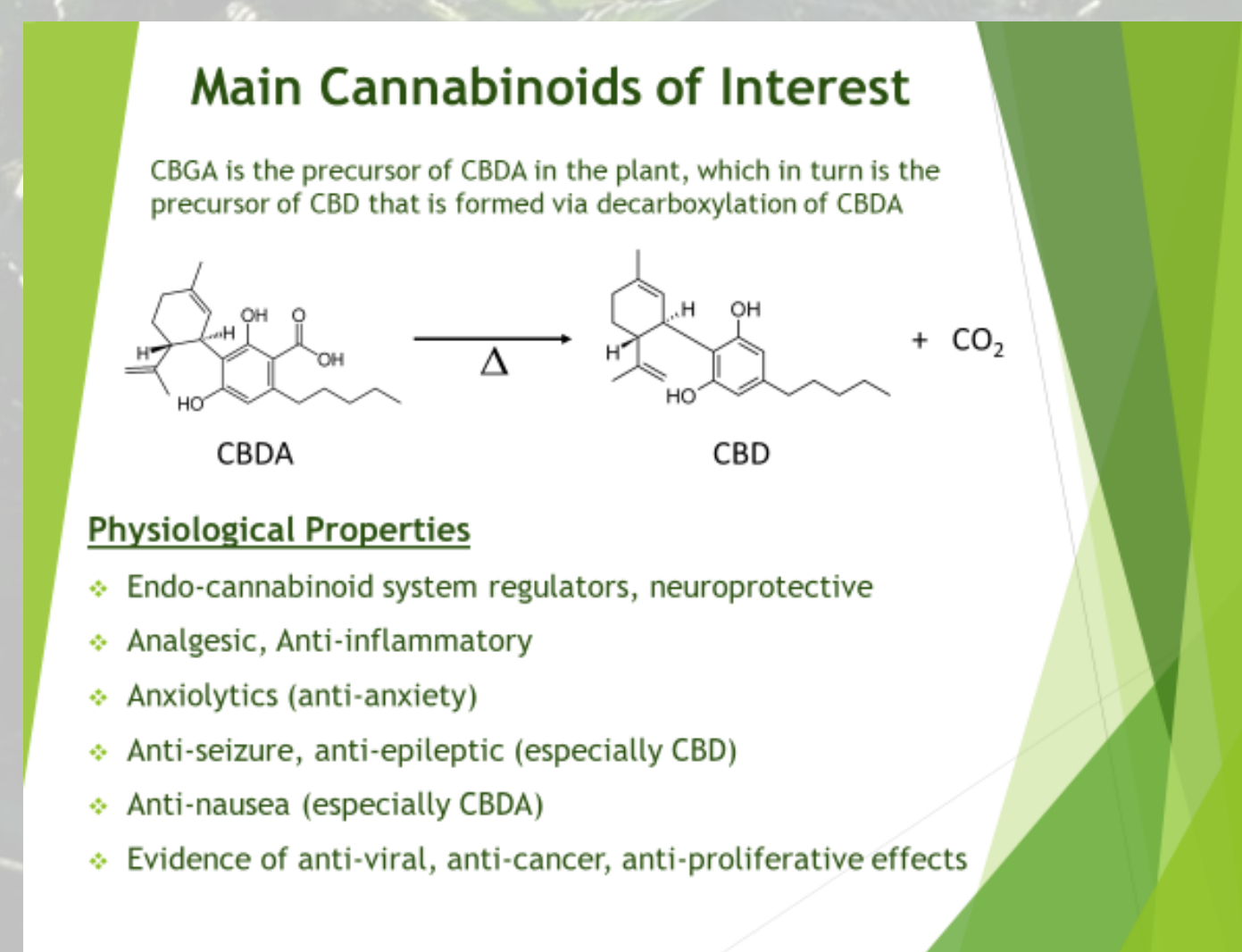


Seedlings Ready for Planting – Early June

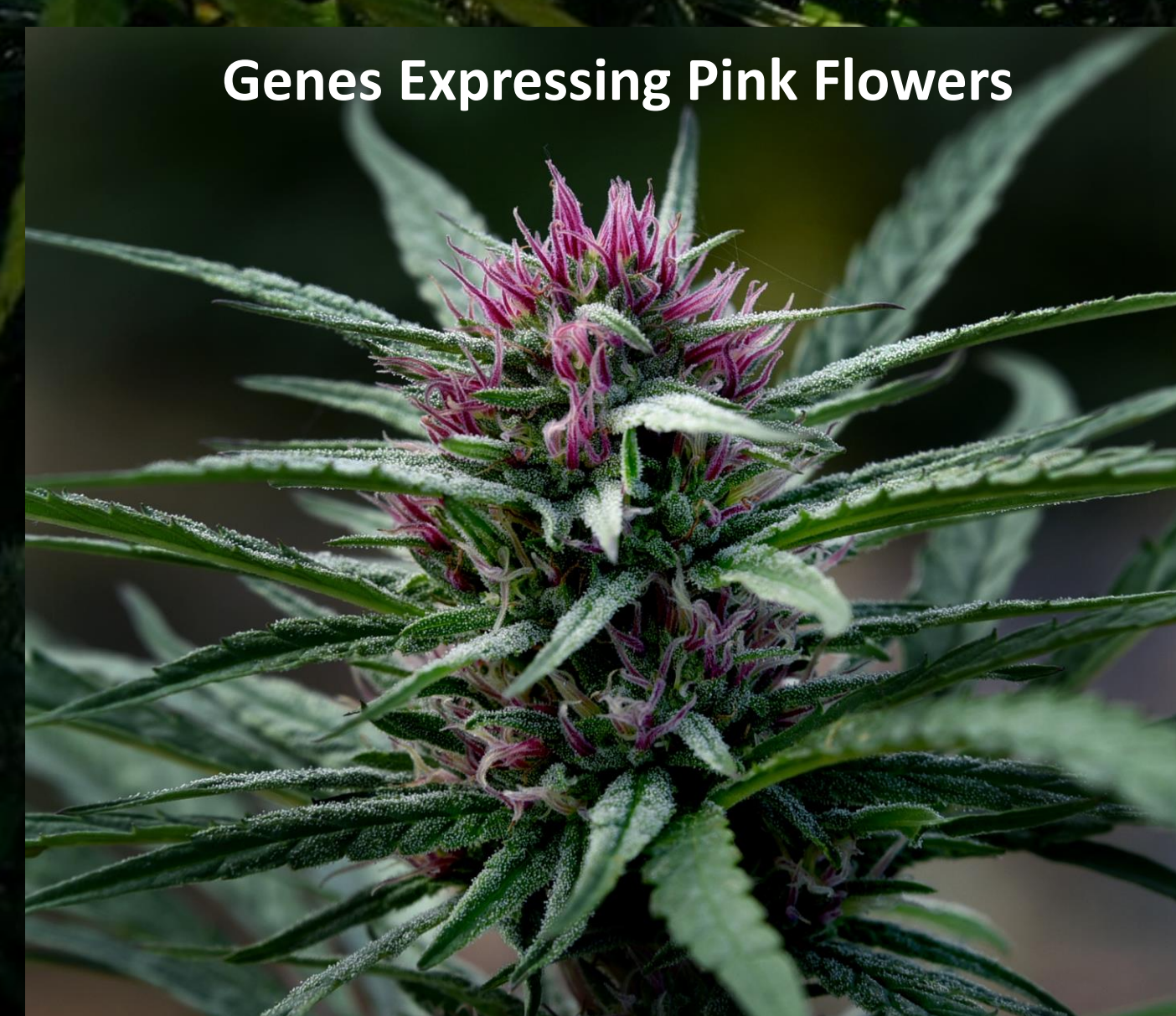


### Hemp Varieties Studied

- Cherry Wine
- River Rock
- Otto II
- Colorado Pine
- Sticky Citrus



Aerial View of Planting



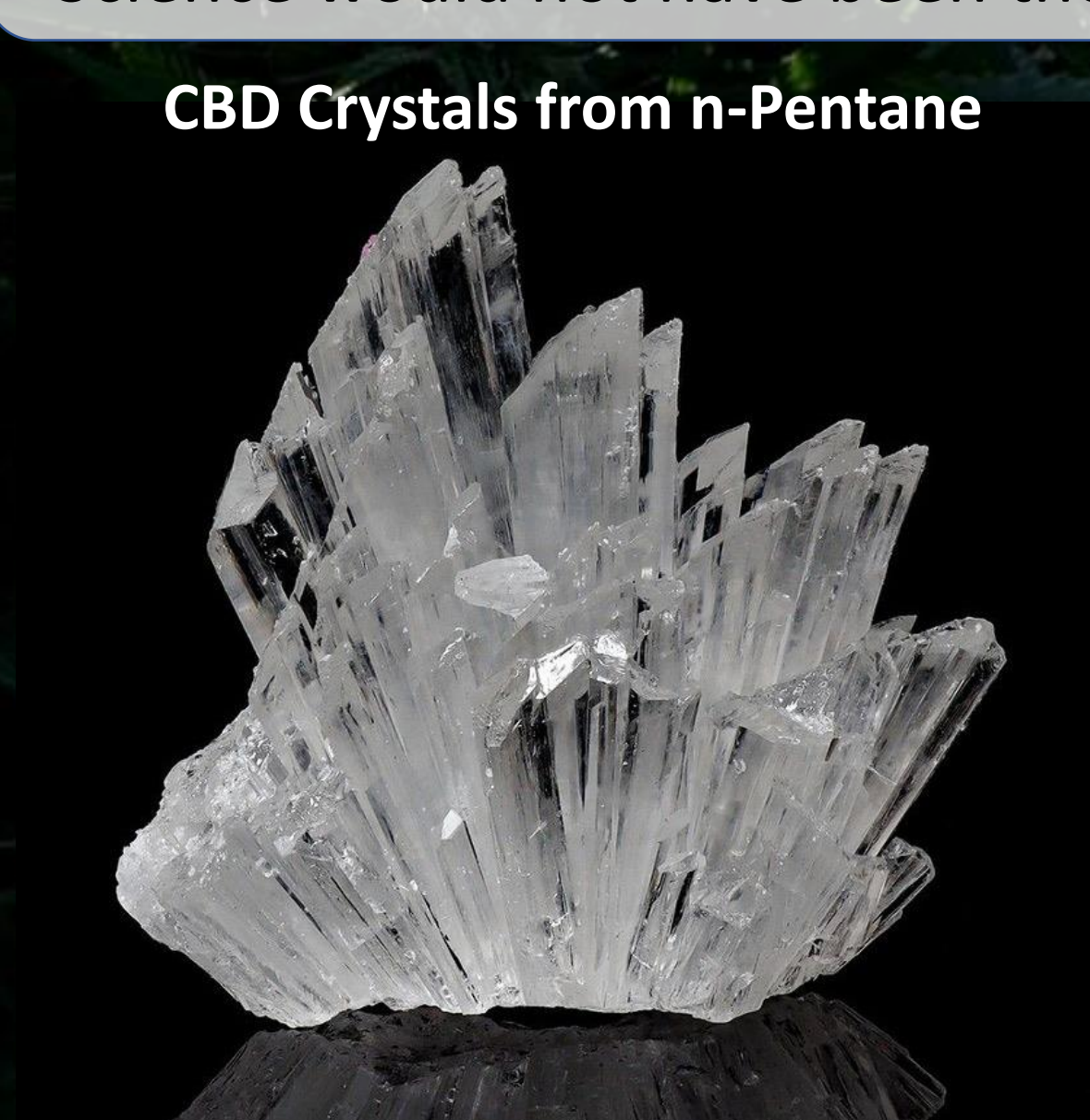
Genes Expressing Pink Flowers



Center for Environmental MS



Commercial CBD Isolate Samples



CBD Crystals from n-Pentane



Scan this QR code for a video about  
Professor Sievers's Field of Dreams

