the Cannabis Scientist™

The Innovation Awards 2020

What tools and applications are making the lives of cannabis scientists easier?

16 – 23

In this issue, we're delighted to announce the winners of our first-ever Innovation Awards (page 16). We're certainly delighted to recognize and celebrate the biggest advances in cannabis science from the past two years. Nevertheless, we approached the awards with some trepidation. Would there be enough products and papers nominated? Would there be enough variety to make it interesting?

Our fears proved entirely unfounded and the cannabis science community came through with nominations ranging from new analytical methods to clever genetics for better cultivation. Special thanks must go to our wonderful (and anonymous) judging panel for sifting through the many entries and giving thoughtful, reasoned scores.

Of course, not everyone will agree with the entries chosen. As a veteran of several Innovation Awards in our sister magazine The Analytical Scientist, I know that any such list will generate some controversy; I look forward to hearing your thoughts on what we got wrong (or right) in the weeks to come...

The thread of innovation doesn't end with the Awards though. From QR codes printed with cannabinoid ink for precise dosage (page 9) to uncovering how lighting affects the cannabinome (page 10) the issue is bursting with cool technology and fascinating studies!

Our In My View articles present two perspectives on how the industry can meet rising demand by moving production out of the plant and into cells (page 12) or chemical synthesis (page 14). Plus, we sit down with Keith Allen, Head of Bioinformatics at Front Range Biosciences. Having previously worked at agriculture giant Syngenta, Keith was shocked to discover how little is known about the cannabis genome – turn to page 27 to find out how his team is untangling the complex genetics of the plant.

There is much still to learn. Indeed, as Allen points out (echoing several other interviewees this year), the cannabis space is a dream for scientists precisely because it is relatively unexplored. But with grant funding for cannabis research at its highest ever level – albeit skewed towards harms rather than benefits (page 6) – and the literature growing year-on-year, the pace of change is rapid. It is an exciting time, and I hope the stories in this issue will spark your curiosity – and perhaps serve as inspiration.

Charlotte Barker
Editor

Cannabinoid Purification

Interchim manufactures chromatography instrumentation to support several cannabinoid applications.

Minor Cannabinoid Purification

Purification of compounds at low concentration is accomplished using smaller particles in the chromatography column.

Pesticide Remediation

Fungicides and pesticides can be removed from either hemp or cannabis distillate using high throughput liquid chromatography.

THC Remediation

Reverse phase chromatography is the primary technique to provide a robust and reproducible manufacturing process.

In this example, 700 grams of distillate is remediated in 48 minutes.
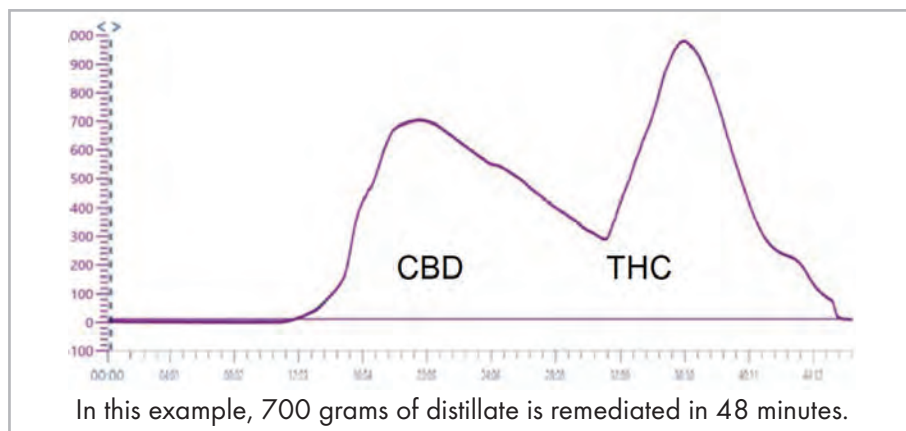
Continuous operation is easily programmed using multiple cycles in the software.

Pilot and process scale instruments are built to specification and utilize hydraulic piston columns with inner diameters from 20 cm to 100 cm.

Interchim is a well established manufacturer of low and high pressure chromatography instrumentation. The company was founded in 1970 to create innovative products for pharmaceutical R&D. Years of experience have been applied to provide the cannabis market with a simple and cost-effective purification solution.

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fMRI image of preteen brain from the ABCD study. Photo by Richard Watts, PhD, University of Vermont and Fair Neuroimaging Lab, Oregon Health and Science University / CC BY

Cannabis Use in Pregnancy: a Dangerous Legacy?

Exposure to cannabis in the womb is associated with psychiatric symptoms in childhood

A large observational study adds to evidence that consuming cannabis during pregnancy could have a negative impact on children later in life (1). The researchers examined data on 11,489 children aged 9–11 years, of whom 655 had been exposed to cannabis prenatally. The children are enrolled in the ABCD study, a long-term study of brain development and child health. After correcting for other risk factors, children whose mothers continued to consume cannabis once they knew they were pregnant were more likely to experience psychotic symptoms or behavioral problems such as ADHD.

In contrast, when pregnant women gave up cannabis on finding out they were pregnant (usually at around seven weeks), any differences in outcomes were accounted for by existing risk factors.

One possible explanation for these results lies in the fact that endocannabinoid type 1 receptors (to which THC binds) typically develop at around 6 weeks gestation, so cannabis use before that time may be less harmful to the developing brain. Alternatively, continuing to use cannabis after becoming aware of pregnancy may be a marker for more intense cannabis use; notably, the study did not record details of dose or frequency.

Principal Investigator Ryan Bogdan told JN Learning podcast (2) that cannabis use during pregnancy has increased in recent years in the US, with 7 percent of pregnant women reporting past-month cannabis use in 2017 (more than double the rate in the early 2000s).

The study does have important limitations (beyond the aforementioned lack of data on amount or potency), including the fact that cannabis use was self-reported by parents based on their memory of a pregnancy from a decade ago. Nevertheless, the work goes beyond previous observational studies in the size of the sample, the age of the children, and the attention given to teasing out confounding factors, such as alcohol exposure or family history of mental illness.

A new study from the group is recruiting pregnant cannabis users and following them (and their child) throughout their pregnancy and beyond in an attempt to answer some of the outstanding questions.

Author Sarah Paul commented on Twitter: "So what should we take away from this? a) Future research needs to better account for frequency, potency, quantity, and mode of ingestion of cannabis use, each of which could lead to different THC concentrations in the fetus. b) Cannabis use during pregnancy should be discouraged."

References
1. *SE Paul et al., JAMA Psychiatry, [Epub ahead of print] (2020). DOI:10.1001/jamapsychiatry.2020.2902*
2. *JN Learning (2020). Available at: https://bit.ly/3idWVIV*

INFOGRAPHIC

Follow the Funding

A project from hellth.com analyzed grants awarded for cannabis research in the USA, Canada and UK

Funding for cannabis research increased significantly from 2000 to 2018 ($)

2018
151 MILLION

2000
31 MILLION

TOTAL FUNDING 2000-2018
1.5 BILLION

US researchers were better funded than their UK and Canadian counterparts ($)

US **1.48** BILLION

CANADA **36.1** MILLION

UK **39.9** MILLION

We catch up with the latest twists and turns in cannabis regulation and policy

- Taking a strong position on emissions. Colorado has mandated heavy metals testing of vapor (rather than the e-liquid/oil) for vaped cannabis products, effective January 1, 2022. Companies will also have to start adding an expiry date to vape cartridges.

- Dearth of diversity in Canadian cannabis. A report on race and gender in Canada's legal cannabis industry found that C-suite executives and directors were overwhelmingly white (84 percent) and male (86 percent). The authors recommend that the Canadian government implement social equity programs to help members of underrepresented groups enter the industry.

- Hemp headaches. The recent US DEA interim final rule on hemp has raised concerns that intermediates in hemp processing (which can go above the 0.3 percent THC limit, even when the final product is legal) could be classified as schedule 1 substances. Hemp companies have filed a lawsuit against the DEA, alleging that the interim rule misinterprets the 2018 Farm Bill.

- Have your say. The US FDA recently published draft guidance to allow abbreviated new drug applications for oral CBD products; public comment open until November 23. Plus, the USDA announced plans to survey 18,000 hemp business to inform future regulatory efforts

For links to the original announcements, visit the online version of this article at tcs.txp.to/PolicyNov20

The Drugs Don't Work?

Cannabis use could make anesthesia less effective

A widely reported study found that patients who had used cannabis in the month prior to fracture surgery required more anesthesia during the op and had more pain afterward (1,2).

Ian Holmen, the lead author and an anesthesiology resident at the University of Colorado Hospital, says, "Myself and several of my colleagues had noticed that some patients who reported cannabis use were reacting differently to anesthesia and seemed to require more pain medications after surgery."

Holmen and colleagues set out to test this hypothesis by reviewing the dose of anesthetic drugs received during surgery by cannabis users and non-users. There was little difference for most drugs, but cannabis users did receive significantly more of an inhaled anesthetic, sevoflurane. Cannabis users also reported higher pain scores post-surgery and received more opioids.

"It is clear that there needs to be significantly more research on the topic and that there is still a dearth of high-quality studies," concludes Holmen.

References
1. *American Society of Anesthesiologists. (2020). Available at: https://bit.ly/34yGiEa*
2. *IC Holmen, J Clin Anesth, 67, 109980 (2020). DOI: 10.1016/j.*

The top funded research areas were:

1 Effects of cannabis use (harms)
2 Endocannabinoid function
3 Cannabinoid therapies
4 Treating cannabis use disorder

15x more funding for cannabinoid medicines vs cannabis as a medicine

**20x** more funding on harms vs therapeutic use

Source: hellth.com

Are Strain Names Meaningless?

What's in a name? When it comes to cannabis... maybe not a lot

"Strain" names failed to correlate with cannabinoid and terpenoid profiles in a study of 2,662 cannabis flower samples from Nevada's medical use program (1) – adding weight to calls for an alternative way of classifying chemovars.

The authors were surprised by the lack of chemical diversity in the samples, with all containing high levels of THC and low levels of other cannabinoids. Terpenes were more variable, forming three clusters. But the (nearly 400) names assigned by breeders were essentially meaningless, giving no indication of chemical composition.

The samples were collected in 2016/17, when cannabis was only available for medical use in Nevada. An earlier study from Washington State, which analyzed a much bigger sample set, found greater diversity and some correlation between name and cannabinoid content (2),
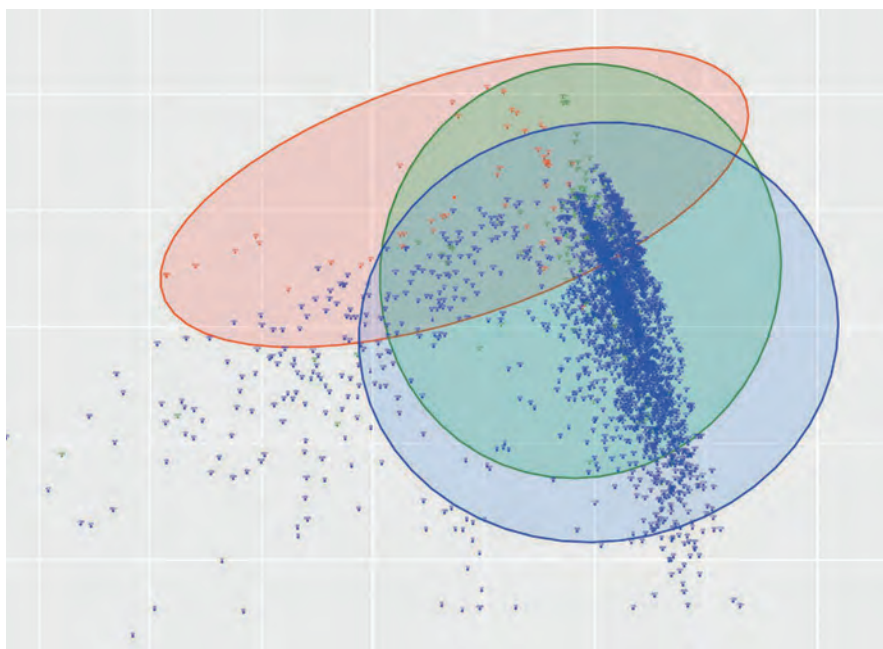
concluding: "These results suggest that strain names can provide meaningful, though variable, signals of the composition of flower samples."

Do strain names provide valuable information to consumers? Joseph Smith, CEO at Florida testing laboratory Canaveral Labs, is skeptical: "My personal experience is that strain names do not have much correlation to terpene profiles. Without DNA sequencing, who knows what attributes a plant really has? Also, flower curing and remediation (e.g, HPI or Ozone) are additional variables and we don't yet know what effects that has on the terpenes. Strain names are mostly a marketing tool until all markets can obtain and cultivate standardized genetics that

will perform in the growers environment."

Pat Reynolds, Operations Director at Confidence Analytics, agrees: "Consumers need more to go on than the THC number. The question is - what? I've seen (and been involved in) many different attempts to characterize cannabis beyond the THC content, but I haven't seen any of them take off. They are still too complex and require too much expert knowledge to grasp easily."

References
1. *U Reimann-Philipp et al., Cannabis Cannabinoid Res, 5, 215–230. DOI: 10.1089/can.2018.0063*
2. *N Jikomes, M Zoorob, Sci Rep, 8, 4519 (2018). DOI: 10.1038/s41598-018-22755-2*

Gut Reaction

The body's own cannabinoids help fight off stomach bugs in mice

Endocannabinoids modulate gut biology and appear to protect mice from becoming ill with certain "stomach bugs" (enteric bacteria) according to a new paper out in biology journal Cell (1). Researchers focused on an endocannabinoid called 2-arachidonoyl

Credit: NIAID

glycerol (2-AG) and found that it blocks virulence pathways in enteric bacteria;

specifically, the bacterial QseC receptor.

Mice with elevated 2-AG (whether caused by genetics or drug treatment) had fewer symptoms and recovered faster than control mice. The researchers hope the findings might lead to new options for hard-to-treat infections in humans, such as drug-resistant E. coli.

Reference
1. *M Ellermann et al., Cell [Online ahead of print] (2020). DOI: 10.1016/j. cell.2020.09.022*

Let There Be Light!

Now a new study will explore the epigenetic and chemical impact of phytochrome manipulation in hemp

Phytochrome manipulation is common in indoor grows and involves using different wavelengths of light to help control the plants' circadian rhythms and maximize yield.

Researchers from the Collaborative Laboratories for Environmental Analysis and Remediation (CLEAR) at the University of Texas at Arlington, University of Texas at El Paso, genetics company ZED Therapeutics, and Curtis Mathes Grow Lights are joining forces to examine how different lighting protocols affect phytochemical profiles in a bid to better understand how phytochrome manipulation works and how we can harness it.

We caught up with one of the project leaders, Zacariah Hildenbrand (Research Professor, University of Texas at El Paso), to find out more.

How did the collaboration come about?
Kevin Schug (Director of CLEAR) and I have been working together since 2011, and we've always focused on unconventional fields of research. We've looked at the environmental implications of fracking, the chemical characterization of cannabis and hemp, and the advanced analysis of beer, to name just a few.

This new project is a continuation of ongoing research efforts amongst the collaborators, in which we are analyzing hemp (<0.3 percent THC) analogs of mainstream cannabis cultivars, created using proprietary genetic technologies developed by Adam Jacques of ZED Therapeutics.

How will understanding the genetic basis for phytochrome manipulation help growers?
Phytochrome manipulation is a very poorly understood phenomenon in the horticultural sciences, let alone in the cannabis/hemp sector. Our hope is that this research will unearth a better understanding of how modulating phytochromes with red light will affect phytochemical content (the expression of cannabinoids, terpenes, and flavonoids).

Our initial set of experiments will involve four different hemp cultivars with precise genetic makeup, each grown under four different lighting intervals (with respect to modulation of red light frequencies). Essentially, we will be able to evaluate how the different lighting intervals affect different hemp cultivars of unique genetic origins.

What will be the role of each partner?
Curtis Mathes is providing four different lighting configurations; ZED is growing the plants, collecting environmental data, and performing the cannabinoid analyses; Kevin (UTA) is overseeing the more advanced chemical analyses; and I (UTEP) am coordinating the study.

When can we expect to see the results?
We plan to conduct the experiments and analyses over the next 6 months and have the results submitted shortly thereafter. Needless to say, we are all very excited about the findings that this research may yield!

Cannabis Cultivation: Is Time Running Out For the Plant?

As the demand for CBD and other cannabinoids expands, paths in cultivation are set to diverge

By Jeremy Friedberg, Chief Science Officer at LAVVAN, New York, New York, USA

Demand for CBD products is exploding, and the industry is a long way from understanding how to scale production to meet future demand. So far, cannabis producers have successfully filled the supply gap using traditional agricultural methods, adapting nimbly to the shift in end-use from dried flower to individual cannabinoids.

However, more change is coming. Now that consumers are more interested in oral or topical use of cannabinoids than they are in smoking the plant, the need for low-cost, stable production is more apparent. These new product applications require both CBD and minor cannabinoids at levels that will strain the scalability and sustainability of traditional agricultural systems.

The rising popularity of cannabinoid products isn't lost on consumer packaged goods (CPG) companies — they all have cannabinoid programs in various states of progress. So why haven't we seen CBD product releases from household name brands? Their top concern isn't safety or purity, it's ensuring a stable supply chain and federal regulatory clarity in the USA.

Until CPG companies are confident that the quantities of cannabinoids they'll need are consistently available across multiple suppliers, they will delay introducing cannabinoid-derived product lines out of concern of potential business disruption and damage to their brand reputation should products be delayed.

The risk of shortages is all too real. Farming the cannabis plant for cannabinoid extraction is an intensive process. The first major hurdle for producers is harvesting and drying plants in the two-week window in which the plant is mature, but before it begins to rot. Recent US field data suggest that growers are only able to harvest approximately 20 percent of the plant, with 80 percent lost on the field or at the processor (1). Due to the resinous nature of oil-rich cannabis varieties (including hemp), mechanized cannabis field harvesting equipment has not been widely implemented. Thus, field cannabis is still commonly harvested by hand, a labor-intensive process that can easily become a bottleneck in supply.

Finding enough laborers for the field as the industry grows will also prove challenging. The farming sector is already facing labor shortages and is further vulnerable to policy changes and destabilizing regional or global influences like COVID-19. In addition, outdoor production only allows for one harvest per year. Indoor growing allows for multiple cultivation cycles with significantly increased yields but comes with vastly higher production costs and greater risk of operational failure.

Turning cannabis plant tissue into pure cannabinoid isolates is a complex multi-step process. Batches may fail due to chemical and biological contamination or regulatory non-compliance (such as THC contamination), making a consistent and stable product supply challenging. With demand growing and large companies planning new product lines, I believe cannabinoid production systems must evolve.

The goal now is to produce large quantities of cannabinoids, some of which are present in such small amounts in the plant that the traditional agricultural model will never be able to yield enough. To move beyond this limitation, a better "factory" is needed for creating cannabinoids, just as historically was the case for vitamins and a variety of other important molecules. Cellular agriculture, a form

of biotechnology involving biosynthesis and fermentation, has been used for over 40 years to produce molecules at scale.

Cellular agriculture employs single-cell microorganisms, where each cell functions as an independent metabolite factory, producing a single cannabinoid. The cells can be grown in large vessels with high efficiency, under sterile conditions, and free from contaminants. Although this approach has its own set of challenges, it vastly simplifies the steps needed to produce a single molecule in comparison to traditional agriculture. Pure isolated cannabinoids can be used independently (in a CBD beverage, for example) or in combination, even recreating the cannabinoid profile combinations observed in the plant. The process removes the risks of crop production systems, generates

a pure raw ingredient for multiple applications, and frees up land for other agricultural activities.

The cannabis industry is faced with a fundamental decision: to supply the burgeoning cannabis market, do we embrace the plant crop system, turn to cellular agriculture, or use both?

In my view, a hybrid system offers the greatest advantage. We can scale focused cannabinoid production by using cellular agriculture to supply cannabinoid-infused products, and reserve agricultural production for the high-quality cannabis flower market. Furthermore, the flower production system can move into greenhouse/indoor production systems, where the yield and flower quality are vastly superior. Cellular agriculture systems can be built anywhere, freeing up prime farmland

for other activities, but also reducing the cost and environmental impact of producing cannabinoids.

Aside from the production process, several regulatory and product validation developments are badly needed to meet consumer demand for cannabinoids. These include unified federal regulatory bodies issuing clear product specifications, a single validated method to accurately and repeatedly determine cannabinoid and residuals concentration, and established nomenclature for definitions of cannabinoid ingredients (for example, the difference between a plant material, distillate, concentrate, or isolate).

Reference
1. *Hemp Benchmarks 2019 Report (2019). Available at: www.hempbenchmarks.com*

Making the Grade

To meet the growing demand for high-quality cannabinoids for pharmaceutical use, the industry is increasingly turning to synthetic production methods

By Kevin Hennessy, Global Director, Business Development and Portfolio Strategy, Johnson Matthey, Philadelphria, Pennsylvania, USA

The application of cannabis-derived medicines in the pharmaceutical sector has come a long way since the 1980s. Back then, the only cannabinoids approved as pharmaceuticals were synthetic tetrahydrocannabinol (THC) analogs, mainly applied to cancer and HIV patients suffering from nausea, vomiting, and poor appetite. Today, we're seeing cannabinoid therapeutics being investigated to treat a myriad of conditions, from epilepsy to multiple sclerosis.

Cannabinoid therapeutics are even being investigated as a treatment for COVID-19. Specifically, the anti-inflammatory and immunomodulatory effects of cannabinoids are being assessed to tackle the "cytokine storm" associated with severe cases of the disease.

The current attitude to cannabis within the pharmaceutical industry is one of liberalization. Across the world, we see regulatory changes that have contributed to the expanding use of cannabis in medicine. In particular, innovators in the cannabinoid space are developing multiple new therapies based on CBD, which does not cause the intoxication that comes with THC analogs, and are therefore not considered restricted drugs in most territories.

The discovery that CBD is an effective medication for Dravet syndrome in children ignited widespread interest from pharmaceutical companies. Today, there is a growing body of evidence to suggest that CBD is beneficial to treat the symptoms of multiple sclerosis, anorexia, schizophrenia, and other conditions.

Despite the growing availability of CBD, many uncertainties remain about the legality, quality, and safety of the drug. One major concern is bioavailability. Cannabinoids are highly lipophilic and exhibit poor oral bioavailability – estimated to be as low as 6 percent (1). As such, developers are forced to design sophisticated drug delivery technologies to help their products get to market, which often requires specific particle-size engineering.

With the growing requirement for GMP-grade material within the pharmaceutical sector, cannabis processors need to seek out expertise in the development and manufacturing of cannabinoids. Recent supply issues caused by the COVID-19 pandemic are likely to drive the development of ultra-pure synthetic offerings through high-quality raw material sourcing and refinement strategies for process chemistry and products.

For a potential active pharmaceutical ingredient (API) to navigate the regulatory minefield, manufacturers must meet strict quality controls on purity and consistency. For instance,

"To ensure regulations are adhered to, and to safeguard the health of consumers, manufacturers increasingly apply optimized, synthetic routes to purify cannabinoids."

drugs using CBD as the API must ensure they meet the extremely low THC threshold, and avoid the psychoactive side-effects, especially for pediatric or other vulnerable populations.

To ensure regulations are adhered to, and to safeguard the health of consumers, manufacturers increasingly apply optimized, synthetic routes to purify cannabinoids. Control over every available parameter will improve yields while avoiding impurities and contamination.

The future for cannabis-based therapeutics is indeed bright. And with new players entering the field, it is vital to ensure that partnerships with established forces are forged. I believe that the right scientific expertise and manufacturing capabilities will play a crucial role in helping the industry fulfil its true potential.

Reference

1. *CJ Lucas et al., "The pharmacokinetics and the pharmacodynamics of cannabinoids." Br J Clin Pharmacol, 84, 2477–2482 (2018). DOI: 10.1111/bcp.13710*

the Cannabis Scientist®
INNOVATION AWARDS

WELCOME TO OUR INAUGURAL INNOVATION AWARDS – CELEBRATING INNOVATIVE SOLUTIONS IN ALL AREAS OF CANNABIS SCIENCE

We launched the Innovation Awards to recognize the new products and processes driving cannabis science forward. Whether in testing, processing, medicine, or botany, innovation is bubbling over in this emerging field. We asked for nominations for the best innovations in two categories – tools and applications – released since January 2019. The entries were then reviewed and scored by an eight-strong panel of expert judges from analytical, extraction, formulation, and plant science. And so, without further ado, we count down the top five winners in each category!

TOP 5 TOOLS

What tools or technologies have made cannabis scientists' lives easier?

5

HEMP ANALYZER

Streamlined analytical toolkit for testing hemp to USDA requirements

Created by Shimadzu

Why is it innovative?

Obtaining consistent analytical results has been a major challenge for the hemp industry. To address this issue, Shimadzu introduced the "Hemp Analyzer" as a means of providing the same results from sample to sample, instrument to instrument, and operator to operator. Similar to the company's earlier Cannabis Analyzer, each Hemp Analyzer is provided with a "Cookbook" detailing sample preparation procedures, premixed mobile phases, and a premixed 11-cannabinoid standard. The hardware is integrated into a single box containing all of the HPLC components, including sample tray, injector, pumps, columns, and detectors. All of the analytical conditions are preprogrammed in the software.

What is the potential impact?

The easy-to-use HPLC device cuts down on time-consuming method development and allows users to start running samples quickly and getting consistent measurements. The aim is to allow anyone in the industry – whether amateur scientist, farmer, or chemist – to produce the same analytical results and meet the USDA's 2018 Farm Bill requirements.

The judges said...

I have used both the Hemp Analyzer and Cannabis Analyzer in the field. This instrument revolutionizes potency analysis and is extremely user-friendly.

It is important for growers to be able to do testing due to the legal and financial risk involved in growing hemp. Simplifying LC is likely a better approach than some of the other technologies, due to the legal requirements involved. Results can also be correlated with laboratory results.

ARCATECH

4

Combining data science, genome editing, and predictive breeding to rapidly prototype and advance cannabis varieties

Created by Arcadia Biosciences

Why is it innovative?

The ArcaTech platform is a combination of several technologies, including screening tools to identify desirable genetic variation (TILLING) and CRISPR-Cas9 gene editing to edit plant genomes. Having worked on various crops over the years, the company decided to apply this technology to the growing hemp market and last year launched a line of hemp seeds promising consistent low THC levels and disease tolerance. However, the company's plans for hemp don't end there. They say that the ArcaTech platform creates an opportunity to address challenges in completely new ways. For example, current high-CBD hemp varieties are not well adapted to tropical latitudes. The company hopes to use genomics and phenomics to identify the genetic circuits that control photoperiodism and use this knowledge to create a continuum of new varieties adapted to various latitudes.

What is the potential impact?

The company says, "We believe that by targeting this powerful technology platform at a semi-domesticated crop like cannabis, we have the opportunity to rapidly solve grower challenges such as disease, yield, and plant architecture." For example, the platform could be used to engineer varieties with custom cannabinoid and terpene profiles for the wellness market, improved flavor and oil profiles for grain, or higher quality fiber for industrial uses.

The judges said...

If the results prove accurate and precise, the potential impact of this technology is endless.

Enabling the easy genetic modification of hemp cultivars to adapt to different latitudes is a game-changer since at present hemp tends to become "hot" if not grown in the appropriate climate.

PULVERISETTE 19 PRECISION CUTTING MILL SYSTEM

Milling re-engineered for the cannabis industry

Created by Fritsch Milling & Sizing

Why is it innovative?
Efficient grinding of cannabis flower to a consistent particle size is important for producing a wide range of cannabis products, from pre-rolls to extracts. This easy-to-use homogenization system aims to make milling easy, with a variable-speed motor (300-3000 rmp) and adjustable, controlled particle size output. The Pulverisette 19 is suitable for large-scale operations, allowing continuous processing of 1-5lbs per minute on average, and is designed with GLP/GMP in mind.

What is the potential impact?
The judges saw this technology as another step towards standardization and efficiency in the industry, allowing greater product consistency and improved extraction yields at greater speeds. Plus, the speed of cutting allows no time for friction to generate heat, so cannabinoids and terpenes are preserved.

The judges said...

This is a fun tool and totally necessary for this industry since "one size fits all" particle size reduction is unacceptable for separate methods of extraction. The variable sizes, speeds, and GMP compliance make this an engaging tool.

Particle size and milling are vital to extraction optimization.

JAMAICAN LION REFERENCE GENOME

A comprehensive cannabis reference genome

Created by Medicinal Genomics, in collaboration with DASH Cryptocurrency, New England Biolabs, Phase Genomics, and Pacific Bioscience

Why is it innovative?
Released in January 2019, the Jamaican Lion Reference Genome is the most comprehensive cannabis reference genome to date. Compared to previous reference genomes it is made up of fewer, longer pieces of genetic code (contigs), which can be more easily arranged, with fewer gaps, creating a more complete genome.
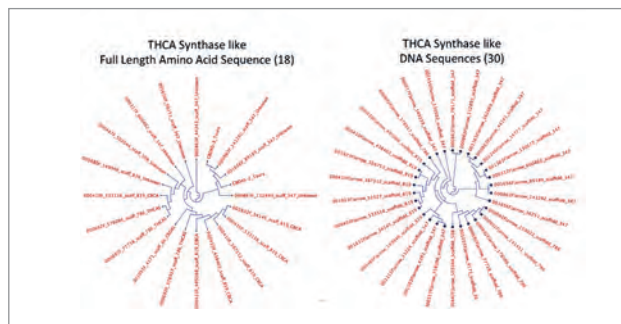
What is the potential impact?
The company says "The completeness of this genome and its publication on its blockchain ushers in a new era of intelligent breeding, with the potential to change the way cannabis is grown, sold, and consumed." It also provides a foundation for others to build upon to fully characterize the genome and understand how it functions. Some of the discoveries made so far include identifying important locations for improved seed production and gene families that regulate the production of specific cannabinoids. In the long term, the company hopes to see a genomics-based approach to pest control that uses the cannabis plant's natural defenses to reduce pesticide use and make farmers more profitable.

The judges said...

Extremely innovative. Having the opportunity to completely characterize cultivars makes personalized drugs a real future application.

This information is fundamental and could have far-reaching impacts in many areas, including medical product consistency.

1

TRIPLOID SEED

Triploid cannabis varieties that cannot be seeded by rogue pollen

Created by Oregon CBD

Why is it innovative?

If female *Cannabis sativa* plants are pollinated (by pollen from a rogue male/hermaphrodite plant on the same site or drifting across from a neighbor's farm) it causes the plant to produce seeds instead of buds and significantly reduces overall biomass – disastrous for farmers.

Oregon CBD claims farmers can avoid this risk altogether. How? Wild cannabis is usually diploid (two sets of chromosomes) but spontaneous mutations can produce plants that are tetraploid (four sets of chromosomes). By crossing diploid and tetraploid plants Oregon CBD scientist Hsuan Chen and colleagues created triploid plants, which have a number of advantages – most importantly, they produce sterile flowers and pollen, eliminating the risk of accidental fertilization.

What is the potential impact?

According to the company, farmers using the triploid seeds will no longer have to worry about crop loss due to pollination events, and can grow grain and fiber crops alongside cannabinoid crops without issue. Tests grows of the triploid plants suggest that the increase in ploidy also makes for more vigorous, hardy, and aromatic (via increased secondary metabolite production) plants.

The judges said…

Cross-pollination is a phenomenon that can ruin entire crops at a time. Avoiding and mitigating this risk through promising technology like triploid seeds will increase revenues and give farmers peace of mind during cultivation.

Pollination of crops, causing the development of seeds and resulting in lower yields and potency, is a huge problem in cannabis, especially in outdoor settings like California and Oregon.

This could change the entire growing portion of the industry so the potential impact is huge.

I would love to work with these!

TOP 5 APPLICATIONS

Just as important as having the right tools is knowing how to use them. Here are our judges' picks for the best application notes or method-based scientific papers from the past two years.

5

DETERMINATION OF PESTICIDE AND MYCOTOXIN RESIDUES IN DRIED CANNABIS FLOWER: LC-MS/MS AND GC-MS/MS METHODOLOGY TO MEET THE RECOMMENDED AOAC REGULATORY REQUIREMENTS FOR US STATES AND CANADA

By Kim Tran, Michael Young, Kari Organtini, Marian Twohig, and Christopher Hudalla

"A sensitive method to meet US and Canada requirements for all regulated pesticides and mycotoxins. Simple extraction and cleanup protocols followed by LC-MS/MS and APGC-MS/MS analysis provide rapid, sensitive, and robust workflows for determination of pesticides and mycotoxins in the challenging cannabis matrix.

Method detection limits meet or exceed AOAC SMPR requirements for all pesticides with regulatory imposed action levels in any US State or Canada. The multiresidue analysis of 105 pesticides plus mycotoxins can be accomplished using the same mass spectrometer (Xevo TQ-XS) for both UPLC and APGC analyses."

Read the article: bit.ly/34AtmNZ

The judges said…

Testing of pesticides is crucial and any method that makes it easier/better is good news.

4

MOISTURE CONTENT DETERMINATION IN CANNABIS INFLORESCENCE

By Zachary Parish, Conner Griffeth, Dennis Acord, Wallace Harvey, Klaus Schöne, and Evelyn Marschall

"Based on the loss-on-drying method, in this study we compare the classical oven method with the infrared method, in which two moisture analyzers from Sartorius are tested with regard to differences to the oven reference, the precision and measurement times depending on the sample quantity and initial moisture.

There is no current approved standard methodology for performing moisture analysis in cannabis/hemp. This was the first published extensive study done in collaboration with the cannabis testing lab industry that indicates the potential for using moisture analyzers as a suitable methodology for determining moisture content in cannabis/hemp."

Read the article: bit.ly/34CEZUN

The judges said…

Determining moisture content is vital in several aspects of the supply chain from curing flower to understanding the chemical profile for extraction.

As someone who is all too familiar with the innate flaws associated with the "loss on drying" technique for moisture content determination, I am very pleased to see that this important parameter in cannabis production is being more thoroughly investigated through collaboration between an established testing lab and instrument vendor.

3

UTILIZATION OF DESIGN OF EXPERIMENTS APPROACH TO OPTIMIZE SUPERCRITICAL FLUID EXTRACTION OF MEDICINAL CANNABIS

By Simone Rochfort, Ashley Isbel, Vilnis Ezernieks, Aaron Elkins, Delphine Vincent, Myrna A. Deseo, and German C. Spangenberg

"Carbon dioxide supercritical fluid extraction (CO_2 SFE) is a clean and cost-effective method of extracting cannabinoids from cannabis. Using design of experiment methodologies an optimized protocol for extraction of medicinal cannabis bud material (population of mixed plants, combined THC:CBD approximately 1:1.5) was developed at a scale of one kg per extraction. Key variables investigated were CO_2 flow rate, extraction time, and extraction pressure. A total of 15 batches were analyzed for process development using a two-level, full factorial design of experiments for three variable factors over eleven batches. The initial eleven

batches demonstrated that CO_2 flow rate has the most influence on the overall yield and recovery of the key cannabinoids, particularly CBD. The additional four batches were conducted as replicated runs at high flow rates to determine reproducibility. The highest extraction weight of 71g (7.1 percent) was obtained under high flow rate (150g/min), with long extraction time (600min) at high pressure (320bar). This method also gave the best recoveries of THC and CBD. This is the first study to report the repeated extraction of large amounts of cannabis (total 15kg) to optimize the CO_2 SFE extraction process for a pharmaceutical product."

Read the article: go.nature.com/37IILzLk

The judges said…

A nice DOE study that will be useful for many in the field.

If CO_2 extraction is going to be competitive with other extraction mediums, focusing attention on optimization is a must.

2

INVESTIGATION OF CHOCOLATE MATRIX INTERFERENCE ON CANNABINOID ANALYTES

By David D. Dawson and Robert W. Martin

"The first known findings of chocolate matrix interference on cannabinoid analytes is reported. Stock solutions of four biogenic cannabinoids (Δ9-tetrahydrocannabinol, cannabidiol, cannabinol, and cannabigerol) and one synthetic cannabinoid (cannabidiol dimethyl ether) are subjected to milk chocolate, dark chocolate, and cocoa powder. A clear trend of matrix interference is observed, which correlates to several chemical factors. The amount of chocolate present is directly proportional to the degree of matrix interference, which yields lower percent recovery rates for the cannabinoid analyte. Structural features on the cannabinoid analytes are shown to affect matrix interference, because cannabinoids with fewer phenolic –OH groups suffer from increased signal suppression. Additionally, aromatization of the p-menthyl moiety appears to correlate with enhanced matrix effects from chocolate products high in cocoa solids. These findings represent the first known documentation of chocolate matrix interference in cannabinoid

analysis, which potentially has broad implications for complex matrix testing in the legal cannabis industry."

Read the article: bit.ly/31PXvqI

The judges said…

I loved reading this paper. It brings awareness to how inaccurate the "dilute and shoot" method is on specific matrices and gives a good framework for how to handle difficult matrices.

It's important to acknowledge that we still don't know everything and that even common methods/matrices still need further investigation.
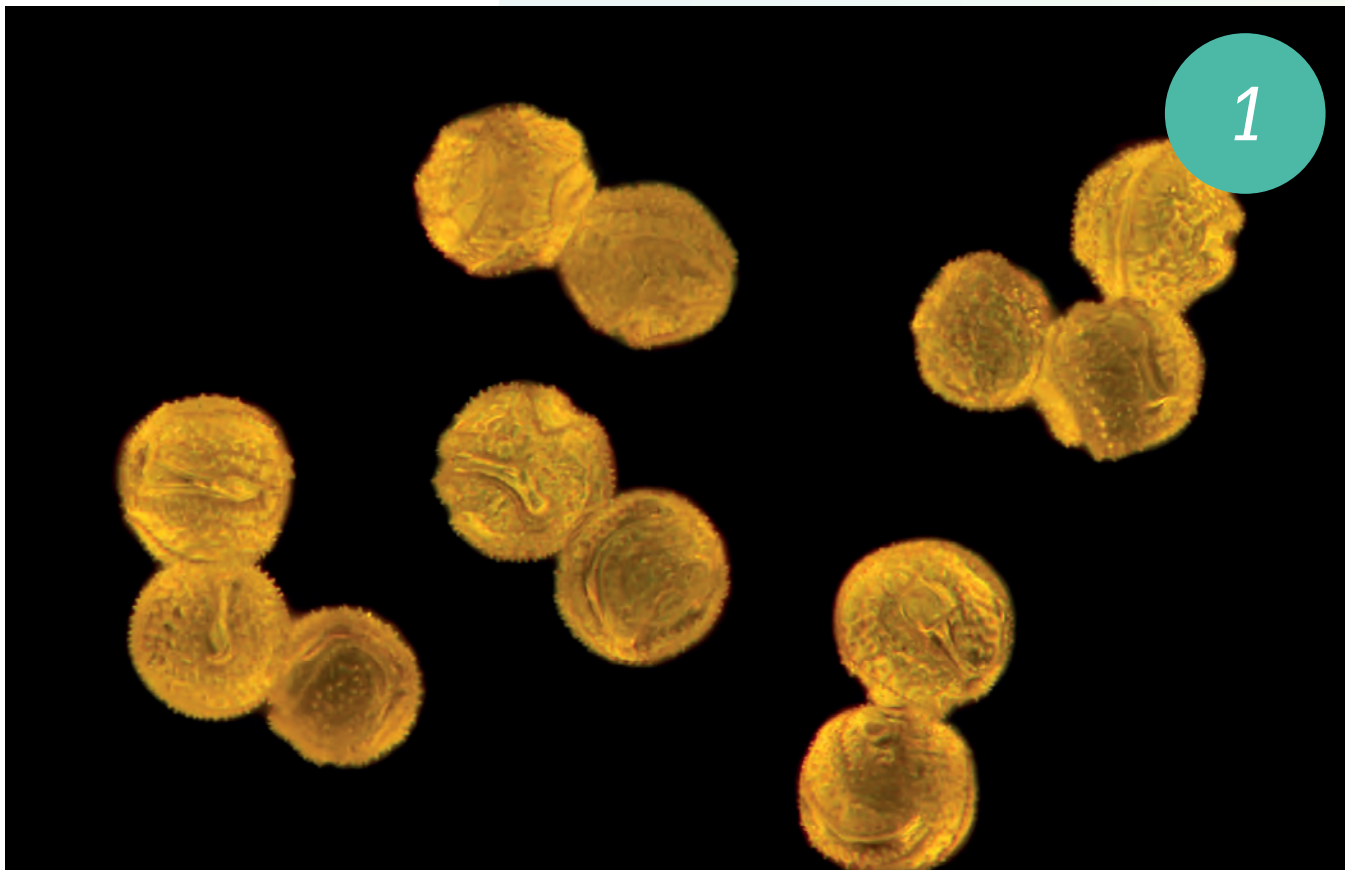
DEVELOPMENT AND OPTIMIZATION OF A GERMINATION ASSAY AND LONG-TERM STORAGE FOR CANNABIS SATIVA POLLEN

By Daniel Gaudet, Narendra Singh Yadav, Aleksei Sorokin, Andriy Bilichak, and Igor Kovalchuk

"Pollen viability and storage is of great interest to cannabis breeders and researchers to maintain desirable germplasm for future use in breeding or for biotechnological and gene editing applications. Here, we report a simple and efficient cryopreservation method for long-term storage of *Cannabis sativa* pollen. Additionally, the bicellular nature of cannabis pollen was identified using DAPI (4′,6-diamidino-2-phenylindole) staining. A pollen germination assay was developed to assess cannabis pollen viability and used to demonstrate that pollen collected from different principal growth stages exhibited differential longevity. Finally, a simple and efficient method that employs pollen combined with baked whole wheat flour and subsequent desiccation under vacuum was developed for the long-term cryopreservation of *C. sativa* pollen. Using this method, pollen viability was maintained in liquid nitrogen after four months, suggesting long-term preservation of cannabis pollen."

Read the article: bit.ly/35E77pG

The judges said…

Loss of genetic diversity in cannabis is a big problem.

This technique and its application could be a game-changer for preserving that diversity. I only wish this had been available while landrace strains were more commonly available.

Saving pollen will be great for my research!

Are You Still High?

THC Metabolites in Urine

By Adam L. Moore

In humans, cytochrome P450 contains three primary enzymes involved in the metabolism of Δ9-tetrahydrocannabinol (CYP2C9, CYP2C19, CYP3A4). These enzymes are mostly found in the liver, but can occur in other lipophilic tissues like brain, small intestine, heart, and lungs. THC elimination in the body is dictated by the accumulation of the compound in both adipose tissue and plasma and, due to the lipophilicity of THC, the determination of the concentration through excretion is difficult (1,2). Likewise, states have passed regulations allowing for the legal use of both medicinal and recreational cannabis while workplaces in those states continue to dictate safety regulations with a zero tolerance drug policy culminating in a urine test to determine compliance. With more than 100 THC metabolites, detection of the most abundant, 11-hydroxy-THC, 11-carboxy-THC glucuronide, and 11-carboxy-THC, hold the most focus (Figure 1). Typically, between 80 and 90 percent of the THC consumed is excreted as carboxylate and hydroxylate metabolites (3,4). The Hamilton PRP-C18 reversed-phase column offers good selectivity and peak shape when detecting the three major components of THC metabolites in urine. As shown in Figure 2, samples spiked with metabolites in urine show good correlation in low concentrations and rapid sample analysis can be achieved in under eight minutes. In addition to good column performance, all Hamilton columns come with value added features that include great chemical and thermodynamic stability and increased lifetime due to their PS-DVB backbone.
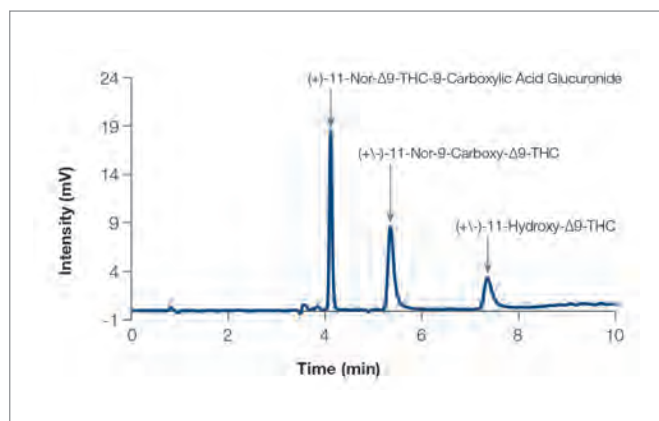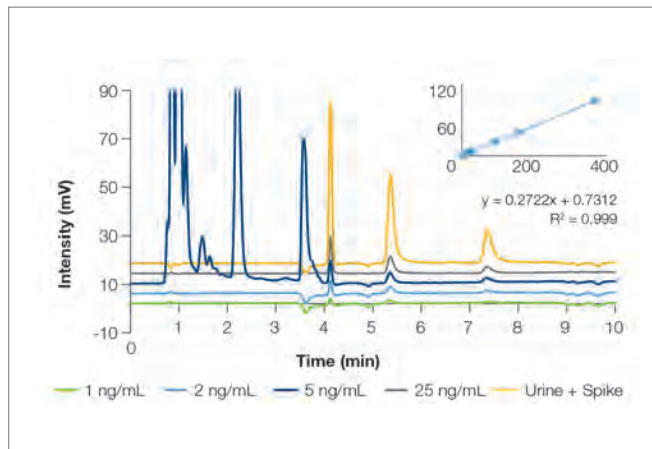

Figure 1. Urine sample spiked with 3 ng/mL of THC metabolites.

Column Information

Packing Material	PRP-C18, 5 μm
P/N	79676

Chromatographic Conditions

Gradient	0.00–2.00 min, 5%B
	2.01–3 min, 5–50%B
	3.01–8 min, 50–95%B
	8.01–10 min, 95%B
Temperature	35°C
Injection Volume	5 μL
Detection	UV at 230 nm
Dimensions	150 x 4.6 mm
Eluent A	10 mM CH_3COONH_4
Eluent B	CH_3CN
Flow Rate	2.0 mL/min

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1. CJ Lucas et al., "The pharmacokinetics and the pharmacodynamics of cannabinoids," *Br J. Clin Pharmacol*, 84, 2477 (2018).
2. F Grotenhermen, "Pharmacokinetics and pharmacodynamics of cannabinoids," *Clin Pharmacokinet*, 42, 327 (2003).
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4. DJ Harvey, "Absorption, distribution, and biotransformation of the cannabinoids," *Marihuana and Medicine*, 91. Humana Press: 1999

For more information on Hamilton HPLC columns and accessories or to order a product, please visit www.hamiltoncompany.com or call (800) 648–5950 in the US or +40–356–635–055 in Europe.



Figure 2. Most abundant THC metabolites.

Spotlight on...
Technology

Introducing the Anden A710 Industrial Dehumidifier – Grow-Optimized with VLGR Technology

The Anden A710 Dehumidifier is designed for cultivation from the ground up. It utilizes innovative Variable-Speed, Low Grain Refrigerant Technology (patent pending) to deliver maximum performance and efficiency throughout the grow cycle. With the A710, you're working with the latest technology to ensure maximum moisture removal and optimal VPD control.

For more information visit:
https://www.anden.com/a710/
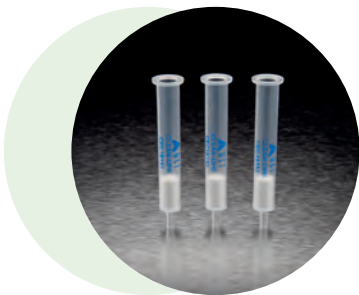
UCT's 30+ Years in the Making, Gold-Standard C18 SPE Column

UCT's rugged Clean-Up® C18 SPE Column has quickly become to go-to cannabis filtration approach to isolate your cannabinoids from your pesticides - available in both SPE and push-thru column formats.

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INTip Filtration

Filtration tips are compatible with all cannabis sample types (plant material, extracts, oils, edibles, etc) to replace the use of manual, tedious and multi-component syringe filters. These products seamlessly integrate with automated liquid handlers and remove particulates down to less than 1 μm to prevent clogging of chromatography columns.

*For more information visit:*
https://bit.ly/DPXCannabis

Cannabis Profiler: One System for Six Cannabinoids

The AZURA Analytical system by KNAUER is dedicated for the analysis of six common cannabinoids of high medicinal interest. Cannabidiol (CBD), cannabidiolic acid (CBDA), cannabinol (CBN), $\Delta 8$-tetrahydrocannabinol ($\Delta 8$-THC), $\Delta 9$-tetrahydrocannabinol ($\Delta 9$-THC), and $\Delta 9$-tetrahydrocannabinolic acid ($\Delta 9$-THCA) can easily be quantified according to the monography of German Pharmacopeia.

Learn more at www.knauer.net

Green Genes

Sitting Down With… Keith Allen, Director of Bioinformatics, Front Range Biosciences, Lafayette, Colorado, USA
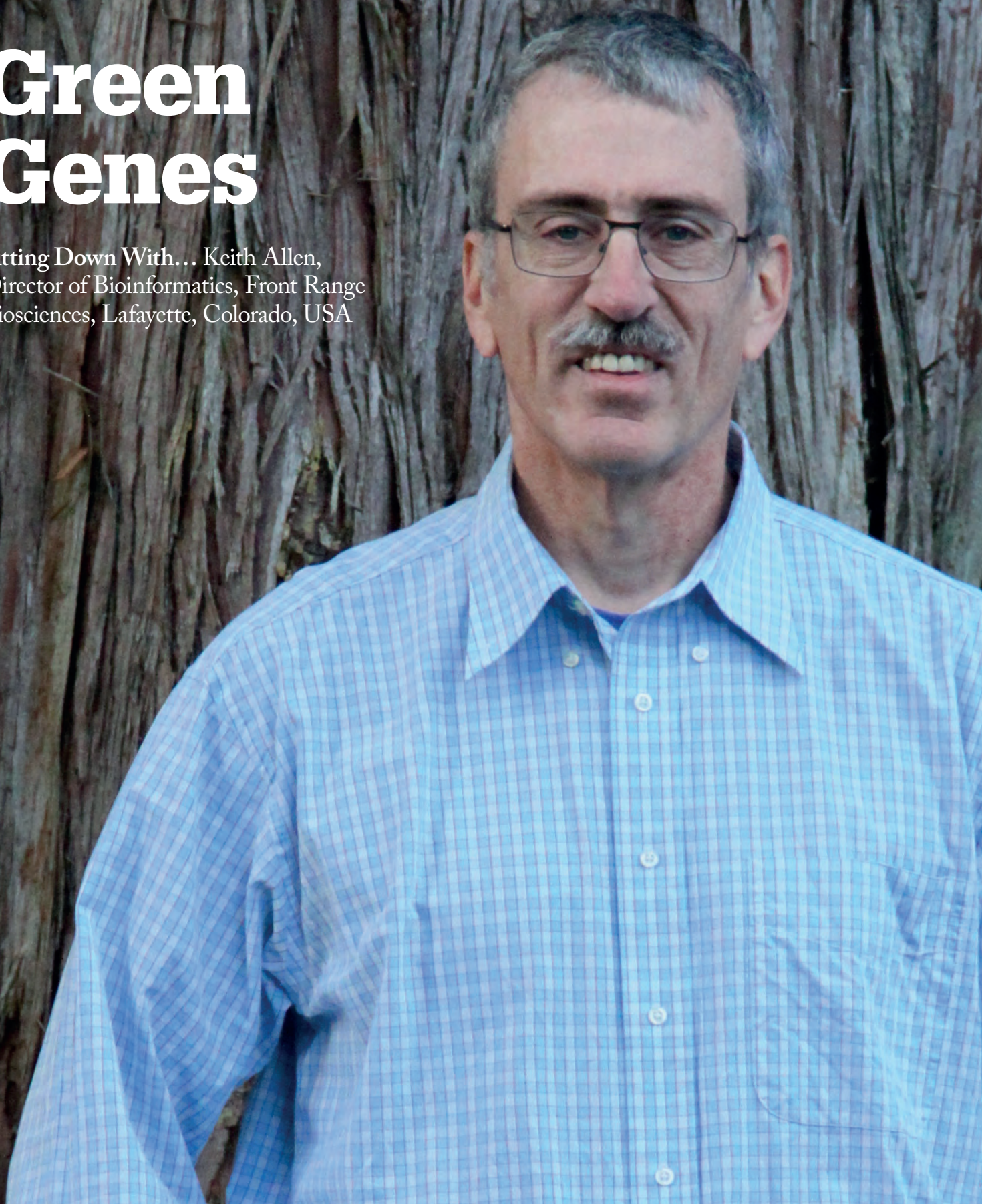
What were you doing before entering the cannabis industry?

I originally trained as a biologist – my undergraduate degree was in biology and I earned my PhD studying photosynthesis in plants. During my postdoc in plant developmental genetics, I discovered the then-brand-new field of bioinformatics and was hooked! I moved from academia into biotech, where I worked on gene discovery, first in mice and later in plants.

In 2005, I was hired by Syngenta to find the genes underlying the traits that are most important to modern agriculture, including drought tolerance, nitrogen use efficiency, and overall yield. Eventually, I was promoted to a research fellow, which was a very cool gig. My job was to advise the company on what was coming down the pipe in my area of expertise – to prepare for a coming challenge or capitalize on an emerging niche. The skills I developed during my time in "big ag" have proved invaluable in my work in cannabis.

How did your move into cannabis come about?

Around five years ago there was a drop in commodity prices and the agriculture sector was hit hard. I was made redundant and there weren't a lot of roles available in traditional agriculture.

I'd been keeping an eye on the emerging cannabis and hemp markets for years, intrigued by the rapidly shifting legal and scientific landscape. I took on some consulting work in the industry and started analyzing published data sets to find interesting new patterns. Often, scientists will generate huge data sets in order to answer a particular question – I looked at the same data but asked different questions. I built a name for myself in the industry and was hired, first by Steep Hill and now Front Range Biosciences.

How does cannabis compare to traditional crops?

Moving into the cannabis industry from big agriculture was both frustrating and exciting. I was used to working in species like tomato, corn, and rice, where we have a huge knowledge base. Whole academic careers have been spent studying tomatoes alone, and the genomes of major crop species are complete and well-annotated. I came into the cannabis field and, frankly, the genome was a mess. We still don't have a full understanding of the genes in the cannabinoid and terpene pathways that lie at the heart of the industry – let alone the rest of the genome.

What does your role at Front Range involve?

An important part of my work is improving our knowledge of cannabis genetics. I published a paper last year on the terpene synthesis gene family (1) and I'm now working on the genes leading up to cannabinoid biosynthesis. What I'm discovering is that every step of the cannabinoid pathway is more complex than we thought at first blush. At the same time, I'm looking at genetic variation across different cannabis cultivars. Together, these two lines of work form the foundation for our eventual goal: creating cultivars with a consistent chemical profile.

There are many cannabis consumers who are only interested in getting the biggest high – that demographic is well served by the current market. But others are looking for something more subtle and specific – the older user who wants to feel stimulated but not stoned or the patient who needs a precise formulation to treat a specific illness. These groups want to go to a dispensary, buy the same strain, and know that they will get the same effects. We want to serve that market.

Where do you see cannabis cultivation heading?

I believe that the future of cannabis, at least for medical use, lies in careful inbreeding to produce largely homozygous strains, supplied with precise growing instructions.

> *"Every step of the cannabinoid pathway is more complex than we thought at first blush."*

The ultimate goal is to understand enough about the plant to breed all the different varieties that there is a demand for, or could be a demand for in the future. Beyond CBD synthase and THC synthase, there are another 16-plus active synthase genes in this family, which we are working to functionally characterize. Some of them are making unknown cannabinoids and – as any pharmaceutical chemist will tell you – if you know that one member of a molecular family is of medical interest you're going to want to explore all the related molecules. It will take a while to bottom out but I think it's worthwhile.

With more and more serious genomics people entering the field, I'm hopeful for the future.

The industry is changing fast…

Many cannabis companies now are high-tech operations with excellent scientists doing really interesting work. The more professional it becomes, the more attractive the field is going to be, and with legalization marching onwards there will be fewer and fewer barriers for people wanting to move into this space.

Reference

1. KD Allen et al., "Genomic characterization of the complete terpene synthase gene family from Cannabis sativa," PLoS One, 14, e0222363 (2019). DOI: 10.1371/journal.pone.0222363.