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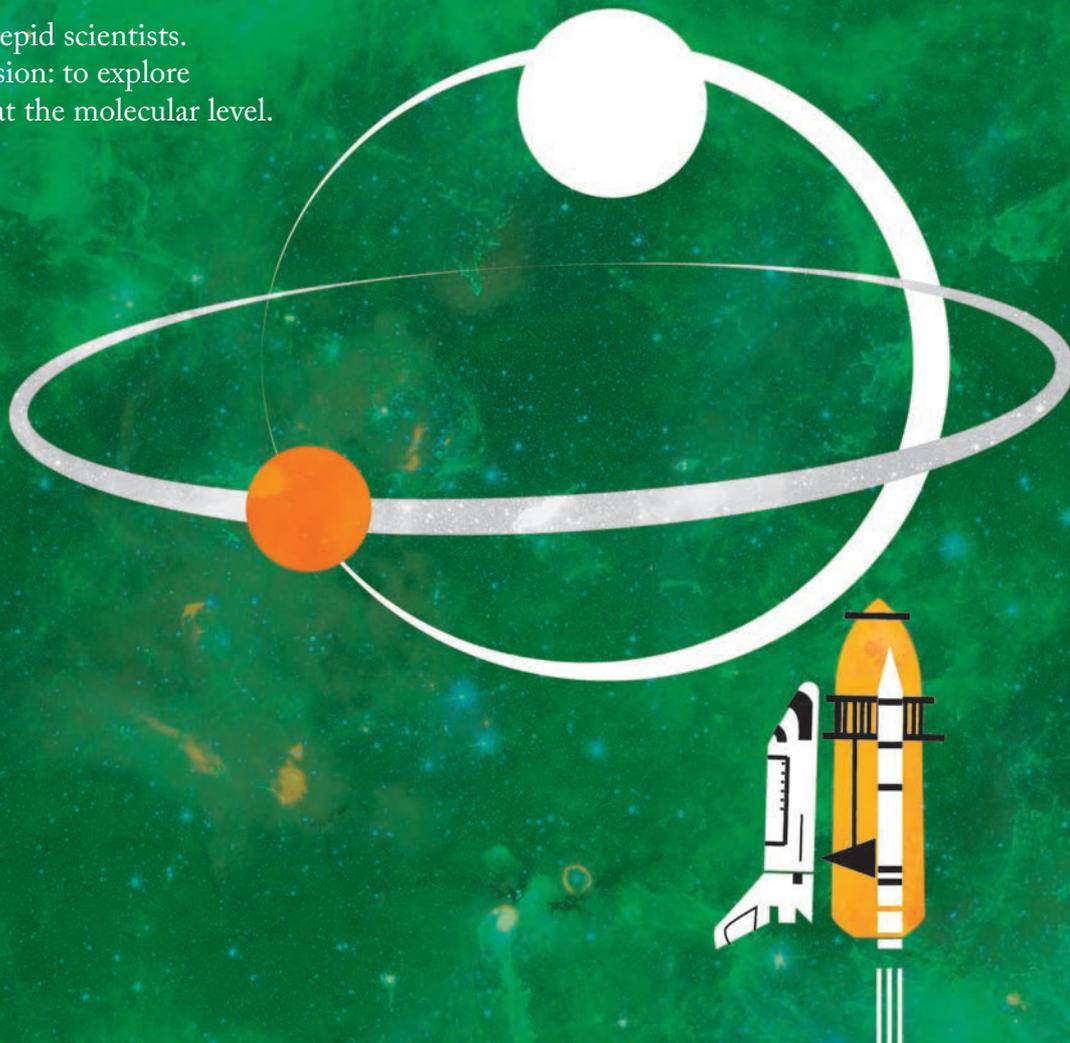
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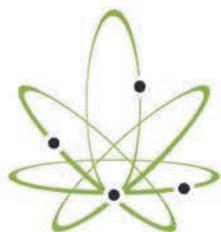
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Meet three pioneering scientists on a mission to explore the genetic and chemical profile of cannabis – from astronomical genetic variation, to a constellation of bioactive compounds.

Sitting Down With

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A Rose by Any Other Name

As the industry evolves, so must the language of cannabis

Editorial



*What's in a name? That which we call a rose
By any other name would smell as sweet*

– William Shakespeare, *Romeo and Juliet* (2.2.42–44)

Juliet assures Romeo that a name is essentially meaningless – a sweet sentiment but, given the eventual fate of the star-crossed lovers, perhaps rather misguided.

Indeed, a name can mean a great deal. For instance, a study by Limb, Limb, Limb and Limb (yes, really) suggests that doctors are drawn to specialties that reflect their name (people called Limb are more likely than most to become orthopaedic surgeons, for example) (1).

And when it comes to forging a modern cannabis industry, names matter. On page 15, Duke Rodriguez argues that use of the term 'marijuana' is outdated, pejorative – even racist. It's a word that reflects the prevailing attitude of the 1930s, when marijuana was seen as "the devil's weed", brought to America by immigrants and driving the country's youth to ruin. In contrast, 'cannabis' is the Latin name of the plant's genus – no more and no less. Will saying 'cannabis' instead of 'marijuana' transform the industry overnight? Of course not. But neither is the choice of name irrelevant to how the industry is viewed – or how it views itself.

As the cannabis industry – and cannabis science – emerges as a vibrant, forward-looking field, the way its members talk about the plant and their work is changing. And we can expect the language of cannabis to continue to evolve. As genetic and chemical analyses reveal the true nature and origin of different strains, the rather haphazard naming of varieties is likely to become more streamlined, as multiple names for the same variety are consolidated (page 18). And with experts predicting the rise of 'personalized cannabis medicine', I wonder if we will see a move away from the more colorful names of today. A prescription for 'Steve McGarretts Hair' may fail to engender confidence in an elderly cancer patient...

The jargon associated with many technical and scientific professions can be frustrating for those not in the know, but using precise language means that specialists can 'speak the same language', avoiding ambiguity and confusion. The cannabis industry is not like any other industry, nor should it try to be. Perhaps a wiser aim would be, as Robert Martin advocates on page 27, to "take the best of the corporate experience, and leave the worst behind". Part of that process will be finding the right terminology.

Reference

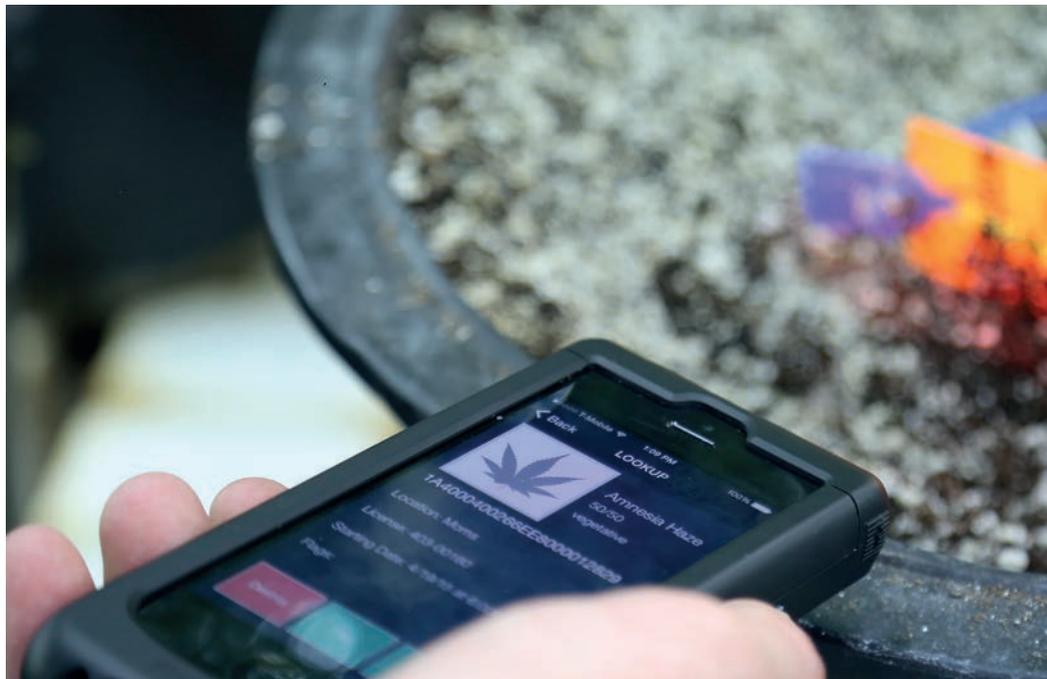
1. 1C Limb et al., "Nominative determinism in hospital medicine", 97, 24–26 (2015).

Charlotte Barker
Editor

Upfront

Reporting on research, personalities, policies and partnerships that are shaping cannabis science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker@texerepublishing.com



Professional Growth

How software could enhance transparency in cannabis cultivation – and help speed up the legalization process

In 2014, digital entrepreneur and former grow-worker Kyle Sherman spotted a niche in the market for tracking software that simplified the cannabis supply chain process. The result was Flowhub (<http://bit.ly/2l1F7TU>), which has since won multiple awards and attracted customers from big retailers in medicinal and recreational cannabis. We asked for Kyle's top lessons learned about the cannabis industry.

Professionalism and transparency are key

When I joined this industry, I realized that while there was a lot of passion, there

wasn't a whole lot of professionalism. There was no custom software platform that could collate mandatory compliance data and report it back to the government automatically – mistakes were being made, issues were reported incorrectly, information was incomplete. I saw that if we built something customized to the industry, we could solve a lot of problems. I started to build prototypes and workflows for the grow I worked at, and quickly realized that this could be used by the entire industry. I felt that if we could increase transparency, governments would be more likely to legalize cannabis in their localities – which means easier access to medicine.

Technology plays a vital role

We noticed early on that lot of people in the industry don't necessarily have supply chain access, but are really passionate about legalization. We've really had to understand how they interact with technology, so that they could get their data out of the system easily, automate



elements, increase efficiencies, and ultimately become better at their jobs. That's why we created the NUG – a handheld device. We went from pencil and paper to a scanner. With Flowhub's Cloud, we're essentially able to take all the data, aggregate it, and send it up to the state government on their behalf. It makes a big difference...

There's still a lot to learn. We're not just building a business; we're building an industry. For us, it's about defining the future by working closely with retailers and growers, and understanding their needs day-to-day. We need to anticipate where the industry is going and drive things in a more efficient direction logistically. It's a collaborative effort, going out and learning these lessons, finding these

problems and solving them. Each day in cannabis is like a month in another industry – it's so fast moving.

Advocacy is the lifeblood of the industry. We've got to push this forward and it starts with the users – people who have been helped by the medicinal side of cannabis. I have been helped by cannabis myself – that's why I'm in this industry. I've met cancer patients, people with arthritis and Parkinson's, and all of them have seen such a huge benefit. I think a lot of companies don't know why they do what they do every day, but with Flowhub we all have a real reason to wake up in the morning: to help bring access to this medication to people across the country, and show people that it's something that we can do responsibly. It's a great thing to be a part of.

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Breaking with Tradition

What does legalizing medical cannabis mean for conventional pharmaceuticals?

Do people change their use of prescription medications when cannabis becomes a legal alternative? Father-daughter research team W. David Bradford and Ashley Bradford, from the University of Georgia, trawled through the data to uncover the truth, and their findings could play a part in the rescheduling of medical cannabis – as well as speaking volumes about public opinion.

The Bradfords analyzed data on prescription drug use in the US from 2010 to 2013, focusing specifically on patients covered by Medicare Part D, a federal government program that subsidizes prescription drugs for people over 65 – an age range thought to be most opposed to using cannabis. “It was a question of robustness,” says David Bradford, Adjunct Professor in the Department of Economics. “We had good data on Part D drug use, and believed that if we found an effect we could be confident that it was real. Ultimately, we were surprised at how

statistically significant and robust the results were.”

The researchers estimated that implementing a medical marijuana law (MML) reduced Medicare Part D spending by about 0.5 percent in nine disease categories. Bradford breaks this down: “In 2013, that represents \$165 million saved for the 17 states and the District of Columbia that had medical marijuana laws in effect. If all states had had a medical marijuana law in effect that year, Medicare Part D spending would have been about \$470 million lower.”

The team has already examined the effect of MMLs on opiate-related deaths in the United States, and found that opiate deaths fell significantly when states implemented dispensary-based MMLs. They have also examined the impact of MMLs on Medicaid prescription drug use, and the findings were even more noteworthy: “The magnitudes in Medicaid were a good bit larger – 2 to 4 percent reductions in Medicaid spending when MMLs go into effect, compared with a one-half percent reduction for Medicare.”

The report certainly has garnered



attention, both in scientific spheres and across the media, with references to the work made in at least one US Senate hearing. But the Bradfords were more interested in another implied aspect of the findings. “Patients and doctors together are treating medical marijuana as a real alternative to pharmaceutical medication,” says Bradford. This, if true, emphasizes the importance of further scientific research – with better health care the ultimate goal. *JC*

Reference

1. AC Bradford, WD Bradford, “Medical marijuana laws reduce prescription medication use in medicare,” *Health Aff*, 35, 1230–1236 (2016).

Treating Opioid Addiction with CBD? TBD...

Could cannabis come to the rescue in the midst of an “opioid epidemic”?

Whether in medical or recreational contexts, questions are repeatedly raised about the addictiveness of cannabis. But studies over the last few years suggest that cannabis could actually assist in the fight against addiction – to opioids.

Prescriptions for opioids in the US are estimated at over 200 million a year, with pain management a major driver. Methadone, buprenorphine

and naltrexone have all been shown to be effective for acute severe pain – but the evidence that such pharmaceuticals are effective in treating chronic pain is far less compelling (1). However, according to a recent paper in *Neuron* journal, the cost of developing new drugs provides a disincentive to develop new therapeutics, leading to what the authors refer to as “widespread retreat of

the pharmaceutical sector” (1). Could cannabidiol (CBD) fill the void?

Research over the past few years supports the use of cannabis – specifically CBD – for treatment of addiction (2). Unlike Δ^9 -tetrahydrocannabinol (THC), CBD is non-rewarding, and modulates feelings of anxiety – a key feature of addiction (3). Combined with the fact that no severe side effects have been reported and its low lethality (3), some argue that this makes it a natural replacement for current pharmaceutically and legislatively approved medications. So why – as opioid addiction hits an all-time high – is this area of research being neglected?

In a paper published in Trends in Neurosciences earlier this year (3), neuroscientist Yasmin Hurd – whose own research indicates that CBD reduces heroin cravings and restores neurobiological damage – blames the reluctance to acknowledge CBD’s effectiveness on the scientific community’s exclusion from policymaking discourse. “Legalization has outpaced the science... This is one of the first times in US history that the question of whether a plant (or any drug) is an effective medicine has been decided at the ballot box.”

Hurd calls for a structure to “fast track” clinical CBD – and, importantly, to make trials looking at this particular cannabinoid a priority. “As the legal landscape for medical marijuana unfolds,” she says in the paper, “it is important to distinguish it from ‘medical CBD’ and other specific cannabinoids, that can more appropriately be used to maximize the medicinal potential of the marijuana plant.”

The situation certainly involves a complex interplay between neuroscience, pharma and the cannabis industry, but one thing is clear – both the barriers to scientific research and the opioid addiction epidemic need to be simultaneously addressed. And, Hurd suggests, until the scientific and medical communities are permitted to take a more leading role, safe and efficacious therapies will remain out of reach. *JC*

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2. YL Hurd et al., “Early phase in the development of cannabidiol as a treatment for addiction: opioid relapse takes initial center stage”, *Neurotherapeutics*, 12, 807-815 (2015).
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The Mother of All Detection

A new analytical approach can detect trace levels of cannabis in breast milk

If knowledge on the long-term effects of medical cannabis is patchy, it is virtually nonexistent when it comes to a less obvious consumer – the breastfeeding baby. Cannabinoids are highly lipophilic, so combine easily with the fats present in breast milk; when breastfeeding mothers consume cannabis, infant exposure is difficult to avoid.

As legalization at the state level grows and as cannabis is increasingly used for medicinal reasons, there are growing calls to assess any adverse impact and raise public awareness. But current analytical methods struggle to detect cannabinoids at the extremely low concentrations found in breast milk, increasing the risk of false negatives – especially in cases of passive exposure.

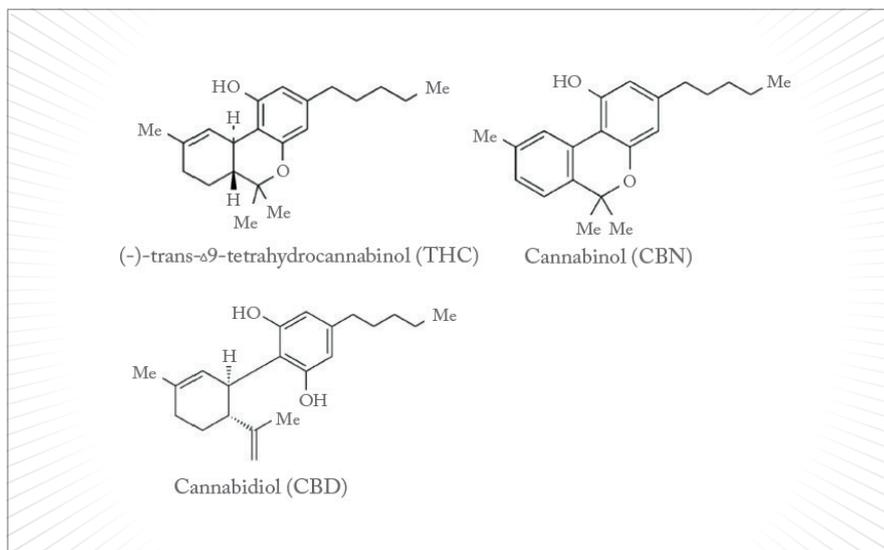
To that end, researchers at the Center for



Disease Control and Prevention (CDC) have developed a more sensitive approach to trace detection: alkaline saponification–solid-phase extraction (SPE) to separate

cannabinoids from the milk fats, followed by isotope dilution UHPLC-MS/MS. They achieved detection levels of 13, 4 and 66 pg/mL for Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN), respectively – a significant increase in sensitivity compared with current methods.

In the published paper (1), the authors state that they expect the new method “to play a critical role in assessing infant exposure to cannabinoids through breastfeeding.” Although we are still a long way off fully understanding the impact of cannabis on nursing babies, being able to measure potential exposure takes scientists one step closer. *JC*



Three key cannabinoids detected in the study.

Reference

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Participants at the 2016 Canna Boot Camp, a full-day, hands-on workshop hosted at Chalice Farms (Portland, OR).

Exhibiting Excitement

Joshua Crossney is President and Founder of jCanna, Inc., (www.jCanna.com) a Maryland-based non-profit organization devoted to the advancement of cannabis science, analytical testing, and personalized (precision) medicine. Josh organizes the newly minted Cannabis Science Conference, and is chairing a symposium on cannabis analysis at Pittcon 2017. Here, he updates us about the events that are getting the medicinal cannabis industry talking.

What was the response to the inaugural Cannabis Science Conference in 2016?

The feedback has been nothing short of amazing. We had over 750 attendees from all over the world, including Israel, Italy, Germany, Canada, Colombia, Germany, Japan, Australia, Denmark and Nigeria! Attendees said that the presentations “gave them goosebumps”, and there was a level of excitement, energy, and enthusiasm that you simply do not see at most tradeshow.

In short, many people from different backgrounds and areas of expertise came together to share their experiences and work together to build cannabis science. There was an air of collaboration, and a sense

that we began to overcome the 80-plus years of suppression and stigma associated with cannabis!

What topics will feature in the Analytical Cannabis Symposium at this year’s Pittcon?

My goal was to create a symposium that not only educates and informs, but also excites attendees to get involved. The focus is definitely more on the analytical testing and technology side, but we have incorporated medical, cultivation and cannabis industry



perspectives into the mix, with speakers chosen as expert representatives from these various disciplines.

Jack Henion, Scott Kuzdzal, Olga Shimelis and Melissa Fauth will speak about cannabis analytical testing challenges and opportunities, explaining the technologies and methods involved in everything from sample pre-processing to analytical testing and research. These experts are leading the way in cannabis analytical instrument and method development. We also have two medical doctors, Kevin Rosenblatt and Uma Dhanabalan, who will cover a variety of cannabis medical topics such as genomics, personalized medicine, and the history of cannabis as a medicine.

Personally, I am most excited to meet more analytical industry experts and talk to delegates. jCanna, Inc. will have an

exhibit booth (#5102) at Pittcon, and I encourage anyone that wants to learn more about the cannabis industry or our annual Cannabis Science Conference to stop by and say hello! I will also be moderating a Pittcon Conferee Networking Session.

How has the industry (and attitudes) changed in the last twelve months? Every year the cannabis industry, and cannabis science in particular, gains momentum. Every day I talk with companies eager to learn how their products or services might fit into the cannabis market. Over the past year I have seen more and more consumables and reagents companies getting involved, and great interest from informatics companies. Not only are these companies becoming successful in selling their products/services to new markets, but they are actually

helping to solve some of the integration and workflow issues in cannabis markets.

What we know about cannabis is merely a drop, and what we do not know is an ocean. Researchers in other countries, especially Israel, are making great strides and I expect to see an explosion of research here in the US soon.

The Analytical Cannabis Symposia (I and II) will take place at this year's Pittcon, on Thursday, March 9th, 2017, 8:30am-5pm, Room W183A.

The Cannabis Science Conference will be held from August 28-30 in Portland, Oregon, at the Oregon Convention Center. Visit www.cannabisscienceconference.com to learn more. Use the code "PITTCON" for 25% off the cost of registration.

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In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of cannabis science. They can be up to 700 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

The Pesticide Problem

To keep cannabis consumers safe, tests for pesticide residues must use multiple methods.



By Jack Cochran, Senior Director of Applications, VUV Analytics, Cedar Park, Texas, USA.

I recently attended the 2017 Emerald Conference in San Diego, California, a meeting devoted to the science of cannabis, where I chaired a session on Analytical Testing and Sampling Schemes (1). I gave the opening presentation, which focused on pesticide testing of cannabis, something I helped pioneer with the QuEChERS sample preparation method, in collaboration with Frank Dorman at the Pennsylvania State University (PSU).

We analyzed illicit cannabis provided by the PSU police (the only kind we could get, since Pennsylvania was not a medical marijuana state at the time) and found numerous incurred pesticides, including fungicides and insecticides at low to high ppb levels, a herbicide, and the pesticide synergist, piperonyl butoxide.

Since our original work, cannabis testing laboratories have been rapidly adopting the QuEChERS approach for analysis of pesticides in medical and

retail cannabis, as US state programs try to determine how to regulate this new industry. Still to be determined: which pesticides can be safely used on cannabis, what pesticides to test for in cannabis and cannabis products, and the maximum levels of pesticides allowed. Since the US EPA has not authorized any pesticide specifically for use on cannabis, regulation falls to states (2). The number of pesticides proposed for determination by any one state is typically limited (3,4). This probably reflects the desire to start small by targeting the pesticides most commonly used on cannabis, while laboratories develop their skills and validate their methods. However, the high cost of capital equipment could also be influencing the list of pesticides tested for, as testing labs seem to be mainly purchasing LC-MS/MS over GC-MS/MS for this work.

The best suggestion I've seen for a cannabis-specific target pesticide list is from Rodger Voelker and Mowgli Holmes of the Cannabis Safety Institute (5). They applied toxicity, availability, and use-case rational and came up with just over 120 compounds. Importantly, the list includes pesticides that can only be monitored appropriately, or are monitored more easily, using GC. Indeed, if a cannabis testing lab only

“If a cannabis testing lab only applies LC-MS/MS, then numerous pesticides could be missed.”

applies LC-MS/MS, then numerous pesticides could be missed. This is significant given that in the same document Voelker and Holmes report that over 70 percent of select cannabis flower samples in Oregon were contaminated with pesticides, some at very high levels.

The title of my presentation at the recent Emerald Conference included the words, “Cannabis is Food”, a nod in part to borrowing QuEChERS from the food safety community. We should remember that one of the most elegant parts of QuEChERS, by design of its inventors (6), was to produce an extract that could be used for LC and GC

analyses, since we need both techniques for comprehensive coverage of pesticides we might encounter in food, and now cannabis.

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2. *United States Environmental Protection Agency, Pesticide Use on Marijuana, 27 January 2016 <http://bit.ly/2kce4V7>*
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Executive Office of Health and Human Services, Department of Public Health, Bureau of Health Care Safety and Quality, Medical Use of Marijuana Program, Exhibit 5, Minimum Analysis Requirements for Residues of Pesticides and Plant Growth Regulators Commonly Used in Cannabis Cultivation. <http://bit.ly/2lwOgGe>

What's in a Name?

As cannabis takes its rightful place as an important botanical medicine, it's time to consign the term 'marijuana' to the history books.



By Duke Rodriguez, CEO and President of Ultra Health, Scottsdale, Arizona, USA.

For nearly 70 years, the World Health Organization (WHO) has defined health as “a state of complete physical, mental and social well-being and not merely

the absence of disease or infirmity.” In the spirit of the WHO definition, 28 American states have approved medical cannabis for the treatment of many debilitating conditions impacting the physical and mental well-being of their communities. Eight states have gone further and addressed the social well-being of their populations by fully legalizing the adult use of cannabis.

The full benefits of cannabis will only be revealed by the advancement of research into therapeutic use and a better overall understanding of the plant. Our knowledge is already developing fast – and it's time that is reflected in how we refer to the plant and its genus. As the cannabis industry progresses, the terminology we use to describe our product needs to evolve as well. While there are many colloquial terms that misrepresent the cannabis plant, there is one term in particular that society needs to leave in the past: marijuana.

The plant's scientific genus is Cannabis. Marijuana, on the other hand, is a racially charged, pejorative term that was introduced in the United States in the early 1900s. Early supporters of prohibition referred to cannabis as the “devil's weed”,

“As the cannabis industry progresses, the terminology we use to describe our product needs to evolve as well.”

and soon after the term ‘marihuana’ (many current US laws continue to refer to the original spelling with an ‘h’, versus the more commonly used version with a ‘j’) was introduced to the public, in an attempt to reinforce a connection between cannabis and the minorities who allegedly introduced the drug.

Researchers studying cannabis do not deserve to be derogatorily identified as ‘marijuana’ scientists. Enlightened members of the academic community and the media are already elevating the discourse on cannabis through proper nomenclature;

“A common scientific language is essential to accelerate the cross-pollination of thought and collaboration.”

after all, a common scientific language is essential to accelerate the cross-pollination of thought and collaboration.

International cannabis collaboration is already evident. Israeli firms, such as our partners Panaxia Pharmaceutical Industries, are exporting proprietary processes, protocols and intellectual properties on scientific dosing, product development and quality standards utilizing Good Manufacturing Practices designed for the US cannabis market.

Such partnerships are helping us go beyond traditional smoke-inhaled delivery to more complex and dose-specific cannabis suppositories, metered inhalers, sublingual tablets, pastilles, concentrates and oils.

As well as international collaboration, a new form of cannabis expansion is emerging through the right of sovereignty of indigenous tribes in North America. In both the US and Canada, Tribal Nations are exercising their government-to-government relationship to accelerate cannabis markets, products, and economic development. The Las Vegas Paiute Tribe of Nevada is currently constructing a state-of-the-art cultivation and retail network for a market with 43 million annual visitors, and cannabis legalization approved for adult use effective January 1, 2017.

Native Tribes engaging in cannabis commerce follows a long tradition of self-sufficiency and self-determination of indigenous people. Evidenced by the success of Native American-owned casinos in the US, we can anticipate that tribal cannabis initiatives will facilitate a new era of cannabis discovery, testing, development and innovation, to be shepherded by individuals culturally

aligned with natural, sustainable and holistic healing.

The scientific truth about cannabis will become increasingly clear going forward. The population trend in attitudes is toward greater understanding and acceptance, and the integration of cannabis protocols in physical, mental and social well-being is becoming a reality. If we are to continue to evolve as an industry, institutionalized stigmas need to be removed through discovery, debate and the introduction of collaborative efforts brought forth by new alliances. In this global effort, there is no room for outdated, offensive terminology like ‘marijuana’.

Worldwide, the art and science of cannabis is embracing a new normal. As observed by physicist Max Planck, “scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it.” A new generation is now actively engaged in the science of cannabis, and will provide the evidence-based truths we need, to rise above the cognitive quagmire of the past.

Towards Mindful Consumption

By improving knowledge about cannabis, we can help consumers make the best decisions for them – and make the most of the benefits.

By Amanda Reiman, Lecturer, School of Social Welfare, University of California, Berkeley, USA.

Drug taking is a social learning experience. We often make decisions



about whether to use drugs, how to use them and how to act after using them from observing those around us. It's about more than just learning the ins and outs of intoxication. For centuries, societies have used drugs to bond, to marginalize, to ostracize, to celebrate and to explore. Group participation and belonging has been tied to choice of intoxicant and a willingness to engage or rebuke opportunities for intoxication.

Growing up going to temple on High Holidays, wine was a common part of the religious service and the celebration that followed. I did not learn about wine in itself, but came to see it as an integral part of the religious experience. People drank to be a part of the community and the celebration, the same reasons that myself and my high school peers chugged cheap, disgusting beer and did shots of syrupy sweet liquors. We were not learning about alcohol, we were learning about intoxication. The result? Too many nights with spinning rooms and too many mornings with splitting headaches.

Like most of my peers, my drinking slowed down considerably in my 20s,

“The more consumers understand the science behind the therapy, the better they will be able to maximize the benefits that cannabis presents.”

partly because of life circumstances, but partly because I moved to California

and started to learn about wine, rather than intoxication. The same social learning processes that had me downing beers in college, had me smelling wine, and asking about growing regions and organic growing methods. When I gained mindfulness around alcohol, I was able to gain mindfulness around consumption.

Cannabis is now legal in eight states for anyone over 21. Unlike the medical cannabis movement, which focused on those who were using cannabis as a prescription drug, legalization made cannabis over-the-counter for adults, opening it up to a wider section of the population. We can help people avoid the ‘teenage years’ of cannabis by stressing the theme of mindful and ethical consumption. Of course, cannabis overdose does not pose the same risks as overdoses on alcohol

and other substances. But, as I am fond of telling the college students in my class, developing a healthy relationship with cannabis as a young adult can ensure its benefits at age 90 and beyond.

The more consumers understand the science behind the therapy, the better they will be able to maximize the benefits that cannabis presents. Cannabinoids, terpenes and flavonoids interact with the individual endocannabinoid system to produce a variety of effects, just as over 200 aromatic compounds give each wine its unique flavor. As regulation makes cannabis testing more rigorous and widespread, consumers will have access to information about the various compounds that impact effect. Part of mindful consumption is taking the time and energy to gain knowledge, which will inevitably enhance experience.



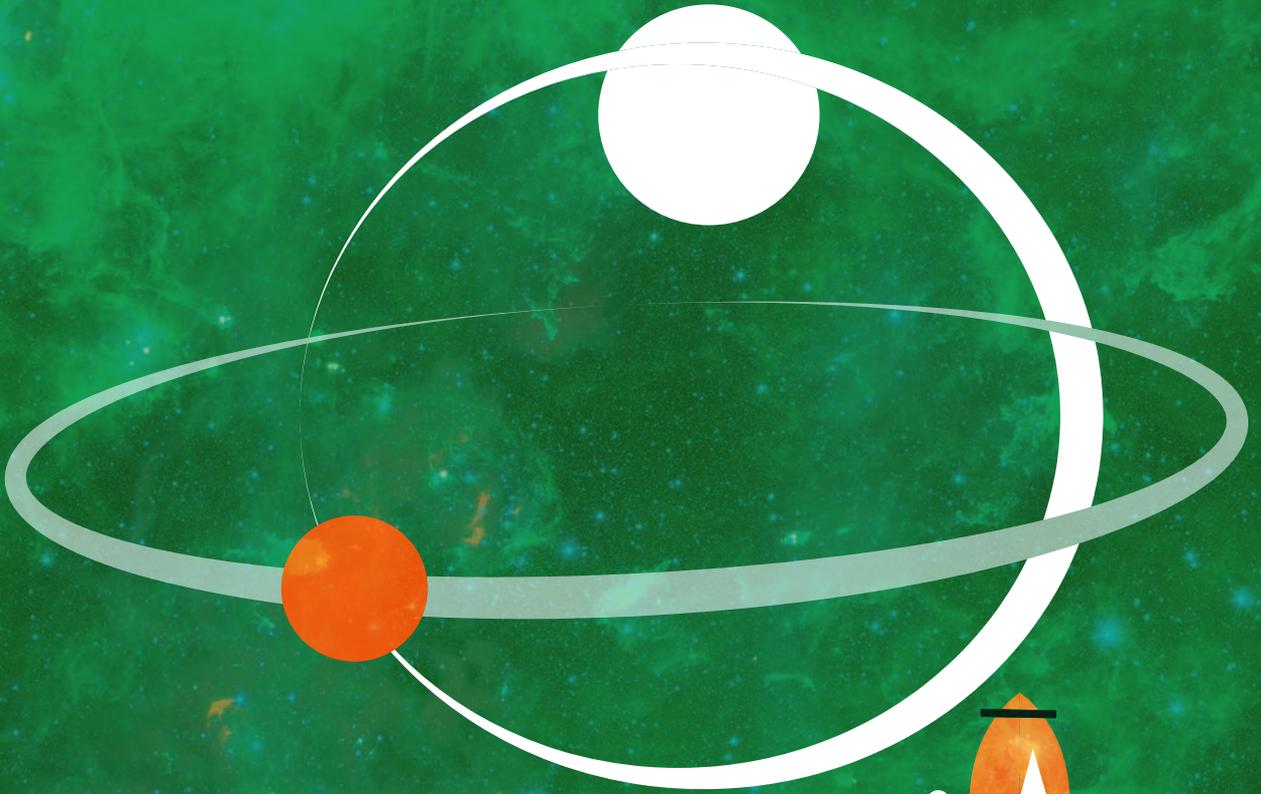
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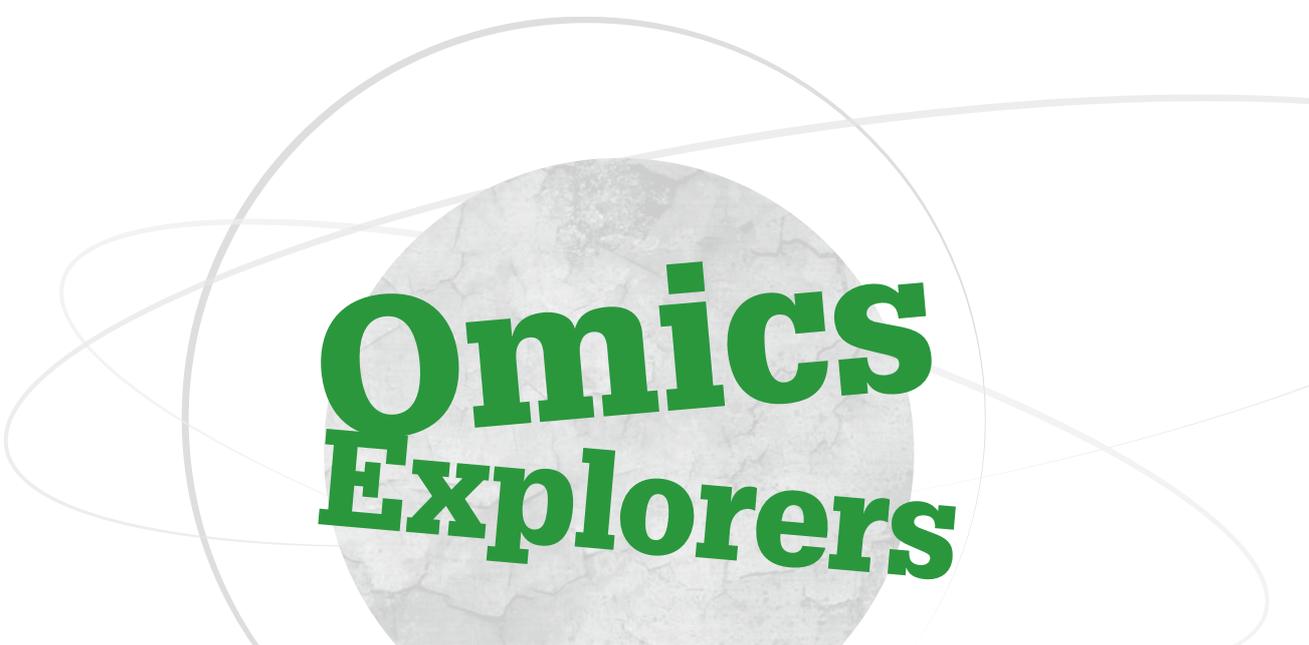
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Omics Explorers

Despite its long history of cultivation, we know surprisingly little about cannabis on a molecular level. As the medical cannabis movement gathers pace, scientists are renewing efforts to build up a genetic and chemical profile of the plant.

We speak with three research pioneers who are intent on applying science to create better plants – and better medicines.

Looking to the Stars

An interview with Alisha Holloway, Director of Bioinformatics at Phylos Bioscience, Portland, Oregon and Assistant Adjunct Professor, Department of Epidemiology and Biostatistics, University of California, San Francisco (UCSF), USA.

I was at graduate school when the human genome sequence was published in 2001, and I decided then to get into data analysis at the whole-genome level. Much as I enjoyed working in the wet laboratory, bioinformatics gives you the opportunity to do experiments very quickly. You can identify a dataset, ask some interesting questions, and get almost immediate answers. It's like solving a puzzle. In my postdoc I investigated genomics evolution in fruit flies, before moving on to medical genomics at Gladstone Institutes and UCSF.

I had been following the legalization of cannabis in many states, and first heard about Phylos at a conference in San Francisco. I was immediately struck by how well their scientific program fit

with my research interests, and soon after joined the company to lead the bioinformatics team.

Phylos is a company using genetic tools to help the cannabis industry. For example, one of our products is a DNA sex test, which allows growers to cull unwanted male plants just seven days after germination, saving significant time and money compared to traditional methods. We have also launched an ambitious effort to identify varieties of cannabis and their relationships to each other. What is the genetic diversity between cannabis varieties? Are varieties always what they purport to be? And how has cannabis evolved over time?

You can go to a dispensary and buy 'Sour Diesel', but it may not be the plant you think you are getting. That's important, because different varieties have amazingly different flavor and chemical profiles, which in large part determines the effect on consumers. By better understanding how varieties are related to each other, and what genetic background produces what chemical profile, we can breed varieties that have beautiful flavor profiles or can treat specific medical conditions.



Male and female plants, determined by a genetic sex test.

We have analyzed thousands of samples from seed companies, growers and collectors in 80 different countries. Some are nonviable seeds, and some are small pieces of plant stem washed in isopropanol to remove any THC and make them legal to ship. In places where we can't ship any part of the plant, people can send us DNA.

We extract DNA from samples, and amplify targeted regions of the genome that have a variable single nucleotide polymorphism (SNP) – a variation in a specific position in the genetic code. We analyze differences in these SNPs using computational tools that allow us to determine identity by descent (familial relationships) and variability (which tells growers how much genetic variation they can expect in any offspring). To present this data in an easy-to-understand way, we use principal components analysis to map each variety in 3D space – the Phyllos Galaxy (<http://bit.ly/2kpz4sl>).

Each star in the Galaxy represents a sample from which we have sequenced DNA. Closely related varieties are connected by lines that are based on the identity-by-descent analyses, to indicate a familial relationship or clone. The color of each node is determined by population subdivision – we've found evidence for six distinct subpopulations so far, and varieties are color coded by their membership in one or more subpopulations.

The Galaxy was released to the public in April 2016 and people are having a lot of fun exploring related varieties and the clusters that form. Growers are using their genetic reports as a marketing

tool, to promote the uniqueness of their strains, and breeders are using the information to decide which varieties to cross. It's useful on a lot of levels, and it's pretty satisfying for us to reach so many people with a cool, interactive tool.

We're trying to be very open about the data that we have – everyone can see every variety that's on the Galaxy, and we're also releasing all of the data (with permission) through the Open Cannabis Project (<http://bit.ly/2k6h9Lo>). There is a lot of work to be done in the field of cannabis genetics and genomics, and the more data we can release in an open format, the more questions researchers can ask and answer. Every time we add a sample to the Galaxy it's a new piece of information – a data point that no-one has had before.

Perhaps surprisingly, a lot of the anecdotal stories about the origins of various strains are really panning out. Growers are often very knowledgeable about the pedigree of their plants and can accurately identify the variety and its parent varieties. As genotyping becomes

more widespread, one positive result is likely to be greater consistency and accuracy in naming of varieties – we'll no longer see ten names for the same variety. That's not to say that growers can't distinguish their product from others of the same variety, as even cloned plants can have significant differences when grown under different conditions.

We have a lot of support in the local community and the industry as a whole. Breeders and growers are excited to learn how varieties are related and what kind of genetic variation there is in their plants.

Often, they have been crossing varieties to create new strains and are fascinated to find out how genetically different or similar the parent plants are, and how that is reflected in the offspring.

Ultimately, to really understand the plant we need genomics, genetics and chemical analysis. If we can combine genetic information with chemical profiles and other characteristics, we'll be able to develop markers for different traits, and so improve the plant – not just in flavor and chemical profile but in ease of cultivation.

Sure, I get a few giggles and jokes when I tell people I'm researching cannabis but, on the whole, they are grateful that real science is being done to understand the plant and how we can use it to our best advantage.

"We have analyzed thousands of samples from seed companies, growers and collectors in 80 different countries."

Breeding Better Buds

An Interview with Daniela Vergara, postdoctoral researcher at the University of Colorado, Founder and President of the Agricultural Genomics Foundation, and co-Founder of the Cannabis Genomic Research Institute, Boulder, CO, USA.

After gaining a PhD in evolutionary biology, I joined Nolan Kane's group at the University of Colorado. The lab works with many agricultural crops, such as sunflowers, chocolate and mustard. Initially, I planned to work on sunflowers, but given that cannabis is an increasingly important crop, we decided to try applying evolutionary biology techniques to cannabis instead.

Cannabis is one of man's oldest crops, and yet we know much less about it than other cultivated plants. What we are proposing to do in cannabis was done long ago in wheat, soy, and tomatoes. All of the techniques and tools we're using have been developed in other crops – the only difference is that cannabis is federally illegal, which makes it challenging to get funding. Our group, known as the Cannabis Genomic Research Initiative (<http://bit.ly/2ltQqoV>), has an associated non-profit, the Agricultural Genomics Foundation (<http://bit.ly/2kploxL>), which supports our work to better understand the biology of cannabis.

We are applying next-generation sequencing to investigate the genetics of cannabis, using shotgun sequencing and genotyping-by-sequencing technology from Illumina. The cannabis genome was published in 2011 by a Canadian group, but we want to add to the sequence data by mapping the physical location of genes across the chromosomes. You could say that the genome sequence on its own is like a book with the pages inserted at random; a genomic map will let us order the pages, and make it much easier to read.

We are also collaborating with analytical scientists at Steep Hill Labs to compare the genomes of different strains with the levels of different cannabinoids – to link genotype with phenotype.

One of our most interesting studies so far was a comparison of the cannabis being sold in dispensaries and the cannabis supplied for research from the National Institute on Drug Abuse (NIDA). We found that cannabis on the private market is much more varied and has much higher potency than NIDA-supplied cannabis (<http://bit.ly/2kP3rd2>). The cannabis industry has been carefully refining their strains and growing methods over time, so that their product is now far stronger than the government weed. Clearly,

NIDA cannabis being used for research across the country is not representative of the cannabis being sold for medical or recreational use. This is a real concern – if the cannabis being used in medical research is not what patients are taking in the real-world, how can we expect reliable results?

Another interesting finding has been that strains are not always related in the way that growers or consumers might expect. For example, you could have two strains with very similar names, which are very loosely related. The plants are often not even the species they are thought to be – *C. sativa* often turns out to be *C. indica*, and so on.

How will understanding the genetics of cannabis help the industry on a practical level? For one thing, it makes it easier to create varieties with specific traits; for example, high CBD or drought tolerance. What makes selective breeding of cannabis so difficult is the immense variation in the cannabis genome – there is more variation between cannabis varieties than there is between humans and chimpanzees.

That means that even two sibling plants can have radically different properties, and simply crossing two plants with desirable

traits may not get the result you want. You might cross an unusually tall plant with one that smells

strongly of lemons, but you have a long wait until the offspring reach maturity, and no guarantee that they will be either tall or lemon-scented! By mapping the genome, we can predict the traits of the offspring and guide breeding efforts.

When we talk about understanding the genetics of cannabis, some people assume we want to create genetically modified plants. In fact, cannabis has so much variation that the traits we want will almost certainly be found somewhere in nature, so there is no need to add genes from other species. We just need to be smart with what plants we're crossing, but without a full understanding of the genetics, that's hard.

It takes time to change attitudes. Most in the industry are very supportive of what we do, but others want immediate results; they don't understand that breeding programs take time. Some growers are quite hostile to our work – I'm a 5'1" Latino woman, and sometimes when I tell a guy who's been growing hemp since the 1960s that we need to do things differently, he doesn't take it too well. They can see it as interfering with nature, and think we are going to produce GMO cannabis. But most people can see that I am passionate about the potential of cannabis as an agricultural crop, and as a medicine. I believe that better breeding programs will ultimately benefit patients – mothers of children with epilepsy, veterans with PTSD, and many others.

“What makes selective breeding of cannabis so difficult is the immense variation in the cannabis genome.”





Into the Cannabinome

An interview with Kevin A Schug, Professor and Shimadzu Distinguished Professor of Analytical Chemistry at the University of Texas in Arlington, USA.

The driving focus of my lab is to develop methods that enable the use of new technologies to address challenging problems. Recently, we have had significant success in the environmental sector, looking at the potential impact of unconventional oil and gas extraction (including hydraulic fracturing – ‘fracking’), and developing methods more suitable for large-scale analysis of air, water and soil. We don’t just want to use routine methods, but create novel methods that provide new information.

Zacariah Hildenbrand works closely with us on some of our environmental analysis work, and was also the Chief Scientific Officer of C4 Labs, a cannabis analysis lab in Arizona. Through Zac, we have developed a partnership with C4 labs – the Cannabinomics Collaborative – with a planned research lab in Oregon. The goal is not just to run a cannabis testing lab, but to advance a research agenda for cannabis, using our experience and advanced instrumentation.

It’s an interesting project for us on several levels. Cannabis attracts a lot of interest, and has some very interesting challenges and opportunities for analytical chemists. We like working with complex matrices, which give us the ability to evaluate new sample preparation and analysis techniques. We have done some natural product discovery before, looking for new antibacterial compounds in marine organisms, so this seemed a natural progression.

There are around 85 known cannabinoids, many of which haven’t been studied to a significant degree. Then there are hundreds of terpenes, which help give cannabis its flavor and fragrance. Terpenes are thought to work synergistically with the cannabinoids to elicit a wide range of therapeutic responses; from the reduction of inflammation and the regulation of blood glucose, to the protection of neurons and gastric cells. Again, many of the putative terpenes have yet to be fully characterized. The breadth of those classes, the potential for synergistic effects, and the different growing conditions and genetic factors growers use to enhance and promote the presence of these compounds, all make for a rich field to explore.

We’re developing methods for discovery and quantitative analysis of cannabinoids, terpenes and other compounds of interest, such as pesticides. There are a lot of people out there looking for the next new bioactive cannabinoid, but we believe we have some unique approaches. We have access to a lot of state-of-the-art instrumentation through our partnership with Shimadzu, plus we have vacuum ultraviolet absorption detectors from VUV Analytics. Vacuum ultraviolet detectors and high-end mass spectrometers allow us to focus on low-level signatures consistent with cannabinoids, while triple-quadrupole gas chromatography-mass spectrometry



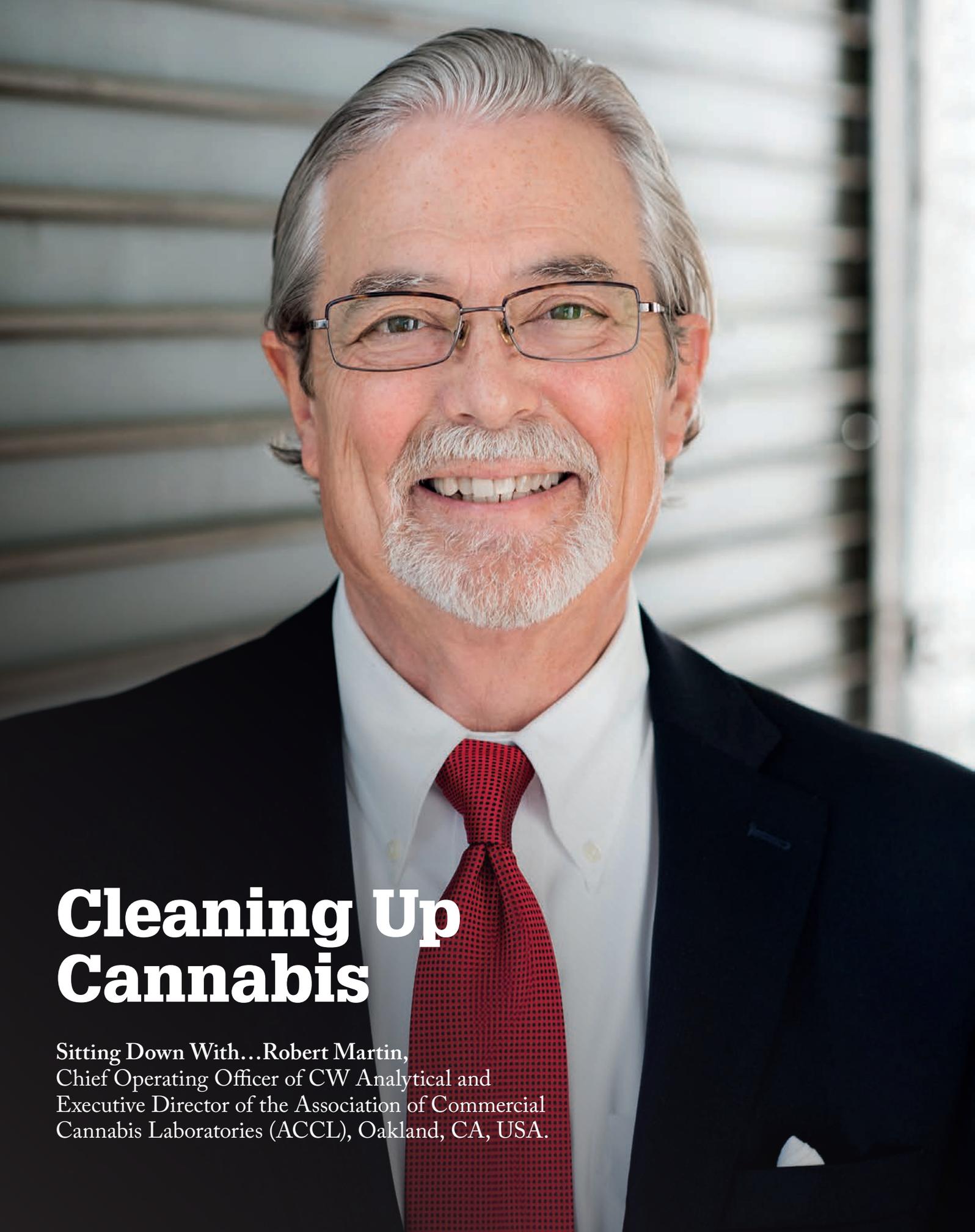
The Shimadzu Center for Advanced Analytical Chemistry, University of Texas at Arlington.

(GC-MS) systems offer additional specificity in targeted, quantitative analysis of both terpenes and cannabinoids. Online supercritical fluid extraction-supercritical fluid chromatography-mass spectrometry systems can, in principle, analyze all the compounds we’re interested in direct from plant matter, with minimal sample preparation. Many of these techniques aren’t yet mainstream in cannabis analysis, and we hope to have some promising results ready for publication soon.

Unconventional oil and gas is a pretty polarising topic. We strive to remain objective and neutral, but that means we don’t get too many Christmas cards in the mail from people on either side of the debate! In comparison, our work on cannabis is fairly uncontroversial, in that the general sentiment is that cannabis represents an exciting new frontier of medicine. That said, Texas is not the most progressive state in this regard, and to date we haven’t been able to work with actual cannabis plants at all. Instead, we’ve been ‘spiking’ matrix from hop plants (a relative of cannabis) with cannabinoids to simulate the type of interference we might find in actual cannabis plants, using DEA-exempt standards.

We’re pursuing DEA-approved avenues to get access to cannabis for the research, but it’s a long process, so we’re also looking at sending a team member over to the Oregon lab once it’s set up. It’s frustrating that we have the ability to contribute a lot of new data to a topic with important health implications, but we’re being held back by regulation.

I am naïve when it comes to hard-core genetics, but it’s exciting to see our knowledge of the cannabis genome grow. There’s a clear path for translation of research – from genetics, to the chemical analyses we’re working on, to bioactivity assays. Plus, findings from each area of study will feedback into the others, creating a circle of discovery.



Cleaning Up Cannabis

Sitting Down With...Robert Martin,
Chief Operating Officer of CW Analytical and
Executive Director of the Association of Commercial
Cannabis Laboratories (ACCL), Oakland, CA, USA.

What did you do before joining the cannabis industry?

I'm a biologist and botanist by training. I earned a PhD in botany/mycology from Ohio University and spent 20 years working for the food industry in quality assurance and R&D. I certainly never dreamed I would one day be running a cannabis lab! After leaving the corporate world, I continued my career in food safety as a consultant, working with some of the largest companies in the world, including Nestlé and Coca Cola.

How did you get involved in cannabis analysis?

As we get older, most of us face some health challenges, and in the 2000s a number of my friends got sick. They knew I was a long-time cannabis smoker, and came to me for advice on safety. I realized I had no answers for them, so I started asking questions at cannabis dispensaries. The answers I received were not reassuring – there was no testing other than for potency, and no quality assurance. I decided to do something about it and, together with environmental chemist John Oram, founded CW Analytical in 2009.

We now have a team of highly qualified scientists, developing relevant analyses and carrying out testing to accredited standards. It's a far cry from the 'stoner' mentality seen in media portrayals of the cannabis industry; you don't need flowers in your hair and painted faces to join our team! I want to bring the best elements of the corporate experience with me – and leave the worst behind.

Is there a market for cleaner cannabis? Absolutely. It was obvious to me in the food industry and it's obvious to me now. Just look at the meteoric rise of Whole Foods Market in the US and you can see that people are willing to pay significantly more for organic, high-quality groceries. The success of organic

food reflects a greater consciousness of just how 'dirty' our agricultural practices are on a large scale.

How can cannabis laboratories help raise standards in the industry?

Cannabis labs have played a key role. At first, we concentrated on microbiological testing – bacterial contamination was rife and when we spoke to growers it was obvious why. Often, they were dragging the harvested plants across farmyards and hanging them up to dry in dirty barns. Once they started to use plastic sheets and stainless steel, it was amazing to see how fast the samples cleaned up. In California, many growers are now beyond that. The new challenge is pesticide contamination. Just in this past 12 months, our laboratories have started to acquire triple quadrupole mass spectrometers to detect trace levels of pesticides that previously may have flown under our radar.

In 2011, we set up the Association of Commercial Cannabis Laboratories (ACCL), which helps bring more professionalism to this emerging industry.

What exactly is ACCL's role?

It's an unconventional business we work in; many of our clients lack knowledge of the basics of running a business (business plans, overheads, and so on). We're trying to make them understand the responsibility they hold when they produce a product for human consumption. All our members pledge to follow strict guidelines on everything from scientific proficiency to professional conduct.

We encourage labs to gain ISO accreditation, and help them stay at the forefront of technology by working with analytical equipment companies. The ACCL has facilitated partnerships between our members and companies like SCIEX and Agilent to test new instruments.

We also lobby for greater recognition of the work of cannabis labs. Right now,

we're busy lobbying to make sure that laboratories are properly represented in new legislation unfolding in California. Otherwise, we could lose the ground we have gained over the past few years in quality standards.

What are the challenges of running a cannabis lab?

People assume that if you are involved in the cannabis industry in any capacity, it's a license to print money. In fact, that is the furthest thing from the truth. Part of my job as Director of ACCL is to help people understand that laboratories are providing a service to our industry, and often for very little reward. We spend a lot of time, effort, and money, but unlike growers and dispensers, we're not getting rich from cannabis.

Qualified staff, high-tech analytical instruments and ISO certification are expensive, but our prices are much lower than most analytical labs, because our customers can't or won't pay more. Most cannabis labs operate on a 'thin ice' principle – as long as you keep moving, the ice won't break!

What keeps you motivated?

The main answer is: the patients. I once met the father of a three-year-old whose cognitive development had been halted by constant seizures. With CBD oil treatment, the child's seizures receded and she was able to speak her first words. That is the type of story that keeps me coming to work. Some people exaggerate the health benefits; but ultimately, if all cannabis does for someone going through a grueling illness is help them have a good day, then I think it's worthwhile.

I'm putting every ounce of my experience and know-how from academia and industry into CW Analytical, and it's thrilling work. If we can succeed, we will leave a template for other cannabis analytical labs to follow – a valuable legacy. If we can instill a clean, rational approach to quality assurance within the industry, I'll be a happy man.

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