

Lab Now Testing For Pathogens, Cannabinoids; High-CBD Strain Becoming Available to Patients

By Fred Gardner

A plant strain relatively rich in cannabidiol (CBD) has been identified by an analytic-chemistry lab recently established to serve the medical cannabis industry in California.

CBD is a cannabinoid with intriguing medical potential that gets bred out of cannabis when the breeder's goal is high THC content (as it has been in California for generations). It has long been assumed that current strains contained at most 0.1 percent CBD.

The availability of cannabis that is approximately five percent CBD by weight will enable doctors and patients to test its effectiveness in treating various conditions.

High-CBD cannabis might appeal to many people who want medical benefit without psychoactive effect.

Because CBD is not psychoactive, high-CBD strains bred to be low in THC might prove palatable to people who dislike the effects of currently available marijuana, or who are seeking medical benefit—say, decreased stress or pain—without feeling stoned.

High-CBD strains might enable patients who need large doses of cannabinoids to ingest them and remain functional. According to Jeffrey Hergenrather, MD, "Patients with cannabis-sensitive cancers, seizure disorders, and inflammatory bowel disease, to name a few, could all benefit with a higher blood level of cannabinoids than is convenient with our high-THC strains. For them, availability of a high-CBD strain could be life-saving."

Steep Hill

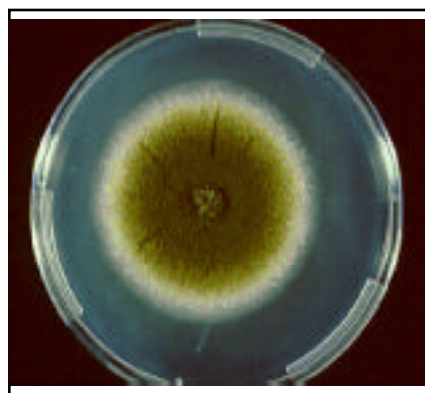
In December 2008, emboldened by the election of President Barack Obama, the founders of a lab in the East Bay doing business as "Steep Hill Medical Collective" notified dispensary operators that they had begun testing cannabis samples for something no one wants to find on their medicine—pathogenic mold—and something everyone wants to find out about—THC and CBD content. The lab also routinely tests for CBN (cannabinol, a breakdown product of THC that indicates time in storage), and is adding a test for bacteria (e coli and salmonella).

Running Steep Hill are two former growers, David Lampach, 33, and Addison DeMoura, 35, who decided to find different niches for themselves



David Lampach (left) and Allan Frankel, MD, examine a chromatogram showing the amount of THC, CBD and other compounds present in a cannabis sample. Lampach is co-founder of the Steep Hill test lab. Frankel, a cannabis specialist based in Venice, CA, intends to track patients' responses to various strains.

Bad News, Good News



ASPERGILLUS FUMIGATUS mold seen growing on a nutrient medium in a petri dish can be harmful to patients with weakened immune systems. *Aspergillus* is present in soil and has been detected in some cannabis intended for medical use.

within the industry. They spent a year learning how use the sophisticated testing apparatus and refining their procedures under the tutelage of a sympathetic university-connected chemist.

Lampach, who put up the original funding, operates the gas chromatograph-mass spectrometer (GC/MS) and flame ionization detector (GC/FID). DeMoura is liaison to the dispensaries, many of whose operators are eager to take part in the testing program.

Throughout the winter and spring of 2008-09 the lab refined its procedures by testing eight to 10 samples a day provided by Oakland's Harborside Health



BUDS HIGH IN CANNABIDIOL (CBD) are from "Soma A+" plants grown indoors in San Francisco. Feedback from analytic lab will enable growers to develop strains with various CBD-to-THC ratios by conventional selective breeding methods.

Center and Sebastopol's Peace in Medicine dispensary. Harborside proprietor Steve DeAngelo has backed the project from its inception. "If you're calling for regulation, you've got to get ready for inspection by public health authorities," DeAngelo says.

Another backer, Morpheus of the Cornerstone Research Collective in Los Angeles, says, "This is a wonderful experiment that is taking place in California—but somebody has to keep an eye on what's being provided to patients."

Promoting quality control is a mission shared by the "Clean Green" organic certification program, the Medi-

Input from the lab has already resulted in growers improving their operations.

cal Cannabis Safety Council, and other industry groups. As longtime activist Michele Nelson puts it, "the whole industry is in a transition towards professionalism."

The Steep Hill lab has found levels of mold, notably *Aspergillus Fumigatus*, that bear witness to unsanitary production methods. Almost 3% of samples tested this spring were found to contain *Aspergillus*, and the pounds from which they came were returned to vendors by the dispensaries.

"Some people will have to clean up their acts," DeAngelo says. "It can't be the whole family and friends sitting around with all the dogs in the living room. We're putting out the message: 'Clean up your trim areas, clean up your storage areas, do not have cannabis curing in an area that's exposed to animals. Set up a clean room and put on different clothes when you go in. Wear gloves. Wash your hands. In other words, remember that your product is medicine and treat it as medicine.'"

Input from the lab has already resulted in growers improving their operations, according to Rick Pfrommer, Harborside's veteran purchasing agent. "Most of the people who have had mold in their

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CBD: a Treatment for Breast Cancer?

By O'Shaughnessy's News Service

When California Pacific Medical Center took a half-page ad in the *San Francisco Chronicle* to announce a public forum on October 7, 2008, it may have been the first time in history that a hospital pitched its cannabinoid research program to prospective patients. "From Water Bottles to Marijuana Derivatives," the text called out, "Latest Discoveries about Breast Cancer."

The ad convinced about 100 women and a few men to skip the second Obama-McCain debate on TV and attend the CPMC forum.

William Goodson, MD, gave a brief talk advising his listeners to avoid carcinogens in the environment—a difficult task, given the quality of our air and water. Goodson singled out Bisphenol A, a hormone-disrupting chemical that can leach out of plastic in water bottles, baby bottles, and the lining of "tin" cans. Glass makes the safest container, he said.

"Marijuana derivatives" referred to work being done by two PhD biologists, Sean McAllister and Pierre Desprez, who have been testing pure, synthetic Cannabidiol (CBD) as a treatment for breast cancer. McAllister said that clinical trials could begin in two years if all went well.

He emphasized that CBD is non-toxic and has no known adverse effects: "That's a really nice starting point when you're trying to inhibit cancer," he said. Many of the women in the audience were all too familiar with the miserable effects of chemotherapy and radiation. Many had used marijuana to fight nausea and restore appetite.

Desprez had spent more than a decade studying metastasis, the process by which cancer cells escape from a primary

tumor and seed secondary tumors at different sites in the body. He found that cells in aggressive tumors—unlike cells in tumors that remain localized—express large quantities of a gene called Id-1.

The normal role of the Id-1 gene is to promote the rapid development and differentiation of embryonic cells; by birth these genes have switched off. But in metastatic cancer, the Id-1 gene somehow reactivates and directs cells to grow and travel throughout the body. Desprez calls Id-1 "the orchestra conductor" of this process.

Desprez proposed [Coppe et. al., 2004, *Clinical Cancer Research*, v10, 2004-2051] that clinicians might use Id-1 level as a "diagnostic marker" to indicate the extent to which a cancer has spread. (When cells are not expressing Id-1, the patient can be advised that the tumor is less likely to spread. When cells are expressing high levels of Id-1, treatment can be planned accordingly.)

The Id-1 gene presented a direct therapeutic target.

Of potentially greater significance, the Id-1 gene presented a direct therapeutic target. Would turning it off block metastasis? Would turning it off destroy cells that had already metastasized? And how do you go about turning off Id-1 expression?

These questions led Desprez to collaborate with McAllister, a CPMC Research Institute colleague who had been testing CBD for anti-cancer effects and



Sean McAllister observes the effects of CBD on aggressive cancer cells.

reported promising results.

McAllister and Desprez observed the effect of CBD on aggressive cancer cells as the cells chewed through an extracellular matrix in a Petri dish. (The cancer cells are trying to reach nutrients on the other side, just as they would try to reach nutrients in the bloodstream by chewing through tissue in the body.) Assays showed that the presence of Id-1 gene diminished as more CBD was applied—and fewer cancer cells survived and invaded.

McAllister said at the breast cancer forum that Id-1 appears to promote the invasiveness of many types of cancer, and treatment with CBD might be a generally effective way to switch it off.

Update Spring '09

In late April O'Shaughnessy's visited McAllister at his lab at the CPMC Research Institute, which is in a renovated industrial building South of Market. He expressed hope that the federal government's stimulus package might improve his chances of getting a grant from the National Institutes of Health to keep test-

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Analytic Lab Project from page 1



VISUAL INSPECTION is conducted when vendor brings cannabis to dispensary. Harborside buyer Rick Frommer (center) also breaks open buds to smell.

cannabis are the people who didn’t have filters on their air intake. They may have had beautiful medicine, but they were pulling in whatever from the air. Now they’ve got filters.”

When the lab begins testing for pesticides, indoor growers who have been using chemicals to kill mites and other pests will have to find organic alternatives or else peddle their wares to dispensaries that don’t adopt safety standards.

“What’s the difference between OG Kush and Afghan? They smell totally different, they taste totally different, they have totally different effects. They’re both relatively high in THC, so we know it’s not the THC...”

The lab also intends to test for terpenes —aromatic compounds that influence the smell, taste and effects of cannabis. “We’re going to map terpenoids,” Lampach says. “What are the terpenoids that make sour diesel sour diesel? What’s the difference between OG Kush and Afghan? They smell totally different, they taste totally different, they have totally different effects. They’re both relatively high in THC so we know it’s not the THC...”

Allan Frankel, MD, who visited the lab in early June, says that “providing decent medical care involves seeing to it that your patients use safe medication and knowing what’s in it. After three years in this field [cannabis therapeutics], I don’t know what truly differentiates a Sativa from an Indica. Genetics, obviously, but what about chemical content?”

Frankel’s ultimate goal is “to match clinical responses to different strains.”



ASSISTANT BUYER Rachel S. selects small cannabis buds from different parts of the bag to get a representative sample for testing. A bud from a part of the plant exposed to full light will differ markedly in cannabinoid content from one that was shaded.



SAMPLES FOR TESTING are culled by dispensary staff from different parts of bags brought by vendors, then picked up by courier from the lab.

His Greenbridge Medical Group uses a software program capable of monitoring patients’ reports. “But until we can accurately define the strains,” he acknowledges, “we can’t do the basic research.”

The knowledgeable Morpheus makes the non-verbal gesture to signify quotation marks when describing a strain as “Indica” or “Sativa.”

We are about to find out what’s what.

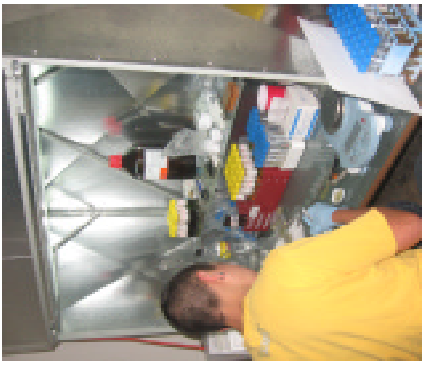
CBD: It’s Not for Stoners

CBD predominates over THC in cannabis that grows wild (ditchweed) and plants grown for fiber (hemp). When plants are bred for psychoactivity THC replaces CBD (because they are encoded by alleles at the same genetic locus).

CBD has anti-inflammatory, anti-convulsant, anti-psychotic, anti-oxidant, and neuroprotective properties, according to a rapidly expanding array of published studies. It also has a direct inhibitory effect on certain cancer cells.

The British company GW Pharmaceuticals has developed a high-CBD strain that it mixes with a high-THC strain to make Sativex, a plant extract formulated for spraying under the tongue that has been approved in Canada and elsewhere to treat neuropathic pain.

In various studies, patients with severe pain have reported getting significantly more relief from Sativex, the mixture, than from GW’s high-THC extract.



PREPARATION OF SAMPLES for testing is done by Steep Hill lab technician. For GC/MS, snippets of dried bud are placed in vials to which a solvent is added.

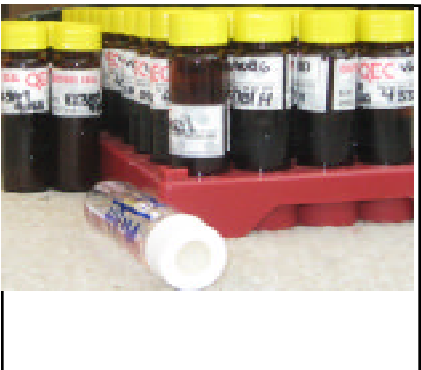
CBD evidently bolsters the pain-killing effects of THC while moderating its psychoactivity.

The Dutch company Bedrocan, which grows medical cannabis for dispensaries in several European countries, provides a 7%-CBD, 7%-THC strain that is preferred by patients seeking to avoid psychoactive effects.

With a few notable exceptions the California cannabis samples tested to date have contained only trace amounts of CBD. The first notable exception occurred in late February when Lampach saw a spike on a computer-generated graph indicating a high level of CBD in a strain that the grower identified as “Soma A-plus.” Additional tests confirmed that buds from this strain are in the 4.5 to 5.5 percent CBD range, with THC in the eight percent range.

Soma A+ is produced indoors in San Francisco by a grower named Chandler who is currently producing six pounds of dried bud every two months. DeAngelo has offered him incentives to rev up production. “We want to provide high-CBD clones to patients ASAP,” says DeAngelo. “It would be immoral to try to hoard the genetic material,”

A second high-CBD strain was identified in March —“Ganja Ma,” grown outdoors in the Laytonville area— but the growers had started it from seed and did not retain a “mother plant” from which



BUD SNIPPETS PLUS SOLVENT are homogenized by a “sonicator” (an ultrasonic bath). Extracts are further diluted before analysis by gas chromatograph.

The attempt to produce and evaluate less psychoactive strains of marijuana will counter the image of stoners paying lip-service to medical use that has been used to tarnish the movement.

to start clones. In other words, it cannot now be reproduced.

A similar false alarm involved sample buds from a strain called New York City Diesel.

On June 14, William Courtney, MD, drove to the Steep Hill lab from Willits with sample buds from 10 strains of interest. They had been preserved over the years, Courtney explained to Lampach, by “an old North Mendocino grower who is a friend of one of my patients.” Plants from these strains would not produce enough weight to interest commercial growers in 2009, Courtney said, but their distinctive effects had induced the grower to preserve some buds and seeds from them.

When Lampach tested the samples, one, “Sug-Red-Dog,” turned out to have a high level of CBD —five percent. Adding to Courtney’s delight, the THC content was about equal —meaning Sug-Red-Dog has the same CBD-to-THC ratio as GW Pharmaceuticals’ Sativex.

The grower did not have another bud for a confirmatory test of Sug-Red-Dog, but he has planted the seeds, Courtney reports, and by August should have flowers that can be tested for CBD content.

A New Stage

With the launching of the analytic test lab and the identification of strains containing substantial levels of CBD, the medical marijuana movement/industry has reached a new stage. Growers will use selective breeding techniques to develop strains with higher CBD-to-THC ratios. Pro-cannabis doctors, who have

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The very loose taxonomy of Soma A+

“Soma” is the name of a plant breeder based in Amsterdam. “Soma A+” is one of 20 seed varieties his company sells. The strain is also known as “Rock Bud.” The Soma seeds website describes Soma A+ as 80% Indica, 20% Sativa and says it was bred from “Super Skunk,” “Big Skunk Korean,” “Afghani,” and “Afghani-Hawaiian.”

Soma A+ seeds presumably gave rise to the 5%-CBD strain that Chandler, who grows indoors in San Francisco, obtained from a friend last winter. Chandler says his friend “got the strain from some young friends in Humboldt who are very into crossing really exotic strains.

“I told him I needed some new strains because I’d been growing just OG and Lavendar for three years. My Headmaster is a low yielder and the Lavendar is a pain to grow.

“He gave me five clones. All of them lived and it took about four or five months of taking care of them until I had enough to do a couple of tables. If a strain grows well it’s about 30 plants per table. This was a pleasant surprise. Most of what I’ve been growing is very tasty but not good yielders. I usually get about three-quarters of a pound per light. But this got me almost one-and-a-half pounds



“Soma A+” plants high in CBD in a San Francisco garage/first-floor flat that Chandler spent a year-and-a-half converting into a secure grow space wired by a union electrician.

per light. Out of rockwool.

“It looked spectacular. It smoked very mellow. A benign sort of high. Very smooth. Not particularly up or down. Just... medicative. Less edgy.”



“Mother Plants” from which Soma A+ clones will be made available.



FEMALE FLOWER glistens with resinous trichomes containing cannabinoids, terpenes, and flavonoids. Weight of resin compared to vegetative structure of bud explains why cannabinoid content of cultivated plants can exceed 20%.

Analytic Lab Project *from previous page*



GAS CHROMATOGRAPH/MASS SPECTROMETER consists of two instruments that work together in tandem. The GC (unit at right with open door) is used to measure quantities of known compounds. As sample is heated very slowly in “oven,” molecules rise into vertical column and cling to walls at identifiable levels. MS unit at left identifies unknown substances.

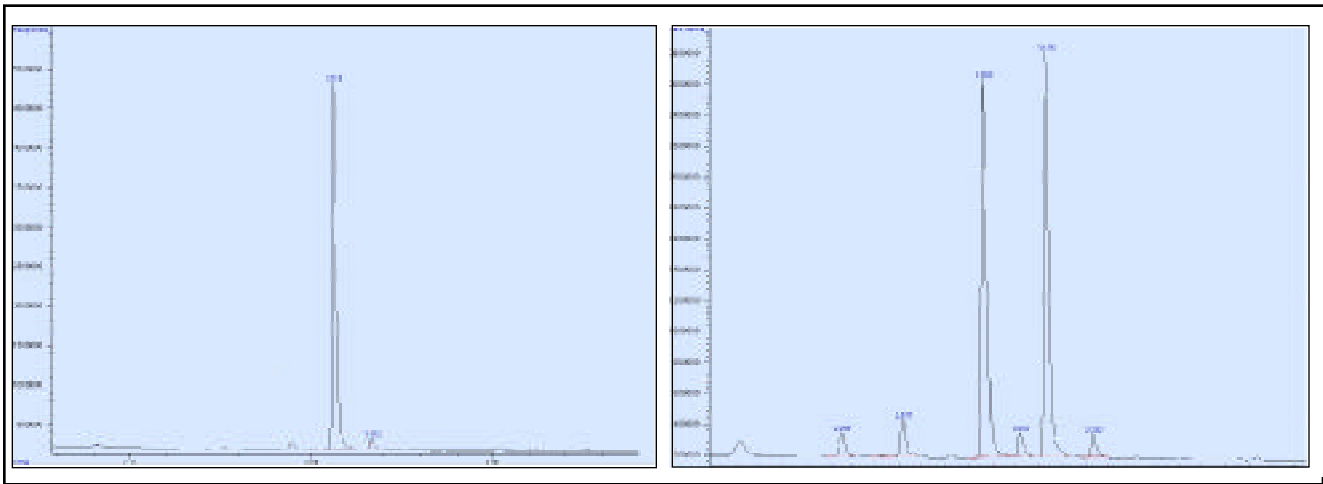
long awaited high-CBD strains, will conduct rudimentary clinical trials to determine whether and in what ways high-CBD cannabis is beneficial.

Whatever the outcome of clinical trials involving CBD, the effort alone—the attempt to produce and evaluate less psychoactive strains of marijuana—will counter the image of stoners paying lip-service to medical use that has been used to tarnish the movement. And if and when the effectiveness of high-CBD cannabis in treating, say, rheumatoid arthritis, can be established, a wave of older Californians will be asking their doctors if cannabis is right for them.

Logistics

Dispensaries using the services of the lab will work out their own procedures and pricing with Steep Hill. The basic testing for mold, bacteria and three cannabinoids is priced at \$70 to \$100, depending on the number of samples being tested. DeMoura expects dispensaries to absorb the expense rather than pass it on to growers.

At Harborside, about 50 vendors—growers and brokers representing them—arrive daily with their wares. Buds are typically offered in three-gallon turkey roasting bags containing pounds. Edibles are brought in packaged units. Purchasing agent Rick Pfrommer and his assistant Rachel S. accept about 30% of



CHROMATOGRAMS OF CANNABIS SAMPLES reveal their chemical contents. Scale at left indicates amount detected in the sample. Scale at bottom is a measure of time as sample is heated from 200° to 325° during a 10-minute run of the gas chromatograph. As samples are heated, compounds separate and are detected at characteristic intervals. Chromatogram at left shows one sharp spike for THC and a very small one to its right for CBD. “This is what 99% of our chromatograms look like—high THC, almost no CBD,” says David Lampach. The chromatogram at right shows contents of Ganja Ma, a high-CBD strain for which no genetic material was preserved. Small spikes include one for CBN and three for compounds that Lampach says, “are unknowns, probably terpenoids, that we will be able to identify” when the lab is fully functional.

the products being offered.

Cannabis from vendors whose material has been tested previously and found to be free of mold is processed for sale (put into sealed baggies containing grams, 1/8th ounces, etc.) and samples are sent to the lab for confirmatory tests and cannabinoid content. Cannabis from sources that have not been tested previously are not processed for sale until test results for pathogenic mold are reported.

Once samples reach the lab they are processed for two different kinds of testing. The testing for cannabinoid content begins with lab technicians (DeMoura and Lampach themselves, until recently) preparing extracts to be fed into the gas chromatograph by an automated process. (See illustrations above.)

The test for mold takes four to five days. A water extract is made from the sample bud and placed on a nutrient medium in a petri dish, which is then incubated. If molds of any kind are present, they will grow out and can be identified. The extent to which the original sample was contaminated can be determined by the number of colony-forming units and their rate of growth.

Steep Hill began by outsourcing its testing for mold, which requires a microbiologist, an expensive incubator, and more space than was available in their original Emeryville location. The lab

moved in June to custom-built quarters in an industrial building in Oakland. New investors materialized at about the same time, making possible several key hires, including a microbiologist. DeMoura expects Steep Hill to be doing its own testing for mold by September.

Three technicians have been hired since the move to Oakland, and candidates for lab manager are being interviewed as we go to press. The business plan is ambitious but logical. The downside is not lack of demand but the looming threat of DEA intervention. “Of course we’re concerned,” says DeMoura. “We can only hope that if and when the government looks at what we’re doing, they’ll see that we’re about safety and public health.”

Steep Hill has found several pathogenic strains of *Aspergillus* in cannabis samples at levels higher than those deemed safe by the World Health Organization for medicinal plants intended for internal use. How serious is the threat to patients who use marijuana as medicine? Wouldn’t pathogens be destroyed by heat when cannabis is smoked or cooked? What health hazard is presented by yeasts, non-sporulating fungi, *Aureobasidium Cladosporium*, *Trichoderma*, and the other unlovely-sounding microorganisms that Steep Hill has detected on samples of White Rhino, etc.?

“For a healthy person the threat is minimal,” Lampach acknowledges. “But for a patient with a compromised immune system, *Aspergillus* in the lungs could be very dangerous—even fatal.” Indeed doctors routinely advise cancer patients on chemotherapy not to work in the garden—to avoid exposure to *Aspergillus* spores in the soil.

Less dramatic gastro-enteric problems can be caused by cannabis contain-

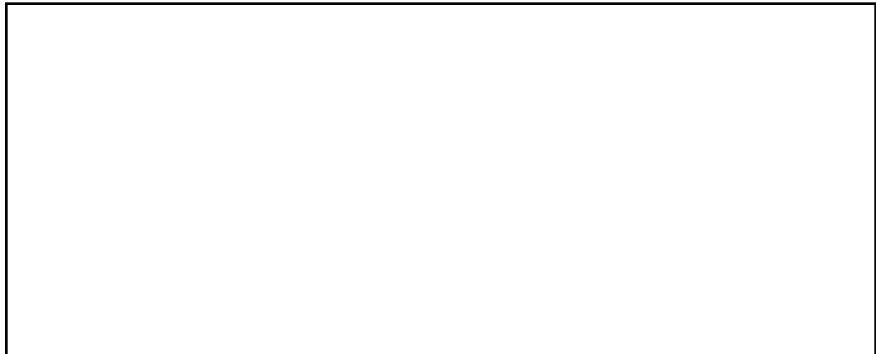
ing *E. coli* bacteria. A patient making brownies might dip a finger in the batter to taste it... To eliminate such risks, Bedrocan in Holland and Prairie Plant systems in Canada—government-authorized cannabis growers—have been required to use gamma irradiation to kill mold and bacteria. In the UK, GW Pharmaceuticals cannabis has passed all bacteriological and fungal tests without recourse to sterilization (or spraying). Clean technique has been the key to their success.

Down the Line

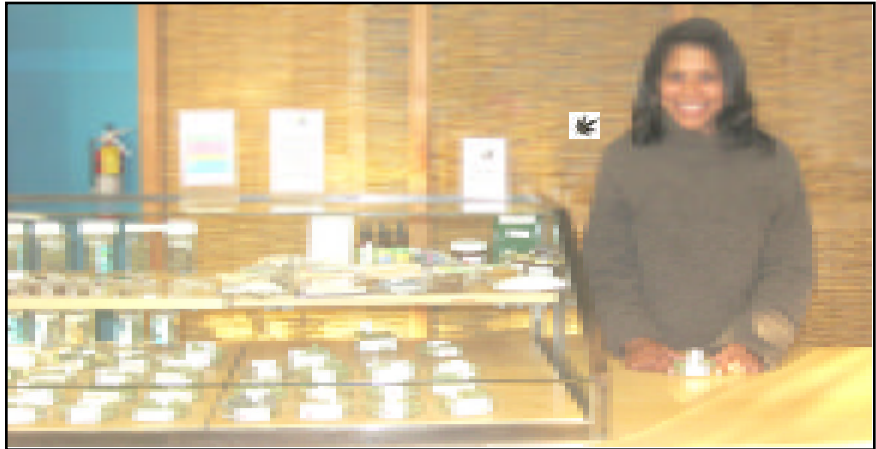
The June 13-14 THC Expo in Los Angeles was a coming out party for Steep Hill, and their booth generated maximum buzz. On a return trip to L.A. in early July, DeMoura finalized arrangements to test cannabis for Herbal Solutions in Long Beach, the Arts District Healing Center, and Cannabis Alternative Medical Systems in Palm Springs. A few days later in Sacramento he signed up the Horizon and Unity Collectives and set up a meeting with the management of Capitol Wellness. After nine months of gestation, things are happening fast.

“We’re not completely dialed in yet,” says DeMoura. “We don’t want to accept more clients than we can report results to on time.”

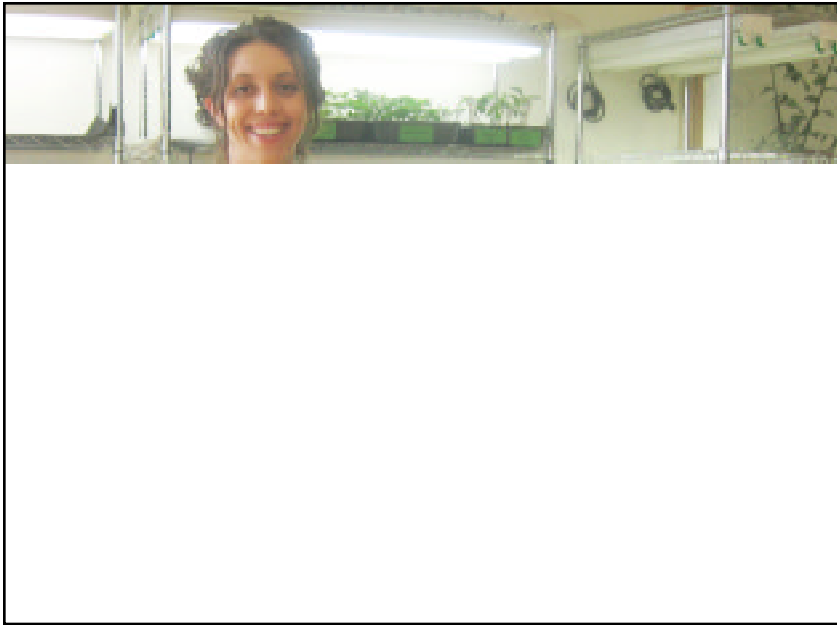
Down the line—in a matter of months, not years, according to the ambitious business plan—Steep Hill will begin testing cannabis for terpenes, which is done with a device called a headspace sampler, and for fungicides and pesticides, which will require the purchase of standards from chemical supply houses for each substance of interest. Lampach and DeMoura seem to have every step flowcharted. And thus far, they’re on schedule.



Prevalence of THC in medical cannabis available in California is exemplified in this photo a Harborside display case. Soma A+ is the lone exception to THC dominance.



CANNABIS BUDS ON DISPLAY at Harborside Health Center are now labeled with safety test results—free of mold or test pending—and cannabinoid contents. Budtender LaShon Mercer-Hursh tells patients which strains other people with their condition have found effective. “People can react differently to medicine,” she reminds them. “You have to figure out what works best for you.”



CLONES available at Harborside will include Soma A+ plants as soon as the grower(s) produce them in quantity.