Cannabis samples grow under specialized lights at Anandia Labs in Vancouver.

JAMES MACDONALD FOR *NATURE*

A GOLD RUSH FOR CANNABIS

As legal weed hits Canada, scientists race to study and improve a once-forbidden plant.

BY ELIE DOLGIN

Jonathan Page has been around cannabis all his life. Growing up on Canada's Vancouver Island in the 1970s, he was surrounded by hippie beachcombers and dope smokers. So after earning a PhD in plant biology and phytochemistry, he felt completely at ease working with the plant *Cannabis sativa* as a postdoc in Germany in the early 2000s.

During that time, Page helped to characterize a pair of genes that some varieties of the plant uses to make fragrant oils responsible for pine- and lemon-like aromas [1]. And during an interview for a position with Canada's National Research Council (NRC), Page proposed similar projects to reveal how cannabis produces pharmaceutically active compounds known as cannabinoids.

He got the job, but was dismayed when he showed up to start his lab group in 2003 at the NRC's Plant Biotechnology Institute in Saskatoon, Saskatchewan. Page recalls his boss saying: "You're not going to work on cannabis here. We're the government."

What a difference a change in policy makes. On 17 October, Canada became the second country in the world, after Uruguay, to legalize cannabis for all uses. And although a few other countries, most notably Israel, have made a concerted effort to support agricultural research into cannabis, full legalization in Canada has brought with it unparalleled access to money for basic research on the plant.

Most of the country's 129 licensed cannabis producers are now clamouring to work with scientists on everything from gene mapping and metabolic engineering to optimal drying techniques and growing practices. And as part of an effort to corner the global legal cannabis market — one that's conservatively forecast to top US$57 billion within a decade — federal and provincial governments in Canada are putting up millions of dollars to support research.

Some researchers, such as Page (who still dabbled in cannabis research during his decade at the NRC), are well prepared to take advantage of Canada's great green rush. But botanists of all stripes are now turning to the plant, for both the funding opportunities and the uncharted science.

"You're talking about a plant that's a century out of date in terms of modern breeding techniques and scientific development," says Ernest Small, a botanist with Agriculture and Agri-Food Canada in Ottawa who has studied cannabis off and on since 1971.

RESEARCH BLUNTED

When Page first moved back to Canada 15 years ago, he initially resigned himself to studying a close relative of cannabis, the hop plant *Humulus lupulus,* which is used in brewing beer. But he doggedly pursued avenues to keep working on pot. Page secured a licence to grow industrial hemp, a variety of cannabis cultivated for its fibre that produces only trace amounts of tetrahydrocannabinol (THC), the mind-altering chemical responsible for cannabis's high. Eventually, he hooked up with the sole company contracted at the time by the government to

JAMES MACDONALD FOR *NATURE*

Many basic agricultural experiments have yet to be performed with cannabis.

produce the plant for medical purposes.

Page unpicked the pathway that leads to the formation of THC and cannabidiol (CBD) — cannabis's other main medically important compound[2]. Together with molecular geneticist Timothy Hughes at the University of Toronto, he sequenced the genome of a potent pot variety called Purple Kush[3]. But, says Page, "the NRC was still totally unsupportive of this work". So, in late 2013, he moved to Vancouver to start a cannabis biotechnology company called Anandia Labs.

In one of Anandia's first projects, Page worked with Sean Myles, a population geneticist at Dalhousie University's Agricultural Campus in Truro, Canada, to genetically characterize 124 samples of cannabis[4]. The analysis showed that the commercial labelling of subtypes indica and sativa rarely matched the plants' DNA profiles. And different samples marketed under the same madcap varietal name — White Widow, for example — often turned out to have wildly divergent genetics. "This is absolutely unthinkable in any other legitimate agricultural crop," says Myles. "You can't put a Mackintosh apple on the shelf and pretend it's a Honeycrisp."

Despite garnering publicity for the research, Page had trouble pulling in capital. Then came the election in October 2015 of Prime Minister Justin Trudeau, who promised during his campaign to legalize cannabis. "It changed attitudes almost overnight," Page says.

Although the Natural Sciences and Engineering Research Council of Canada doesn't have a dedicated cannabis initiative, the agency has funded dozens of projects focused on cannabis biology and cultivation. Genome Canada and other government-backed organizations have made research funds available, too. More substantially, private investment dollars have come pouring into the country's cannabis industry (see 'A smoking-hot sector'). Last year alone, Canadian cannabis companies raked in close to Can$2 billion (US$1.5 billion) — more than half of all the funding raised by legal cannabis firms worldwide — and the industry is on track to triple that number in 2018.

Anandia was one of the many beneficiaries. After securing more than Can$13 million (US$10 million) in private investment, the company got snapped up earlier this year by industry heavyweight Aurora Cannabis in Edmonton, for Can$115 million. "That is a major gesture of confidence," says Cam Battley, chief corporate officer at Aurora, adding that science and innovation are key to growing "a globally competitive company that will be built to last".

The sentiment is a relatively new one, says Michael Ravensdale, a plant pathologist who leads production at the firm CannTrust in Vaughan. "Science was in short supply, but it's going to be very important for the next chapter of the cannabis industry."

That's why many companies investing in research are starting with the fundamentals. "There are these super basic, huge questions that need to be answered," says Greg Baute, who used to breed tomatoes at Monsanto in Woodland, California, and moved north this year to head breeding and genetics at Anandia's new research facility in Page's home town of Comox. "You can do these really straightforward experiments and get these huge results."

JOINT VENTURE

In a suburban Toronto mall, nestled alongside a paint store, sits a nondescript brick building, home to TerrAscend, a cannabis producer that has been shipping its product for about a month in anticipation of 17 October. Inside, past a barbed-wire fence and several layers of electronic security, are grow rooms full of Shishkaberry, CBD God Bud and Cold Creek Kush, cultivars valued for their sleep-inducing, antidepressant and stress-relieving properties, respectively.

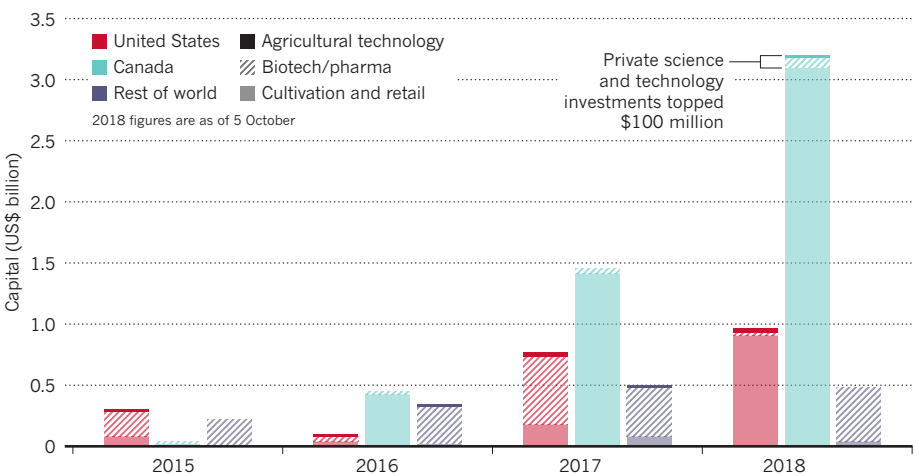
Nearly all the plants are unpollinated females, called 'sinsemilla' (meaning 'without seed'), that produce the highly potent flowering tops, or buds, rich in THC and other cannabinoids. Males, with their pollen-filled sacs, are not only superfluous — the female plants are all propagated through cuttings — but also avoided for fear of scrambling genes in an uncontrolled way.

Yet, TerrAscend has a small space in the back reserved for males. Back in June, the company launched a research and development arm in conjunction with scientists at two Ontario universities who will use the sequestered plants for experiments that are standard in agriculture but have rarely been done with cannabis. The scientists will mate the plants, coax them to produce seeds and then expose the seeds to chemical mutagens in the hope of finding new desirable traits — pest resistance, say, or increased tolerance to environmental stresses such as drought.

Scientists involved with the TerrAscend

A SMOKING-HOT SECTOR

Business deals in the cannabis industry have spiked in Canada since the nation decided to legalize recreational use. But most of the capital has gone to companies involved in growing and selling. Canadian companies' investments in research and development are catching up to the levels seen elsewhere in the world.



SOURCE: VIRIDIAN CAPITAL ADVISORS

THE WIDE WORLD OF WEED

Legal restrictions haven't completely stymied cannabis research.

Although cannabis is now legal in some form in all US states but one, the plant, including hemp, is still illegal at the federal level — and all research conducted on cannabis at any university must abide by federal regulations, or else jeopardize government funds for the institution.

Extracted DNA samples are permitted, and the government is gradually becoming more permissive of hemp cultivation. But any work on the basic biology of cannabis with higher levels of the psychoactive substance tetrahydrocannabinol (THC) is strictly off-limits, and there's no funding available for non-health-related research on the plant. "Everything we've done has been absolutely shoestring," says plant biologist George Weiblen at the University of Minnesota in Saint Paul.

That's led to a few creative workarounds. In Colorado, where cannabis has been legal for recreational use since 2014, evolutionary geneticist Daniela Vergara, at the University of Colorado Boulder, created a foundation to support projects she can't do on campus. Unlike her university, the foundation can accept funding from the cannabis industry — money she then spends on sampling DNA from cannabis plants bred at local dispensaries and growers. Although she can analyse DNA sequence data at the university, "I don't touch the plant on campus or during my working hours," Vergara says.

Even at the University of Mississippi in Oxford, which is licensed by the federal government to grow cannabis for health studies, researchers are operating under restrictive rules. The university can procure plants only from federally approved vendors, and so it has no access to the THC-rich varieties commonly found in recreational and medicinal cannabis shops. Instead, scientists there are using chemical stimulants to boost THC levels in moderate-strength plants. "My hands are tied," says Mahmoud ElSohly, who oversees the operation. "I have to work with what I have."

Even in Uruguay where cannabis is completely legal, hurdles still exist. In 2013, the country became the first to legalize cannabis for recreational use, but researchers have faced bottlenecks in gaining access to the plant, according to an analysis[12] in March, and there's no public money to foster research. "I get very, very little funding," says Astrid Agorio, a plant molecular geneticist at the Clemente Estable Institute of Biological Research in Montevideo who is attempting to exhaustively profile the genetic structure of two varieties of Uruguayan cannabis with a grant of about US$6,000.

In Australia, where medical use is allowed, plant geneticist Graham King at Southern Cross University in Lismore has found both public and private support. He obtained a licence from the state of New South Wales to grow cannabis, and financing from an industrial hemp company to chemically characterize a global collection of cannabis varieties[13]. "We want to understand what the scope is for metabolic engineering," King says.

But nowhere has been as supportive of cannabis research as Israel. It was here that Raphael Mechoulam, an organic chemist, isolated THC and cannabidiol and determined their chemical structures in the 1960s. The country has since sought to establish itself a world leader in the study of medical cannabis. And unlike in some countries, including Canada, where government scientists still essentially can't access high-THC varieties of the plant, Israel's agriculture ministry last year built a national centre for medical-cannabis research.

Housed at the Volcani Center in Rishon LeZiyyon, the government's cannabis farm includes thousands of plants spread across several greenhouses and indoor growing facilities. There, plant biologist Nirit Bernstein is trying to perfect cultivation and weed out bad practices. "We have to develop science-based protocols for optimizing the cultivation of this magical plant," Bernstein says. "There's very little scientific information that's available." **E.D.**

spin-off, including plant geneticists Peter McCourt and Shelley Lumba at the University of Toronto, plan to mutagenize six varieties of cannabis with the aim of obtaining improved versions of some of the company's go-to stock. "Our main goal," says Lumba, "is to make cannabis into a real horticultural crop."

Another decades-old practice for improving agricultural plants involves intentionally doubling or tripling their genomes, which tends to give plants bigger cells, larger structural features and greater yields of chemical compounds. Domesticated wheat species, for example, have 4–6 copies of their genome; sugar cane can have as many as 16. And although most modern farmed plants had their DNA multiplied simply through hundreds of years of cultivation, there are ways to speed up the process. All cannabis varieties characterized so far have only two sets of the genome — all, that is, except for a handful of plants growing at Canopy Growth Corporation in Smiths Falls.

There, plant molecular geneticist Shelley Hepworth at Carleton University in Ottawa and her former graduate student used a cell-cycle-disrupting herbicide to trigger five varieties of cannabis to double their normal chromosome count. At first blush, says Hepworth, "the plants are definitely bigger". But the scientists still need to finish their analyses to determine whether the 'tetraploid' cannabis lines have elevated levels of THC, CBD or other cannabinoids.

A more modern plant-breeding technique — one that dates back to the 1980s — is known as marker-assisted selection. It involves finding genetic signatures associated with a desirable trait — high essential-oil content, say, or automatic flowering under any light condition. Scientists can then use DNA analyses to quickly 'preview' which seedlings should have optimal properties instead of waiting months for the plants to mature.

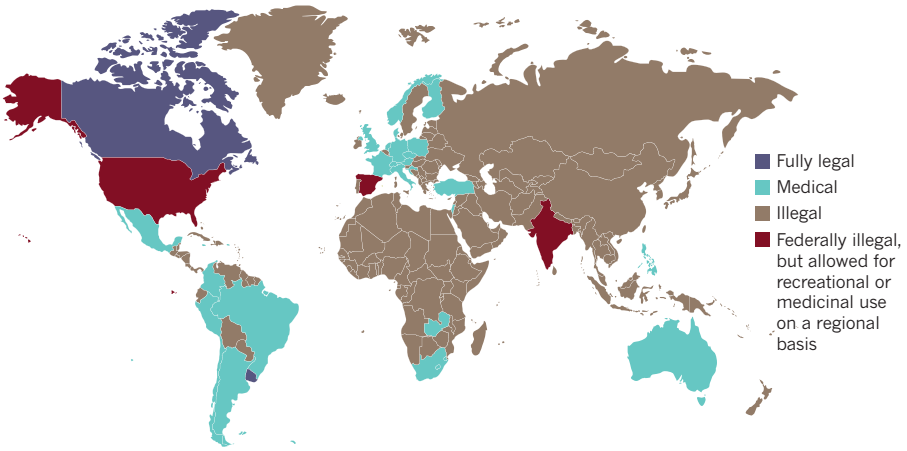
"THIS YEAR IS A TURNING POINT FOR CANNABIS MOLECULAR GENETICS."

Only a small number of markers exist for cannabis, however, in large part because few researchers have ever looked for them. One that has been described comes from George Weiblen, a plant biologist at the University of Minnesota in Saint Paul and one of the few academics in the United States who has a federal licence to grow cannabis — but he's restricted to just 50 plants at a time (see 'The wide world of weed'). It took him 12 years to determine the inheritance pattern of genes that affect drug content and to identify a genetic marker linked to the THC-to-CBD ratio[5]. That's longer than it took Gregor Mendel to work out the laws of heredity, Weiblen says. "Our programme is a poster child for the absurdity of cannabis research in the United States."

A complete cannabis genome would make it easier to identify informative DNA markers. But early efforts have yielded maps that are patchy and incomplete, says Kevin McKernan, chief science officer and founder of Medicinal Genomics in Woburn, Massachusetts, who did some of the early work. He (see go.nature.com/2kpzgkc) and Page[3] released maps

A BUDDING TREND

Political movements to decriminalize cannabis for medical and recreational use have been gaining in popularity. The result is a slowly shifting patchwork of regulations as many countries (and sometimes smaller jurisdictions) start to allow limited use and increasingly liberalize their stance.



SOURCE: ARCVIEW MARKET RESEARCH/BDS ANALYTICS

independently in 2011. "They're train wrecks," says McKernan. But that's changing. And thanks to an international trend toward less restrictive laws around cannabis (see 'A budding trend'), many weed companies are now investing in genetics research.

DANK DNA

"This year is a turning point for cannabis molecular genetics," says C. J. Schwartz, founder and chief executive of Sunrise Genetics in Fort Collins, Colorado — one of at least six companies that say they have come up with fine-scaled genome maps. They have not yet been published yet in a peer-reviewed journal, but McKernan posted a preprint of his map on 10 October[6] and Schwartz expects to make his sequence public by the end of the month.

Then there are scientists who are hoping to engineer new properties into the plant. At Canopy Growth, research and development manager Katya Boudko worked with molecular biologist Douglas Johnson at the University of Ottawa to develop a gene-silencing technology that prevents expression of the THC-synthesis gene. Boudko expects plants to compensate by boosting their levels of CBD — or, she says, "it could potentially produce other cannabinoids that the world doesn't even know about yet".

Boudko has yet to fully test this theory, however. That's because she hasn't managed to grow fully fledged plants from genetically modified tissue — and nor have many others. Because seeds or clippings cannot be genetically modified in a consistent and predictable way, scientists need to culture plant tissue and coax it into producing roots and shoots after the genes have been manipulated. Often, scientists can get the cellular masses to produce fine root hairs, but the shoots have proved particularly problematic.

In 2010, a team from the University of Mississippi in Oxford, where for 50 years researchers have grown all the cannabis used for government-backed health studies in the United States, described a hormone recipe for inducing shoot formation that, it says, works more than 80% of the time[7]. Yet, others say that they can't get the protocol to work on their own varieties. "There are tens of labs that are now working on making the protocol more efficient," says Leor Eshed-Williams, a plant developmental geneticist at the Hebrew University of Jerusalem.

For many in the industry, however, any suggestion of genetic modification is an anathema. Besides, says Ethan Russo, director of research and development at the International Cannabis and Cannabinoids Institute in Prague, "this plant is so malleable that a lot of these genetic modifications are really unnecessary". Modern selective-breeding strategies, he argues, should suffice — and those methods needn't require genetic markers. In collaboration with Mark Lewis, president of Napro Research in Westlake Village, California, Russo has used chemical profiling to create dozens of cannabis varieties with unique properties and elevated yields[8].

> "THE PLANTS, AT THE END OF THE DAY, NEED LOVE."

HASHING OUT THE BASICS

Elsewhere, researchers are looking to control and fine-tune environmental conditions at various stages of the growth cycle. Such tweaks could prove important for maximizing profits from costly indoor growing operations that are a major source of high-end cannabis in Canada. At CannTx Life Sciences in Puslinch, operations head Jeff Scanlon and his colleagues developed a system for air circulation.

Scanlon showed that the fans found in most companies' grow rooms move air over the plant crowns. But in the thicket of leaves and branches beneath, the air remains stagnant, leading to pockets of elevated temperature and humidity that breed fungal pathogens. The solution: a pressure gradient from floor to ceiling that ensures airflow along every surface of the plant. "It's a very simple innovation," Scanlon says.

Deron Caplan, director of plant science at Flowr in Lake Country, completed a PhD this year in which he systematically determined optimal rates of fertilizer supply at various stages of cannabis production[9,10] and best practices for propagating the plant clonally through cuttings[11]. "It's very crude, incremental advancements that we're making," says Mike Dixon, an agricultural scientist at the University of Guelph and one of Caplan's graduate advisers. But it's slowly helping to phase out the homespun practices that persist throughout much of the industry, he says — "what I kindly refer to as anecdotal bullshit".

Some of the holdovers of illegal cultivation are on display at Beleave, a cannabis producer in Hamilton, where master grower Shane Whelan-Stubbs has persevered with some practices for 20 years, first in a bedroom cupboard, then in basements, warehouses and now a legitimate business. Whelan-Stubbs is open to the science, and Beleave will soon start collaborating with a team at Guelph, where scientists hope to open the country's first dedicated academic centre for cannabis research some time next year. Still, Whelan-Stubbs continues to water his plants by hand. "The plants, at the end of the day, need love," he says.

Page would argue that they also need science. But the burgeoning research that he's been a part of likewise would have long ago withered without cannabis at its centre. "We think of it in many ways as a drug or a pharmaceutical," he says, "but can't forget that it's the plant that's at the heart of this revolution. It all comes down to a plant." ■

Elie Dolgin *is a Canadian-born science journalist in Somerville, Massachusetts.*

1. Günnewich, N. *et al. Nat. Prod. Commun.* **2**, 223–232 (2007).
2. Gagne, S. J. *et al. Proc. Natl Acad. Sci. USA* **109**, 12811–12816 (2012).
3. van Bakel, H. *et al. Genome Biol.* **12**, R102 (2011).
4. Sawler, J. *et al. PLoS ONE* **10**, e0133292 (2015).
5. Weiblen, G. D. *et al. New Phytol.* **208**, 1241–1250 (2015).
6. McKernan, K. *et al. OSF Preprints* https://doi.org/10.31219/osf.io/7d968 (2018).
7. Lata, H., Chandra, S., Khan, I. A. & Elsohly, M. A. *Planta Med.* **76**, 1629–1633 (2010).
8. Lewis, M. A., Russo, E. B. & Smith, K. M. *Planta Med.* **84**, 225–233 (2018).
9. Caplan, D., Dixon, M. & Zheng, Y. *HortScience* **52**, 1307–1312 (2017).
10. Caplan, D., Dixon, M. & Zheng, Y. *HortScience* **52**, 1796–1803 (2017).
11. Caplan, D., Stemeroff, J., Dixon, M. & Zheng, Y. *Can. J. Plant Sci.* **98**, 1126–1132 (2018).
12. Hudak, J., Ramsey, G. & Walsh, J. *Uruguay's Cannabis Law: Pioneering a New Paradigm* (The Brookings Institution, 2018).
13. Welling, M. T., Liu, L., Shapter, T., Raymond, C. A. & King, G. J. *Euphytica* **208**, 463–447 (2016).