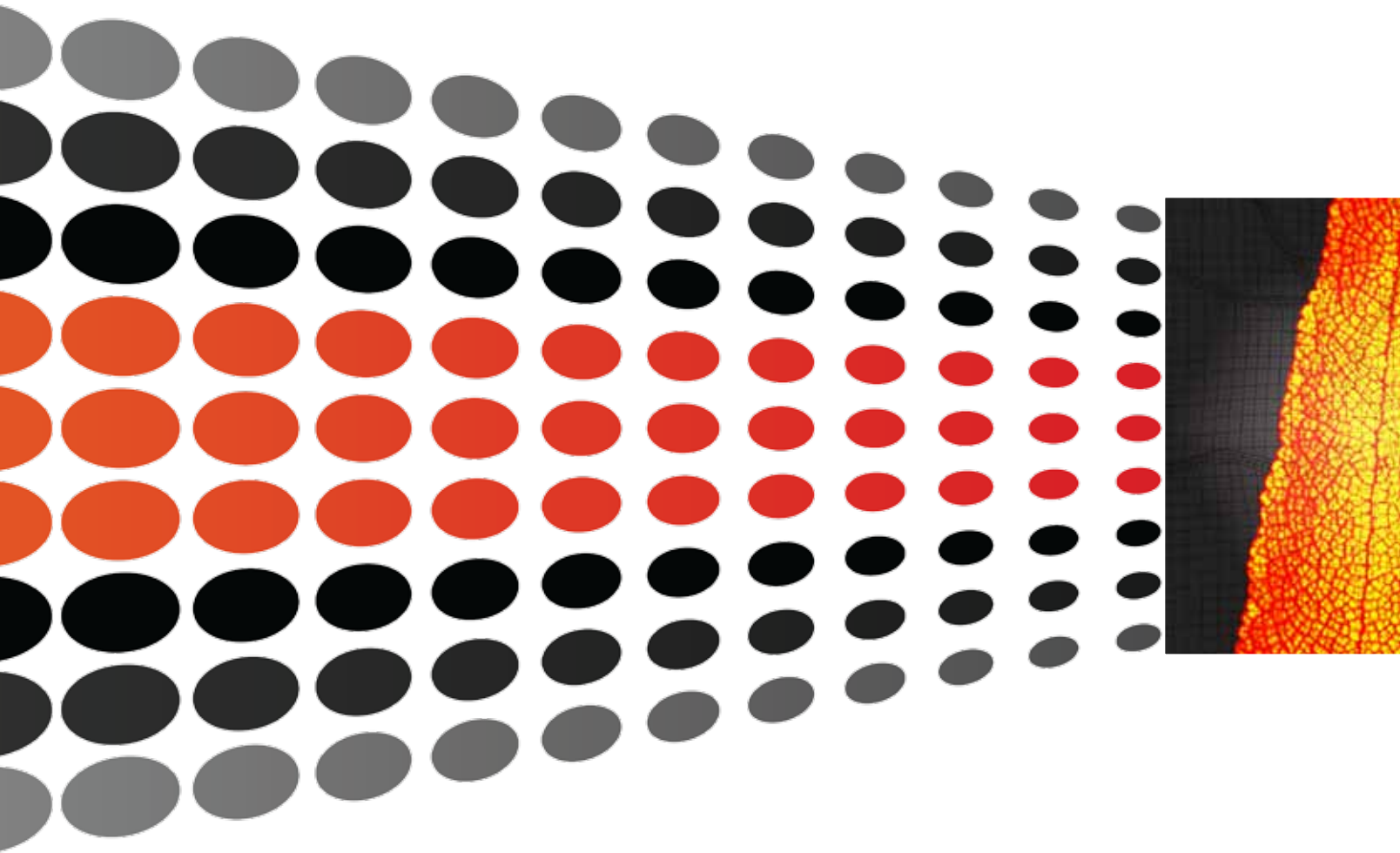# Taking the

Phenomics Revolution

into the Field

By Karl Haro von Mogel

The genomics revolution is racing ahead at full speed. Complete genome sequences now cost a fraction of what they were when the Human Genome Project

started in 1990. The DNA in any plant variety influences how it will develop and grow in the face of multiple environmental challenges including soil type, weather, nutrition, and pests and diseases. Plant breeders can use this DNA information to associate the performance of plants, both in the greenhouse and the field, with the specific pieces of DNA carried by different varieties, even predicting performance before a seed is planted.

But with all the power of current genotyping technologies, there is a limit to our ability to make strong connections between a plant's genes and its traits—our ability to measure the traits themselves.

This situation is changing rapidly. Scientists around the world are making new connections among disciplines to develop and test technologies that can bring a data revolution to

collecting plant phenotypes. And they are doing it in the most difficult and important environment—the field.

Balancing Technologies

Whether you are studying the basic biology of a plant, or breeding new crop varieties for farmers, the phenotype is the center of attention.

Phenotypes are the physical characteristics of an organism, which result from its genes, environment, and how these two factors interact. A phenotype can be everything from the height of a plant, to its chemical composition, yield, or response to specific environmental factors.

Long before the discovery of DNA and its role in heredity, farmers, breeders, and horticulturalists selected desirable plants based on the phenotype. Even with today's sophisticated genetic research, which regularly sees whole genomes published, it is what

the genes do to the phenotype that matters.

Measuring phenotypes of plants can be challenging. The subtle effects of genes that add up to determine quantitative traits can be easily missed and can require many repeated measurements to uncover. In addition, the structure of an entire plant can be difficult to quantify. Edgar Spalding, a professor in the Botany Department at the University of Wisconsin–Madison, says that compared with DNA sequencing technology, phenotyping technology is still rather crude.

"It is not much of an exaggeration to say that a ruler is used, and that's not high throughput."

Spalding is a principal investigator for the Phytomorph project, which seeks to turn delicate developmental phenotypes into quantifiable measures. One of his new tools is a robotic camera that photographs growing seedlings and roots at regular intervals, with micron-level precision. His team tracks the root growth of *Arabidopsis*, maize, and tomato seedlings, looking for changes in their response to gravity.
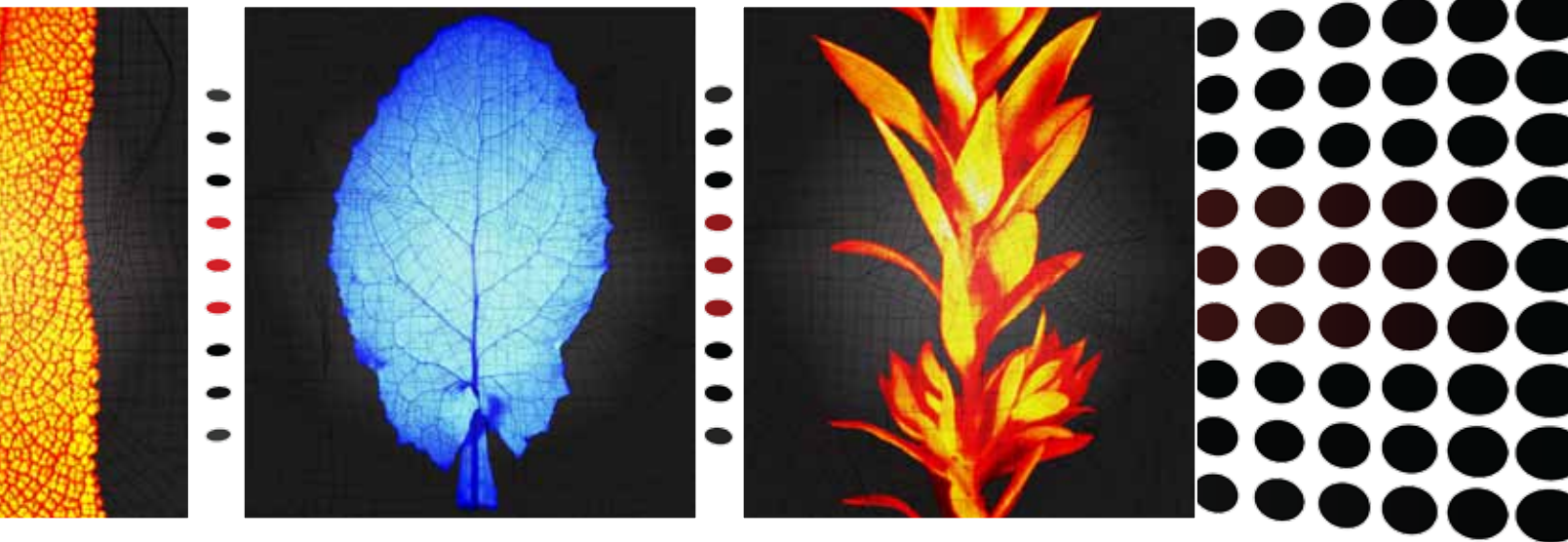
Center images above by Erica Seccombe, courtesy of the High Resolution Plant Phenomics Centre. Backdrop image: iStockphoto.

One of the new tools used by Edgar Spalding, principal investigator for the Phytomorph project at the University of Wisconsin–Madison, is a robotic camera that photographs growing seedlings and roots at regular intervals, with micron-level precision.

"Through electronic image capture and computer analysis, we can see how long even the tiny *Arabidopsis* root grows in five minutes," Spalding says.

"It may not be obvious, but in my view, when we have rich, high-throughput phenotyping, we are going to find out which component of that phenotype set has predictive power over something in the field."

Spalding stresses the importance of raising the capabilities of phenotyping tools. "Anything we can do to make the phenotyping activities more automated and more high throughput will make studies of genotype–phenotype relationships more effective.

"When was the last time you heard someone say that their phenotype data was wonderfully large and complex, but their genotype information needs more detail?"

The Phenomics Frontier

Phenomics, the study of complete phenotypes and how they change over time, is already underway. Companies such as Germany-based LemnaTec have developed systems for phenotyping individual plants in large, robotic greenhouses. Using technologies combining photography, fluorescence imaging, 3D image analysis, and data handling, thousands of individual plants can be grown and

automatically tracked through their development. A conveyor constantly moves potted plants around the greenhouse and through a series of scanning chambers.

With tools like these, physiologists can study how different genotypes respond to stressful environmental conditions and efficiently observe the life cycle of each plant. There are many such greenhouse facilities around the world. However, there are many differences between the controlled conditions of a greenhouse and the variable nature of the field. Studying crucial traits in the field will require turning it into a laboratory.

"It's one thing to use a glass house for a trait that is expressed early in development and at the individual plant level, but a lot of traits that we are interested in are expressed at the community scale, which means you have to be working in field plots," says ASA Fellow Jeffrey White, a research plant physiologist at the USDA Arid-Land Agricultural Research Center in Maricopa, AZ.

White was the lead author on a review of field-based phenomics for plant genetics research recently published in *Field Crops Research* and organized a symposium on field-based high-throughput phenotyping at last year's ASA, CSSA, and SSSA International Annual Meetings in Cincinnati, OH. He explains that many researchers are developing ways to gather phenotypic data in the field, using a variety of approaches. These vary from tractor-based sensors to cameras mounted on irrigation cranes that roll

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across the field, imaging a crop like a giant flatbed scanner, or robots that crawl along cables above the plants. From the air, cameras have been mounted on blimps, full-sized aircraft, and remote-controlled helicopters.

The array of tools that can be deployed is just as impressive. Technologies can include visual light and infrared cameras as well as ultrasonic sonar and its laser-based equivalent known as LIDAR.[1] Geographic Information Systems (GIS) can help align data to the right plots, and infrared thermometers can measure plant temperatures. White raises the possibility of low-power X-rays being tested. He likens these developments to being in the Wild West, where there are almost no rules.

"There are so many great technologies coming out all the time. Who would have thought you could have a 3D camera?"

Wading through the available technologies is the first hurdle, White acknowledges.

"How do you decide what is intellectually neat but not useful? There's going to be many years of research figuring out what works and what doesn't."

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[1] Short for Light Detection and Ranging. It's an optical remote-sensing technology to study canopy architecture.

He adds that a given phenotyping approach may work better for one crop than for others.

"Soybeans and corn are easy targets, but it would be more challenging to examine every wheat plant because of the tillers."

Next, it takes testing the limits of a system with real-world experiments.

"It's one thing to have a proof of concept, and another thing to have something that will work robustly in the field."

White is also part of a team at the University of Arizona that is developing a way to analyze phenotypes in the field from the seat of a tractor, which is already having good results.

Tracking Plants with Tractors

ASA, CSSA, and SSSA member Michael Gore was formerly a research geneticist at the USDA Arid-Land Agricultural Research Center in Maricopa, AZ and has recently accepted a faculty position in the Department of Plant Breeding and Genetics at Cornell University. He worked with Pedro Andrade-Sanchez, an assistant professor in the Department of Agricultural and Biosystems Engineering at the University of Arizona, on developing a high-clearance tractor outfitted with an array of sensors to study drought and heat tolerance in cotton. When Gore first came to Arizona, he was faced with the challenge of quickly and accurately measuring many phenotypes in the field.

He needed to record several kinds of data at once, which he realized would require an army of technicians—each with expensive equipment. One day, he saw a high-clearance tractor outfitted with a handheld device that can record the "greenness" of the crop based on its reflectance, a trait called normalized difference vegetation index (NDVI), and a light bulb went on in his head.

"The more I drilled into it I realized it could be entirely possible to build a high-throughput platform to measure canopy temperature, reflectance, and height," Gore recalls.

Companies such as Germany-based LemnaTec have developed systems for phenotyping individual plants in large, robotic greenhouses. Using technologies combining photography, fluorescence imaging, 3D image analysis, and data handling, thousands of individual plants can be grown and automatically tracked through their development. A conveyor constantly moves potted plants around the greenhouse and through a series of scanning chambers. *Photo by LemnaTec*.

This high-throughput phenotyping system developed at the USDA Arid-Land Agricultural Research Center in Maricopa, AZ is being used to collect plant height, canopy temperature, and canopy reflectance data from cotton plants. *Photo by Michael Gore*.

The tractor they built can measure the temperature of the plants themselves with infrared thermometers and use ultrasonic proximity sensors to get the height of the plants. They are also testing out LIDAR, an optical remote-sensing technology to study canopy architecture.

Driving the tractor through a field of cotton with more than 600 plots that are 28 ft high takes less than an hour and allows Gore to precisely measure how the plants in his experiment respond to heat and drought under sufficient and limited irrigation regimes. And the system allows him to capture this data four times a day throughout the growing season—a feat never possible before.

"Its greatest power is in visualizing traits that cannot be easily scored. Canopy temperature can change very rapidly, so there is a narrow window for robust measurements," Gore says.

He adds that his data give him a true average of the whole plot, rather than measuring a few representative plants. Since every data point is attached to its geographical position, the effects of edges and alleys on plants can be eliminated by trimming it out of the data.

"We have pretty good confidence in what we're doing," Gore says.

With his recombinant inbred population and his phenotyping platform, Gore says these high-throughput traits are heritable, and the quantitative trait loci for these traits can be mapped. He is excited about the prospect of using this technology to measure phenotypes in many environments and answer some fundamental biological questions.

"What I would love to do is to have a high-throughput phenotyping platform that can be cloned and then used by breeders all over the United States. We can use the same platform to target the same traits and really investigate genotype by environment interactions on a national scale."

Flying High with the Phenocopter

On the other side of the world, researchers at CSIRO (the Commonwealth Scientific and Industrial Research Organisation) are also interested in capturing field phenotypes *en masse*. CSIRO's approach takes them into the realm of automated flying robotics. By modifying a remote-controlled gas-powered model helicopter, the researchers have developed a versatile autonomous platform called the "phenocopter."

ASA and CSSA member Scott Chapman is a principal research scientist at CSIRO and adjunct professor at the University of Queensland's Queensland Alliance for Agriculture and Food Innovation. Leading a team working on breeding for adaptation to climate change, he is studying wheat and its response to environmental stresses such as heat and drought. While CSIRO colleagues at the Australian Plant Phenomics Facility[2] work mainly on ground platforms, Chapman wanted to measure the temperatures of an entire field of plants quickly.

"The only way I could think about how to do it was to do it from the air," he says.

Hiring a piloted helicopter to fly over the field is expensive and disruptive to the plants. But Chapman's colleague, Torsten Merz from the CSIRO ICT Centre,[3] had already developed an autonomous helicopter-based system for surveying power lines, so they modified it for applications in crop research. Underneath the helicopter, they installed several cameras—visual, near-infrared, and far-infrared (thermal)—and triggered them in pre-planned repeatable missions.

The phenocopter flies by itself, making multiple passes over a 1-ha field in just six minutes, providing data that can help Chapman measure plant height, canopy cover, lodging, and temperature throughout a

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[2] See www.plantphenomics.org.au/HRPPC.

[3] See www.csiro.au/ICT.

Researchers at CSIRO use a remote-controlled gas-powered model helicopter called the "phenocopter" to measure plant height, canopy cover, lodging, and temperature throughout a day. Pictured here are Scott Chapman (left), a principal research scientist at CSIRO, and Torsten Merz, developer of the phenocopter.

day. Along with temperature sensors placed throughout the field, he says that he can see how plants respond to stress.

"It allows us to see which plots are heating up faster, so we can see which plants are modulating their water use. Over time, the ones that can't modulate their water use will get hot because they run out of water supply."

Working with autonomous helicopters takes some additional software, Chapman notes.

"You need software to plan missions when you arrive, to gather the images, and to extract data for plots within the images."

But this approach, he says, can afford some advantages. Phenotype-gathering helicopters can be pro-

grammed to measure experimental trials in farmers' fields and return the needed data. Because it is a fully automated robot, the CSIRO helicopter can fly "beyond visual range."

In the near future, Chapman believes that small aerial robots will make their own decisions about when to fly based on the weather or follow a breeder's instructions to fetch live photos across the field and beam them to a field tablet.

Sorting Out the Data

Handling large amounts of data and making sense of it presents a big challenge for high-throughput phenotyping. The phenocopter, for instance, only generates about three gigabytes

of data per flight. Spalding has taken hundreds of thousands of images of developing seedlings with his robotic camera, adding up to terabytes of storage. And while it currently gathers data in the gigabyte range, Gore could easily see his tractor platform produce a terabyte of data per run.

"A major problem is that right now we don't have a good data management system in place," White says.

He is looking to the iPlant Collaborative[4] and CGIAR's Integrated Breeding Platform for assistance with managing phenotypic data.

Spalding has an interest in developing the cyberstructure necessary to handle the data being generated in the field. He says scientists have to think about how to move data from where it is collected to where it will be stored and processed for the investigator. "Thumb drives are not the way to do this efficiently. We have to conceive of a set of technologies that will support phenotyping activity in high throughput, robustness, and automation," Spalding says.

As a principal investigator on a grant from the Office of Cyberinfrastructure at the National Science Foundation, Spalding is seeking to identify the most limiting steps for high-throughput phenotyping.

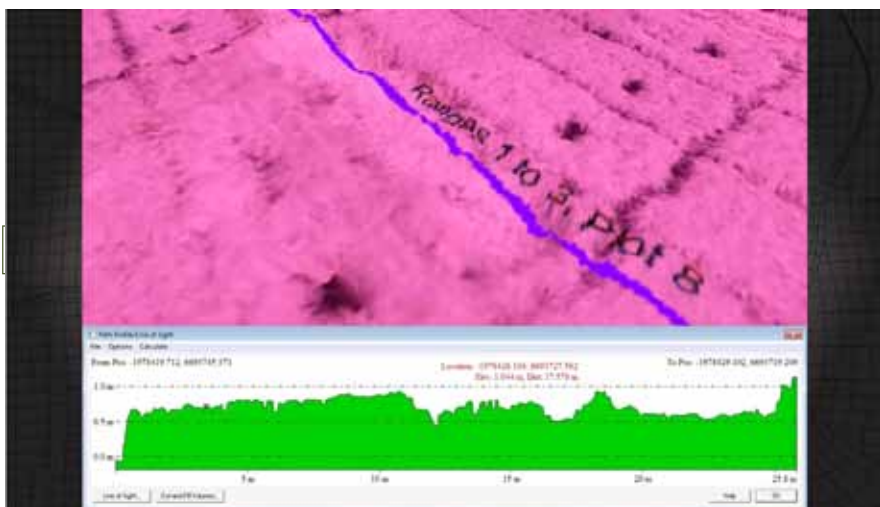"Our goal is to come up with a blueprint for the kind of cyber infrastructure that will make it more efficient."

Thumb drives are not the way to [manage data] efficiently. We have to conceive of a set of technologies that will support phenotyping activity in high throughput, robustness, and automation.

[4] See www.iplantcollaborative.org.

Plant height data collected by the near-infrared camera on the pheno-copter can be used to estimate lodging across plots. *Images courtesy of Scott Chapman, CSIRO*.

By collaborating with researchers working in labs and fields, and with different types of data, Spalding hopes to find patterns in what is rate limiting in this process.

"If you have ideas, I would like to invite you to participate in this project!"

Ultimately, Chapman would like to see the phenotype results right away.

"With the many current systems, it takes hours or days to process the data, whereas if we can process it live and extract all the data for each plot, then we can make a decision right there whether to fly the phenocopter again for more measurements. We generate even more phenotypes by running computer simulations of plots based on the image data."

Analyzing the data can also present a significant challenge. Chapman is currently experimenting with extracting wheat flowering time and lodging scores from aerial photographs, and White's colleagues in Arizona are working on transforming LIDAR data into a useful description of canopy architecture. These require a heavy investment in computer science.

With new sensors for measuring plants, we can derive new traits that were not considered before because they were not seen, Gore explains.

"If you were to correlate a spike in reflectance with an important trait, that is a new trait you can use for breeding," he says. "It changes your concept of what is a phenotype."

As computer science is increasingly used to assemble and describe the data, some associations will seem strange and non-intuitive. Spalding describes how this transition takes place when he measures the phenotype of a seed with a three-dimensional array of CCD (charge coupled device) cameras.

"Imagine that seed now has 40 numbers associated with it from our three-dimensional image of the seed. We describe that seed with a vector. We don't even have a physical concept of what some of those numbers mean other than length, width, and color. They're all just mathematical transformations of numbers, but perhaps some linear combination of them will actually, for reasons we don't understand, have some correlation with important traits such as leaf angle, planting density, and so on.

"That's the mind shift that phenotyping technologies are bringing. There's value in measuring phenotypes together to get what we want, and later if you want to, you can figure out why. It will lead to new hypotheses. It is going to cause people to come up with new ideas for explanations. What drives it will be its utility in improving selection."

Sifting through all this data will be no easy task though, Gore points out.

"Soon we will have hundreds to thousands of traits gathered by technologies such as fluorescence, imaging, and spectroscopy. What do you do with that next? How do you capture the most information content from that and apply it to your breeding program? That question needs to be answered sooner rather than later before high-throughput phenotyping can have a big impact on breeding."

Universally, they agree that developing these technologies requires connections not only between plant scientists, but also with agricultural engineers and computer scientists. For these pioneering efforts, a little creativity goes a long way.

Chapman, for instance, had once considered being an engineer, but now he is able to bring that interest into his work in the field.

"I get to see the most modern, cutting edge of wireless and robotics applied to food production, which is the most ancient technology humans developed. The molecular labs do this all the time, but to do it 'live' in the field is something different."

Gore adds, "The whole world is in a sense wide open. I think that in the next few years, we will see some really interesting advances in high-throughput phenotyping that people haven't even thought about yet and that will be really fun."

Karl Haro von Mogel, contributing writer for CSA News *magazine*